Genetics Applied to Forestry
An Introduction

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Cover photos. Above: A *Pinus sylvestris* stand in Central Sweden, photograph Britt Ekberg-Eriksson. Below: A profusely flowering *Tilia cordata* tree in Central Sweden, photograph Inger Ekberg.

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Preface

This book is a follow-up of An Introduction to Forest Genetics. It is somewhat expanded compared to the book printed in 2007. We were encouraged to ”publish” the revised version of the textbook on the internet. Undergraduate students are the target group as well as graduate students with limited experience of forest genetics. Without the advice and help from Kjell Lännerholm, Björn Nicander, Johan Samuelsson and Hartmut Weichelt the editing would have been more troublesome. We express our sincere thanks to them.

A generous grant from Föreningen Skogsträdsförädling, The Tree Breeding Association in Sweden, made this printing possible. A web version of this book may be found under http://vaxt2.vbsg.slu.se/forgen/Forestry_Genetics.pdf

Uppsala December 2013
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Content

Chromosome cytology

In this chapter we deal with chromosomes and basic concepts of the Mendelian genetics. We present further the two main types of nuclear divisions, the asexual nuclear division - mitosis - occurring in the somatic cells and the sexual nuclear division - meiosis - occurring in the gamete-forming tissues. Different types of chromosomal aberrations will be presented. Finally, the time of meiosis during the year and weather induced injuries in meiosis will be highlighted.

Chromosome cytology deals with microscopic studies of chromosome number, size, morphology and chromosome behaviour during nuclear divisions.

Already in the middle of the 19th century, the chromosomes were dicovered. They got the name chromosome because they became visible when stained with basic dyes, usually red. Cytology advanced rapidly during the latter part of the 19th century thanks to the improved light-microscope technique. More knowledge of the chromosomes could be acquired. The main features of fertilization at the cell level in animals and plants were revealed at that time. The divisions of the cell nucleus were described, both in the somatic cells - mitosis - and in the germ cells - meiosis.

Very soon after the rediscovery of the laws of Gregor Mendel, it became clear that the chrosomomes were the potential carrier of the genes. The final proof was presented by the American geneticist Thomas Morgan and his co-workers Calvin Bridges and Alfred Sturtevant during the 1910s and onwards. Thus, in 1916 when studying the inheritance of the eye colour in the fruit fly (*Drosophila melanogaster*), Bridges observed that rare exceptions from the expected segregation in the progeny appeared when a large number of individuals was studied. He could verify that the exceptions were caused by the formation of abnormal egg cells with two X chromosomes (sex chromosomes, see below) instead of normal egg cells with one X chromosome. This provided an unequivocal evidence that genes are located on the chromosomes. Morgan's group further observed that genes were not always inherited independently but sometimes behaved as if they were linked. Morgan's group also revealed that exchanges of chromosome segments between two homologous chromosomes occur, a phenomenon called crossing-over (for the meaning of homologous chromosomes see Fig. 1-2).

Because of their size, the chromosomes have mainly been studied in the light microscope, using preparations of very thin sections from various tissues, or squash preparations where the cells are pressed (squashed) into a unicellular layer. Thanks to these light-microscope studies, chromosome number, size and morphology in a large number of plants and animals are now known. Studies in the 1960s and onwards using electron microscopy, have provided important information on the fine structure of the chromosomes.

Our understanding of the nature of gene action at the biochemical level is based on studies initiated already during the 1930s. In the beginning of 1940s, the American geneticists George Beadle and Edward Tatum launched the one gene - one enzyme hypothesis. Today we prefer to speak about one gene - one polypeptide since later studies have shown that many enzymes contain two or more polypeptides each being a product of a specific gene (see also Chapter 2). A polypeptide consists of amino acids.

The identification of the macromolecule, deoxyribonucleic acid, DNA, as the carrier of the hereditary information meant a great breakthrough for the genetic research in the middle of 1940s. The next great breakthrough came in 1953, when the double-helical structure of the DNA molecule was elucidated, see Chapter 2.

Karyotype

The karyotype of a species describes its chromosomes including chromosome number, size and morphology. In some instances, the karyotype can provide information on the relationship between species (Fig. 1-1).

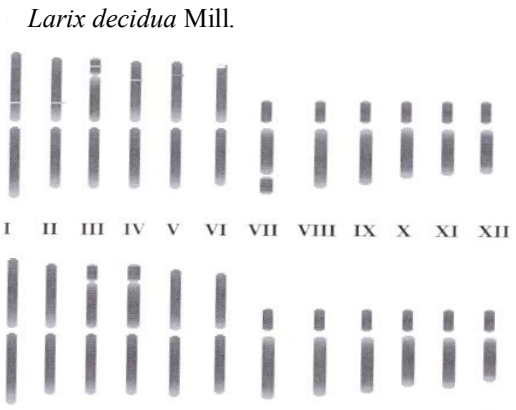


Figure 1-1. Ideograms, diagrammatic illustrations of chromosome morphology of two Larix *species.*

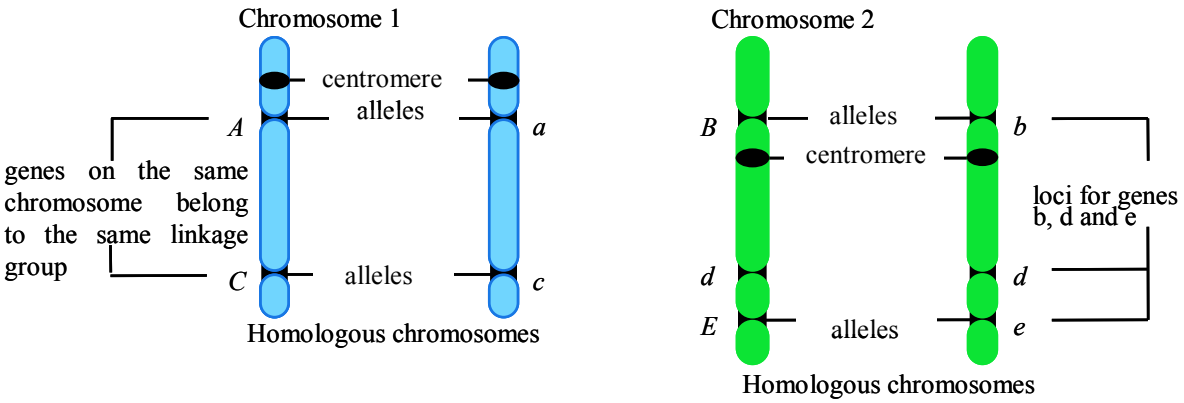


Figure 1-2. Two pairs of homologous chromosomes are shown. Genes with dominant alleles (capital letters) and genes with recessive alleles (small letters) located at loci in the same or different linkage groups are indicated.

In gymnosperms, nearly all species have two sets of chromosomes in their cells. The chromosomes appear in pairs. The chromosomes of such a pair are said to be homologous (Fig. 1-2). On every chromosome there is a centromere which can usually be seen as a constriction at a specific site on the chromosome. The centromere pulls the chromosome to one of the two spindle poles of the cell during the nuclear division. The centromere can be found anywhere on the chromosome and it divides the chromosome in two arms (except when the centromere is located at the end of the chromosome). If the two arms are similar in length the chromosome is said to be metacentric, if the two arms are of unequal length the chromosome is acrocentric and if the centromere is located at a terminal position, at the telomere, the chromosome is telocentric. Some chromosomes have so-called secondary constrictions or nucleolar organizers associated with nucleoli (involved in ribosomal RNA synthesis) that in some species serve as useful landmarks for the identification of individual chromosomes.

Typically, pine and spruce chromosomes are metacentric and their length very similar, and they often lack other landmarks that can be useful in cytogenetic studies. Because of this, the individual chromosomes are difficult to identify in common squash preparations. Recently, however, new techniques have been developed using *in situ* hybridization combined with fluorochrome staining that

Table 1-1. Chromosome number in some woody plants in Scandinavia
x = monoploid number, 2x = diploid , 3x = triploid, 4x = tetraploid

Hardwoods			
Acer platanoides	2x = 26	*Populus tremula*	2x = 38
	3x = 39	*Quercus robur*	2x = 24
Alnus glutinosa	2x = 28	*Salix* sp	2x = 38
Alnus incana	2x = 28		4x = 76
Betula nana	2x = 28	*Sorbus aucuparia*	2x = 34
Betula pendula	2x = 28	*Sorbus intermedia*	4x = 68
Betula pubescens	4x = 56	*Tilia cordata*	2x = 82
Fagus silvatica	2x = 24	*Ulmus glabra*	2x = 28
Fraxinus excelsior	2x = 46		
Conifers			
Juniperus communis	2x = 22	*Pinus sylvestris*	2x = 24
Larix decidua	2x = 24	*Taxus baccata*	2x = 24
Picea abies	2x = 24		

allow the identification of all 12 pairs of chromosomes (*e.g. Pinus elliottii*). Behind this newly-awakened interest in karyotype studies lies the need to assign linkage groups to physical chromosomes to be able to integrate physical and genetic maps.

In most conifers native to the northern hemisphere, the chromosome number is 24, but also 22 or 26 exist. The chromosomes are large and therefore easy to study in a light microscope. In contrast, the hardwoods often have very diminutive chromosomes. In Table 1-1, examples of chromosome numbers in some Scandinavian forest trees are given.

Locus, genes, alleles, homozygosity, heterozygosity, dominant and recessive traits

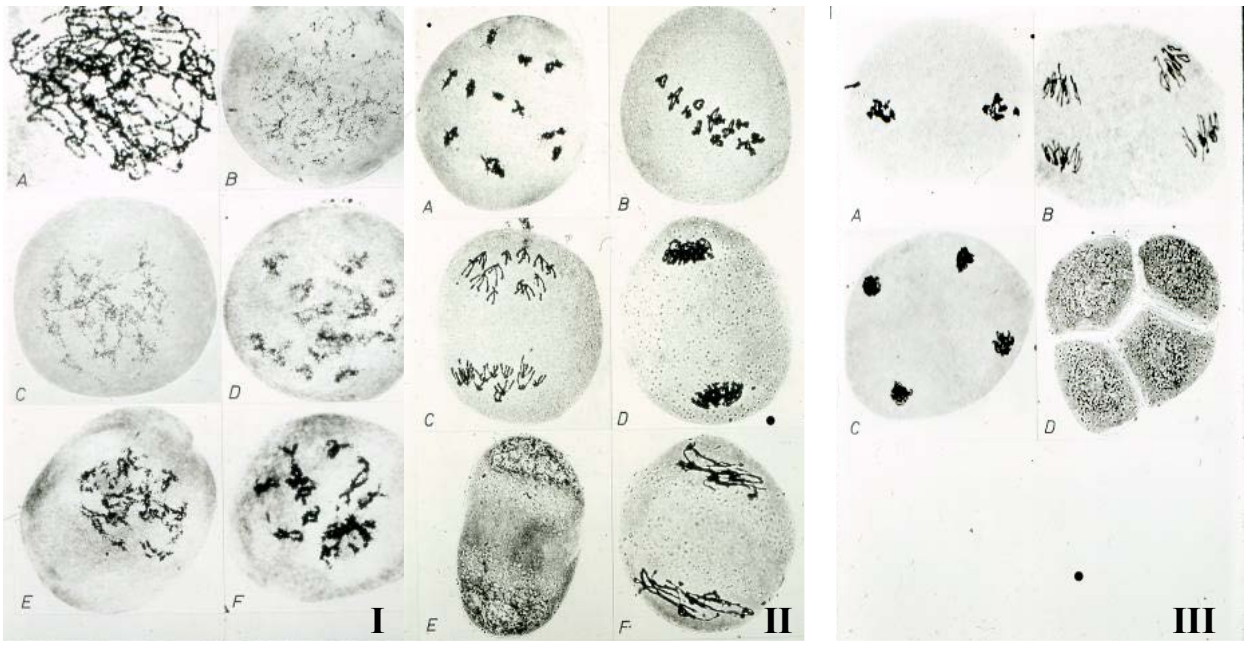
The hereditary units, the genes, are sited linearly on the chromosomes (cf Fig. 1-2). The site on a chromosome, where a certain gene is located, is called a locus (plural loci). Letters are used for symbolizing the genes in accordance with the suggestion of Gregor Mendel. Genes existing in more than one alternate form at the same locus are called alleles. **AA, Aa** or **aa** symbolize genotypes where **A** and **a** are alleles. An individual that carries both the recessive (**a**) and the dominant allele (**A**) is said to be heterozygous. Individuals that have allele **A** or **a** in a double set are said to be homozygous. Individuals with the genotypes **AA** and **Aa** have the same appearance or phenotype if **A** is completely dominant over **a**, while individuals with the genotype **aa** show another phenotype than **AA** or **Aa**. A diploid individual cannot have more than two different alleles at the same locus. But in a population of individuals more than two alleles belonging to the same locus can be found. This situation is termed a series of multiple alleles. We can also talk about polymorphism, *i.e.* when two or more alleles exist at the same locus in a population. With three alleles $\mathbf{a}_1$, $\mathbf{a}_2$, $\mathbf{a}_3$, the following six genotypes will be formed, $\mathbf{a}_1\mathbf{a}_1$, $\mathbf{a}_1\mathbf{a}_2$, $\mathbf{a}_1\mathbf{a}_3$, $\mathbf{a}_2\mathbf{a}_2$, $\mathbf{a}_2\mathbf{a}_3$ and $\mathbf{a}_3\mathbf{a}_3$.

If all these genotypes can be separated phenotypically, the alleles are said to be codominant. The alleles that determine the human ABO blood groups are examples of multiple alleles.

In higher animals and in those plants with male and female flowers on separate individuals, sex is determined by specific so-called sex chromosomes. In plants the sex chromosomes are not always discernible. In humans, there are one X chromosome and one Y chromosome. If an egg cell is fertilized by a sperm cell and both cells carry the X chromosome, this will give rise to a girl. Normally, a boy has the constitution XY and has received the Y chromosome from his father and the X chromosome from his mother. The Y chromosome is much smaller in size and lacks most of the genes located in the X chromosome. This explains why recessive defects determined by genes in the X chromosome, so-called X-linked genes, for example red-green colour blindness and hemophilia, are more common in males than in females. The recessive allele is expressed although the allele occurs in a single dose, because the Y chromosome lacks this locus and therefore there is no wildtype counterpart of the allele. This further explains why such defects omit one generation since a boy affected with red-green colour blindness has an allele, which originates from the X chromosome of his mother's father.

Mitosis

Mitosis is the division of the cell nucleus, that ensures that the two daughter nuclei receive the same number and type of chromosomes as the parental nucleus. It is usually accompanied by the division of the cell, cytokinesis. Before entering mitosis, the chromosomes have duplicated and consist of two sister chromatids. In mitosis, the two chromatids separate and move to opposite spindle poles of the cell. The cell divides producing two daughter cells, each with an identical set of chromosomes. We use to divide mitosis into five stages called prophase, promethaphase, metaphase, anaphase, and telophase. The intervening stage between two mitoses is called interphase. The synthesis of DNA takes place during this stage.



Picture 1-1. The main features of the different stages in meiosis in Larix. *The first division consists of prophase I - leptotene, zygotene, pachytene IA, diplotene IB-F, diakinesis IIA, prometaphase 1 (not shown), metaphase I IIB, anaphase 1 IIC, and telophase I IID. Interphase is the stage between the first and second meiotic division, IIE. Prophase II IIF, prometaphase II (not shown), metaphase II IIIA, anaphase II IIIB and telophase II IIIC are stages in the second division. The final product is the tetrad IIID. Each of the four cells in the tetrad will give rise to a pollen grain while only one of the tetrad cells will give rise to an embryo sac with an egg cell, the other three will degenerate. It should be noted that the meiotic division in pollen mother cells of* Larix *with its diffuse diploten stage (IB-D) differs from many other conifers.*

Meiosis

It is obvious that the organisms must have a mechanism that prevents a doubling of the chromosome number after each generation. The formation of embryo sacs and pollen is preceded by a special process - meiosis - during which the nucleus divides twice but the chromosomes replicate only once, so that the chromosome number is halved (Picture 1-1). Meiosis occurs in specialized cells. The male cells of a tree are known as pollen (or microspore) mother cells while the female cells are called megaspore (or macrospore) mother cells.

It is in the first cell division, that the two homologous chromosomes separate, resulting in two daughter nuclei, each with only one of the two homologous chromosomes. For example, in Norway spruce, which consists of 12 pairs of homologous chromosomes, each daughter nucleus receives one chromosome 1, one chromosome 2 and so on up to one chromosome 12 (Table 1-1). This means that the chromosome number is halved but the DNA content is the same as in a diploid nucleus. This halving of the chromosome number is accomplished by the lateral pairing or synapsis of the homologous chromosomes during the early stages of the first cell division. An associated pair of homologous chromosomes is called a bivalent. The bivalents exist until the onset of anaphase I, when the two chromosomes in the bivalents separate and move to opposite poles. In addition to the random recombinations of chromosomes occurring in anaphase I (see Box 1-1), exchanges of segments between homologous chromosomes, so-called crossing-over, take place during pachytene - one of the early stages of meiosis - resulting in additional recombinations between genes.

The second cell division resembles a normal mitosis except that it is not preceded by chromosome duplication and the two separating sister chromatids have not an identical set-up of genes owing to crossing-over events.

In summary, the function of meiosis is:

to halve the chromosome number so that a pollen grain or an embryo sac will only contain half the chromosome number

to recombine genes from different chromosome pairs

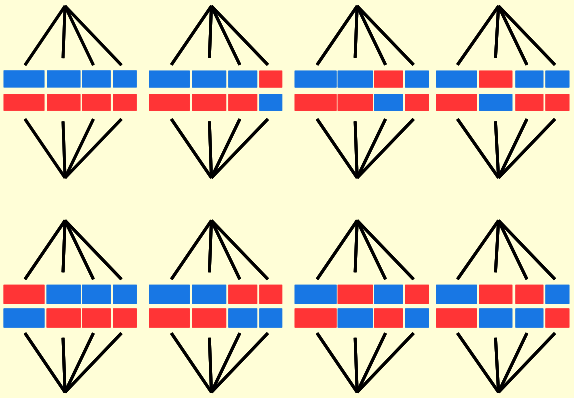
to recombine genes from the same chromosome pair.

Recombination is an essential function of meiosis. Therefore, the mechanisms behind recombination will be discussed in more detail below.

Recombination. Recombination means that genes from the male parent and the female parent are mixed in the gametes. The course of events, when genes in an individual with four chromosome pairs are mixed, is illustrated in Box 1-1. This individual, will show 16 different configurations of the maternal and paternal chromosomes in metaphase I of meiosis. In the box the number of possible recombinations in species with 12 chromosome pairs is given. Those who are interested can themselves calculate this number for species with a different number of chromosome pairs. The general formula for recombination that is also valid for loci with multiple alleles is given in Chapter 3, Box 3-1.

It seems as if *Tilia cordata* with its 41 chromosome pairs has tremendously more possibilities of recombination than *Picea abies* or *Pinus sylvestris*. However, in *Picea abies* and *Pinus sylvestris* many crossovers per chromosome pair can increase the number of possible recombinations and thus compensate for a lower chromosome number. Depending upon how many crossovers per chromosome pair that take place, species with a low chromosome number can attain the same level of recombinations as species with double or triple the number of chromosomes. The number of crossovers is estimated at 2-3 per chromosome pair in *Picea abies* and *Pinus sylvestris* and thus compensate for a lower chromosome number.

Box 1-1 Recombination



The figure illustrates all possible combinations of maternal (red) and paternal (blue) chromosomes in metaphase I in an individual with 4 chromosome pairs and without any crossing-over. The combinations of chromosomes are completely random. In anaphase I, the upper four chromosomes will move towards one of the spindle poles while the lower four will move towards the other pole. In a germ cell the probability of formation is therefore the same for each of the 16 combinations. When there are 4 chromosome pairs, the number of possible combinations is 2^4. In general, the formula is 2^n, where n is the number of chromosome pairs. This means that the possible number of of recombinations in *Picea abies* and *Pinus sylvestris* is very large $2^{12} = 4096$ different gamete types. This means further that the number of possible genotypes that may be formed is even larger, $3^{12} = 531{,}441$. Additional combinations are added through the exchange of chromosome segments – crossing-over – between homologous chromosomes in meiosis. The number of possible recombinants is therefore infinitely large.

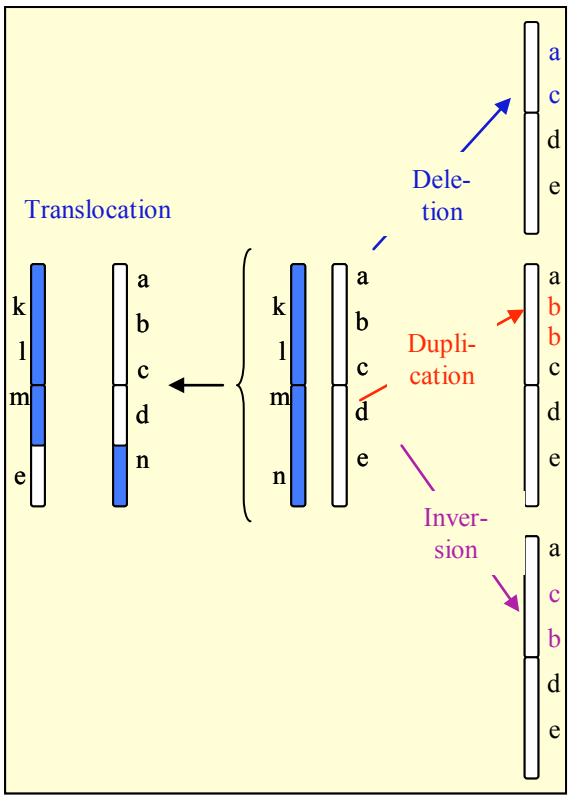


Figure 1-3. A schematic illustration of the different types of chromosome aberrations such as deletion, duplication, inversion and translocation.

Chromosome aberrations

Different types of chromosome aberrations can arise resulting in changes in the sequence of genes or chromosome structure (Fig. 1-3). A loss of a chromosome segment is a deletion while a repetition of a segment is a duplication. A rearrangement of a segment in such a way that the segment order is turned 180 degrees is called an inversion. These three types of aberrations occur within homologous chromosomes (except for certain types of duplications). A fourth type implies an exchange of segments between nonhomologous chromosomes and is called a translocation, the reciprocal type being the most common (Fig. 1-3).

The prime cause of these aberrations is that at least two breaks have occurred in one or two chromosomes. The chromosomes can be restored by joining the broken ends. If the broken ends are joined in an incorrect way, aberrations are generated. One break in each of the homologous chromosomes is needed to generate a duplication, while two breaks in one of the homologous chromosomes are needed to induce a deletion or an inversion. A duplicated segment can also be found on a nonhomologous chromosome as well as at its original location. This means that the segment will be present in three copies, in the two original homologous chromosomes and in the nonhomologous chromosome. In addition to double-stranded breaks, duplications can also arise after errors in DNA replication or after unequal crossing-over owing to mistakes during the homologous pairing in meiosis. This results in duplications present in tandem arrangements. Duplications can also be induced by transposons. The evolutionary significance of duplications will be discussed further in Chapter 2. The exchanged segments in translocations can be of very unequal size. Furthermore, in some species all chromosomes can be involved in segmental exchanges as in the genus *Oenothera*.

All these aberrations can cause problems in meiosis. Individuals, heterozygous for an aberration, show a varying degree of sterility, because some of the gametes will be lethal. The larger the aberrations the greater the probability of producing lethal gametes. It can be of interest to mention that some of these aberrations are easily recognized cytologically, for instance in the meiotic divisions of the pollen mother cells.

There are very few reports of how common chromosomal aberrations are in forest trees. Minor inversions, however, have been observed in American pine species. Many plant species are polyploids, which means that they have more than two chromosome sets. An example of this is *Sequoia sempervirens* native to the coastal region of western North America, which has 6x=66 chromosomes. It is plausible that such species can tolerate deletions easier than their diploid relatives with only two sets of homologous chromosomes.

Development of egg cells and sperm cells

The final result of meiosis is the formation of four haploid daughter cells, a tetrad. In the male strobili each of these cells will give rise to a pollen grain. In the female strobili, on the contrary, only one of the four megaspores in a tetrad will continue to divide mitotically. This megaspore will give rise to an embryo sac and after further divisions of the nucleus, an egg cell is formed. The further development of the megaspore differs considerably between conifers and angiosperms (Fig. 1-4).

Conifers. The formation of the female gametophyte takes place within the remaining megaspore in the ovuliferous scale. The megaspore grows to a large size and free nuclear divisions take place resulting in a large haploid megagametophyte or prothallium. The haploid prothallium is sometimes incorrectly called endosperm. At the pole of the prothallium a number of archegonia are formed that contains the large egg cell.

The formation of several archegonia and the possibility of fertilization of the egg cell in each of them result in a competition among the embryos formed. Embryos, which are less competitive may degenerate and disintegrate. This is one means to avoid formation of selfed seeds in conifers. Self-sterility genes, which in one way or another prevent selfing, have not been found in conifers. The mechanism

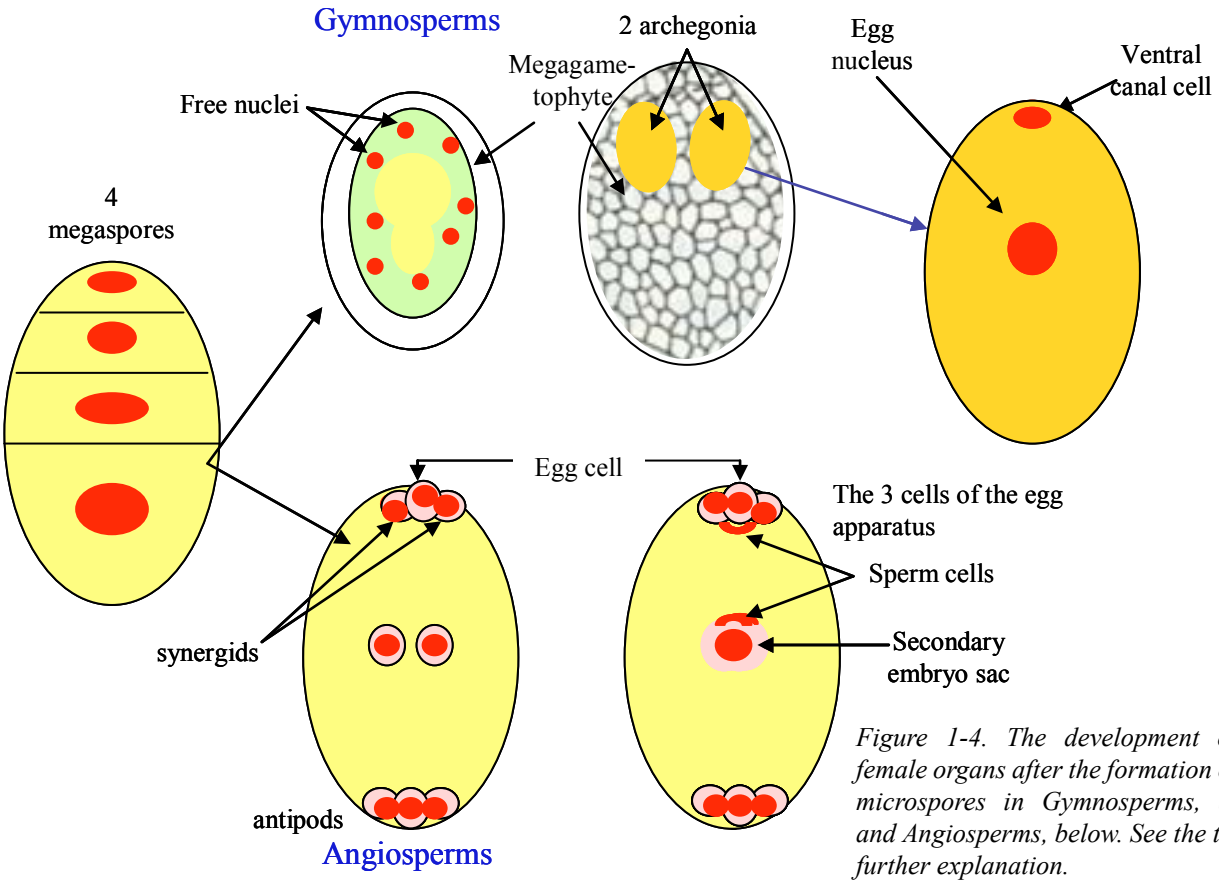


Figure 1-4. The development of the female organs after the formation of four microspores in Gymnosperms, above, and Angiosperms, below. See the text for further explanation.

with fertilization in several archegonia has the same function as self-sterility genes. Especially in Finland analysis of number of archegonia and pollen production were carried out in *Picea abies* and *Pinus sylvestris*.

At the time of pollination the pollen contains two prothallial cells, a generative cell and a tube cell (*Pinus* species). The pollen tube grows and the generative cell divides into a stalk cell and a spermatogenous cell. In *Picea abies* the mature pollen grain contains 5 cells; two prothallial cells, a tube cell, and a stalk cell. The spermatogenous cell divides under formation of two male nuclei.

After fertilization several free nuclei divisions takes place and a proembryo is formed at the distal pole of the former archegonium.

Angiosperms. The mononuclear embryo sac grows considerably in size and its nucleus starts to divide in 3 consecutive divisions. The result is 8 nuclei. Six of these 8 nuclei move to the poles of the embryosac and become enclosed in cell walls. The upper ones are referred to as egg apparatus, one of them tightly connected to the cell wall becomes the egg cell. The two others are coined synergids. The 3 cells at the bottom are called antipods. The remaining two so called pole nuclei move to the center of the embryo sac and unite to a diploid nucleus, called secondary embryo sac.

Already in the pollen sacs of the anthers, the division in the pollen grain may start. One lens-formed nucleus is formed, which becomes the generative nucleus. The other nucleus is a vegetative nucleus. The pollination frequently takes place at this stage and when the pollen has reached the stigma the pollen tube starts to grow and another division of the generative nucleus starts and the two sperm nuclei are formed. The formed nuclei are always in the lower part of the pollen tube and the upper part is frequently degenerated as the pollen tube grows to the embryo sac. When the pollen tube reaches the egg apparatus the pollen tube releases its nuclei into the embryo sac but never directly into the egg cell but into one synergid cell that lateron disintegrates. Also the vegetative nucleus of the pollen tube disintegrates. The sperm nuclei have spiral form and these nuclei probably have an own possibility to move. One of the nuclei enters the egg cell and units with the egg nucleus under formation of a zygote. The other sperm nucleus unites with the diploid secondary embryo sac nucleus under the formation of a triploid endosperm nucleus.

Picture 1-2. Male strobili of Norway spruce, Picea abies.*Photograph Kjell Lännerholm*

Picture 1-3. Female strobili development of Scots pine. Furthest left below is a receptive strobilus. Photograph Kjell Lännerholm.

As seen from Figure 1-5 three archegonia per prothallium was the most common number in *Picea abies*. Figure 1-6 demonstrates that the pollen production in an *Pinus sylvestris* stand varied between years and that the peak distribution occurred at different dates. Initiation of generative organs, female and male strobili, and time for pollen dispersal and receptivity in female strobili are weather dependent.

Time of meiosis

A summary of the time of meiosis in a large number of conifer genera and in individual species in which a variation in this trait exists, is given in Table 1-2. As is evident from this table, there are three main types:

start and completion in autumn
start in the autumn and completion in spring
start and completion in spring

During the sixties Gösta Eriksson and coworkers detected that meiosis in pollen mother cells (PMC) of different *Larix* species starts in autumn and is completed during spring. The previous opinion that meiosis occurs either in autumn or in spring, had to be revised. Furthermore, detailed investigations of the situation in *Pseudotsuga menziesii, Thuja plicata* and *Tsuga heterophylla* showed that even these species exhibited the same type of timing of meiosis as *Larix* species. Both female and male meiosis take place in spring in the majority of species investigated. In most *Larix* species, the time of meiosis is dependent on the weather conditions.

Once the diplotene stage of larch PMC is reached a rest, usually called dormancy, is initiated. To break the rest a certain amount of chilling is required. This is a general phenomenon in woody plant species from the temperate

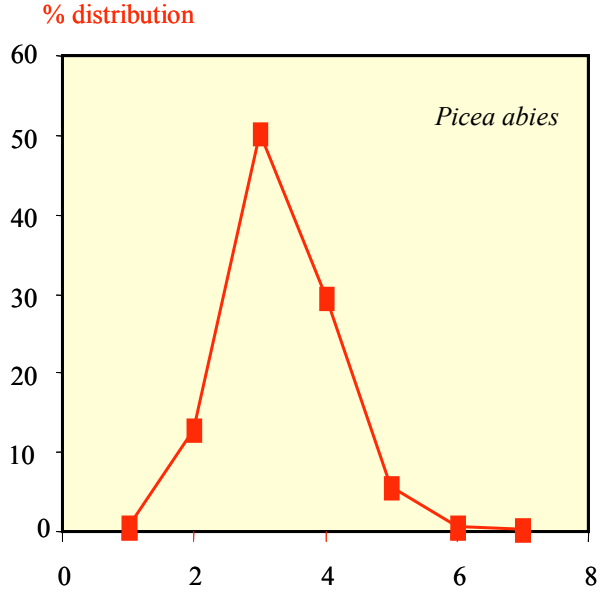


Figure 1-5. The percentage distribution of number of archegonia per ovule in Picea abies.

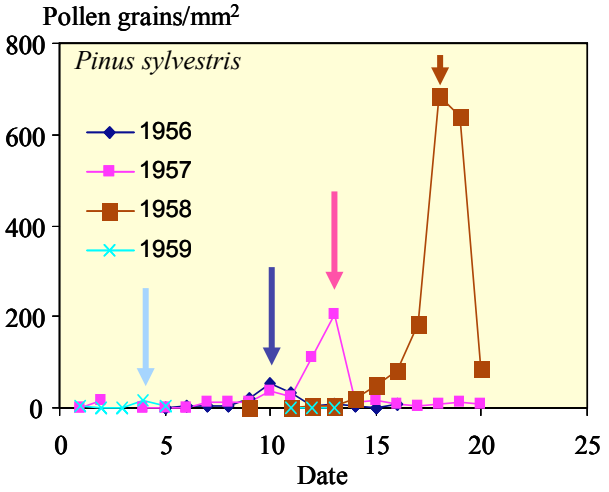


Figure 1-6. Pollen catch during four years in stands of Pinus sylvestris. *The date for the peak of pollen production is indicated for the four years of observation.*

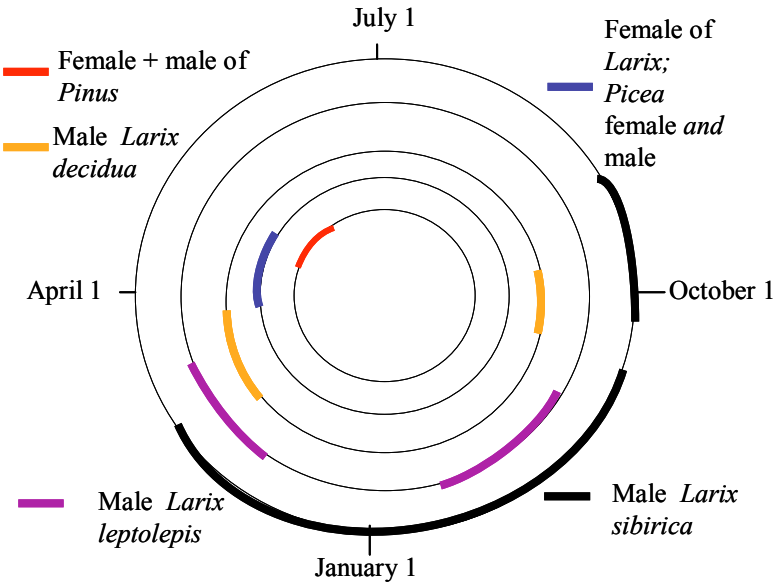


Figure 1-7. The time of meiosis in pollen (microspore) mother cells and megaspore mother cells during the annual cycle in three Larix *species*, Picea abies *and* Pinus sylvestris *studied in southern Sweden.*

and boreal zones. The chilling requirement varies among the three larch species. Increased chilling is required in the order: Siberian - Japanese - European larch. Dormancy in Siberian larch pollen mother cells is easily broken. Under Swedish conditions the breakage of dormancy in PMC of this species frequently takes place already during autumn. In European larch cultivated in Sweden the dormancy is normally broken in February -March when continuation of meiosis takes place. These stages of the meiotic division are probably the most frost sensitive during the life cycle of an individual. The maximum time span for meiosis in some conifers in Sweden is illustrated in Fig. 1-7.

In birch, hazel and alder, meiosis occurs in late summer. In elm, aspen and oak it takes place in spring. In those species in which meiosis starts in late summer or autumn the night length is probably the environmental factor that initiates meiosis. Thus, there is a continuous variation in time of initiation from northern Finland to southern Finland, with the earliest start in the northerly populations of *Betula pubescens*. In those species in which all or most of meiosis occurs in spring, the heat sum is the main factor that determines the timing of initiation of meiosis. However, there is a variation in heat-sum demand, so that the northern populations need smaller heat sum for initiating meiosis than the southern populations. But in spite of this, meiosis takes place later at the northern latitudes because of a much later spring.

Table 1-2. Time of meiosis in some conifer genera and species

Meiosis starts and is completed during autumn	**Meiosis starts during autumn and is completed during spring**	**Meiosis starts and is completed during spring**
Cedrus *Cryptomeria* *Juniperus chinensis* *J. horizontalis* *J. virginiana* *Taxus*	Meiosis of pollen mother cells in *Larix, Pseudotsuga, Thuja,* and *Tsuga* usually show this pattern	*Abies* *Athrotaxis* *Cunninghamia* *Juniperus communis* *J. rigida* *Keteleria* *Picea* *Pinus* *Pseudolarix* Megaspore mother cells in *Larix* and *Taxus*

Injuries and irregularities during meiosis

Certain stages of meiosis are known to be very susceptible to environmental factors during the lifetime of an individual. Especially, the effects of low temperature on meiosis have been studied in forest trees but also the effect of very high temperatures have been elucidated.

Long before climatically controlled cultivation facilities came into use in forest genetics, Enar Andersson professor at the Royal College of forestry in Sweden, was able to carry out very ingenious experiments for studying the effects of low temperatures on meiosis in pollen mother cells of Norway spruce. In 1948, in the end of April, he collected twigs with male strobili from trees growing at various levels along an mountain slope in Dalecarlia (Sälen), in central Sweden, and transferred them to different elevations. As the temperature decreases gradually during clear nights when approaching the bottom of the valley, it is possible to get information about how strongly the different temperatures affect meiosis. The results of such a transfer are illustrated in Fig. 1-8. The figure shows that the percentage damaged pollen mother cells was higher after a transfer to the level of 350 m than if the material was left at the 775 m level. Performing different transfers, Enar Andersson concluded that no injuries occurred at temperatures above -2°C, but at -11°C meiosis was so irregular that no pollen grains at all were produced. Large temperature fluctuations between day and night occur occasionally in the Alps resulting in damage to meiosis in pollen mother cells in Norway spruce. From these and other results it was concluded that also high temperatures, above +20°C can induce disturbances of the susceptible stages of meiosis.

The most extensive investigations of temperature-induced injuries were performed in Swedish studies of the three *Larix* species, *Larix decidua, L. leptolepis* and *L. sibirica*. From these studies we have learnt that the most susceptible stages of meiosis are diakinesis - telophase I and prophase II - telophase II and that injuries will appear at temperatures below -2°C. Some years the pollen formation in *Larix sibirica* collapsed completely owing to frost injuries. Meiosis in the pollen mother cells of larch will also be discussed further in the section Darwinian fitness and domestic fitness in Chapter 7.

Summary

At the chromosome level, each species is characterized by its karyotype, the number, size and morphology of its chromosomes. The chromosomes are the carriers of the hereditary units. The genes are located linearly on the chromosomes at specific sites, loci (sing. locus) More than one alternative form of the the genes - alleles - can exist at a locus. A gene can be dominant (**A**) or recessive (**a**). A heterozygous individual, **Aa**, carries both **A** and **a**, whereas a homozygous individual is either **AA** or **aa**. With complete dominance, individuals with the genotypes **AA** or **Aa** have the same performance or phenotype, which differs from individuals with the genotype **aa**. All genes on the same chromosome belong to the same linkage group. In a diploid organism, there are two homologous chromosomes of each type. They appear in pairs and contain the same loci in the same order.

When a somatic cell divides, the preceding division of the cell nucleus, mitosis, ensures that the two daughter cells receive the same number of chromosomes and thus the

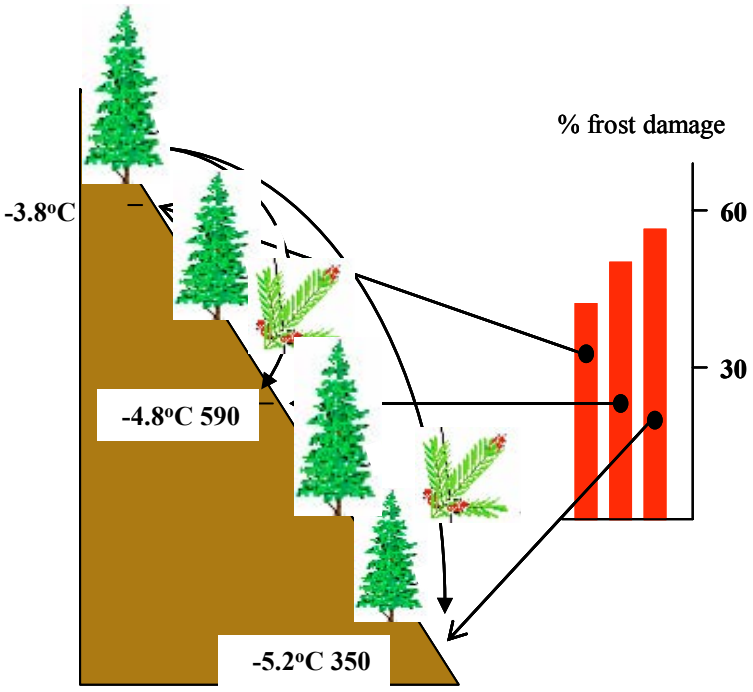


Figure 1-8. Transfers of male strobili of Picea abies *to different elevations along an alpine slope, to determine at which temperature injuries of meiosis appear.*

same genes as the parental cell. When a cell involved in gamete formation divides, two divisions of the cell nucleus, meiosis, result in halving of the chromosome number. Simultaneously recombination of genes between homologous as well as non-homologous chromosomes occur. Four haploid cells are formed, each with one chromosome set. During fertilization, the original chromosome number is restored.

In species such as *Picea abies* and *Pinus sylvestris* with 12 chromosomes pairs, an infinitely large number of possible recombinants can be produced.

Several types of chromosome aberrations occur such as
deletion: loss of segment
duplication: repetition of a segment
inversions: a segment is inverted 180 degrees
translocation: exchange of segments between non-homologous chromosomes

Chromosome aberrations usually cause irregularities in meiosis that are lethal to some of the gametes.

The time of male meiosis in conifers during the annual cycle shows three types of pattern. It starts and is completed during autumn, e.g. *Juniperus* species. It starts in autumn and is completed in spring, *e.g*. pollen mother cells in *Larix sp*. Start and completion of meiosis during spring is the most common type, e.g. *Picea* and *Pinus sp*. Female meiosis in *Larix, Picea,* and *Pinus* takes place in spring only. In angisperms such as birch, meiosis occurs in late summer, whereas in oak it takes place in spring.

Injuries and irregularities occurring during male meiosis are caused by sub-zero temperatures or very high temperatures.

Further reading

Borzan, Z. Z. and Schlarbaum, S.E. (eds). 1997. Cytogenetic studies of forest trees and shrub species. Proc. First IUFRO Cytogenetics Working Party S2.04-08., 1993. Brijuni National Park, Croatia.

Eriksson G. Ekberg I. & Jonsson A. 1970. Further studies on meiosis and pollen formation in Larix. Stud. For. Suec.87:1-65.

Hartl, D.L. and Jones, E.W. 1998. Genetics: Principles and Analysis. 4th ed. Jones and Bartlett Publishers Sudbury, Mass. USA.

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

Genes, DNA, RNA, molecular evolution, and genetic engineering

The hereditary material must have great stability so that it can be transferred unchanged from one generation to the other in the overwhelming number of cases. On the other hand, the hereditary material must not be so stable that no changes whatsoever can take place. Heredity calls for a high degree of perpetuity but with opportunity for variation so that adaptation to new environmental conditions can take place. The molecule that fulfil these demands is deoxyribonucleic acid - DNA. In addition to DNA, we shall also deal with the structure of the genes, regulation of gene activity, the molecular clock, and possible applications of genetic engineering.

DNA structure

As early as in the 1930s, it was known that DNA was a giant molecule much larger than a protein molecule. The four nucleotides of DNA were also known, each composed of one phosphate group, one sugar molecule and a purine or a pyrimidine base. As the result of microbiological experiments in 1940s, it was shown that DNA was the molecular bearer of heredity. The great break-through, however, did not occur until the American James Watson and the Englishman Frances Crick in 1953 published their theory about the three-dimensional structure of DNA. At that time, Watson and Crick knew that the DNA molecule is composed of two long polynucleotides forming intertwined chains. The constant diameter of the DNA molecule was also known. But how these two chains were orientated relative to each other and kept together was unknown. By building three-dimensional models of DNA in such a way that the energetically most stable configurations were favoured, they soon came to the conclusion that the sugar-phosphate part forms the backbone on the outside of the DNA molecule and the purine and pyrimidine bases are on the inside. The bases are oriented so that they can form hydrogen bonds, *i.e.* weak covalent bonds, between each other in the opposite chains. This is the way the two polynucleotide chains are kept together. When they built the model in such a way that a purine base always bound to a pyrimidine base, they also fulfilled the requirement that the diameter should be constant.

The DNA molecule is thus a double helix of two nucleotide chains running in opposite directions. The DNA molecule is like a helical ladder on which the two purine and pyrimidine bases are the rungs and the sugar-phosphate complex forms the backbone (Fig. 2-1). The purines consist of the two bases adenine (A) and guanine (G) with a double-ring structure including five or six atoms, respectively; two of the atoms in each ring are nitrogen, the others are carbon. The two pyrimidines, cytosine (C) and thymine (T), have a single ring with two nitrogen and four carbon atoms. To fulfil the rule of Chargaff, that the proportion of adenine in DNA equals that of thymine and the proportion of cytosine that of guanine, Watson and Crick assumed that adenine can pair only with thymine and cytosine only with guanine. This is called the base pairing of the DNA molecule which means that the two helices are complementary. If the sequence of the bases in one chain is known then the sequence in the other chain is known. The weak hydrogen bonds facilitate the split of the double helix. This in turn facilitates the replication of DNA.

DNA replication

Since the two strands of the double helix are fully complementary, they can serve as templates for generating two daughter double helices identical with the original double helix. Evidence for this model of replication was demonstrated by cultivating *E. coli* bacteria for several generations on a medium containing heavy carbon and nitrogen isotopes. In this way, the original double helix was labelled with these isotopes. The bacteria were then grown for one generation on a normal light medium. It was observed that the weight of all DNA molecules was intermediate between a heavy and a light double helix. The only possible interpretation of this result must be that the newly generated double helices consisted of one old, heavy strand (parent) and one new, light strand (Fig. 2-2). This is a semiconservative model of replication as opposed to a conservative model in which one of the two daughter helices is built only of two old strands while the other daughter helix consists only of new strands. The result of the experiment described above with *E. coli* bacteria demontrates that the conservative model must be rejected. Furthermore, the structure of the DNA molecule proposed by Watson and Crick became quickly accepted by other researchers in the field. James Watson and Francis Crick were awarded the Nobel prize in physiology or medicine in 1962 together with the English physicist Maurice Wilkins.

The Watson-Crick model of the DNA molecule

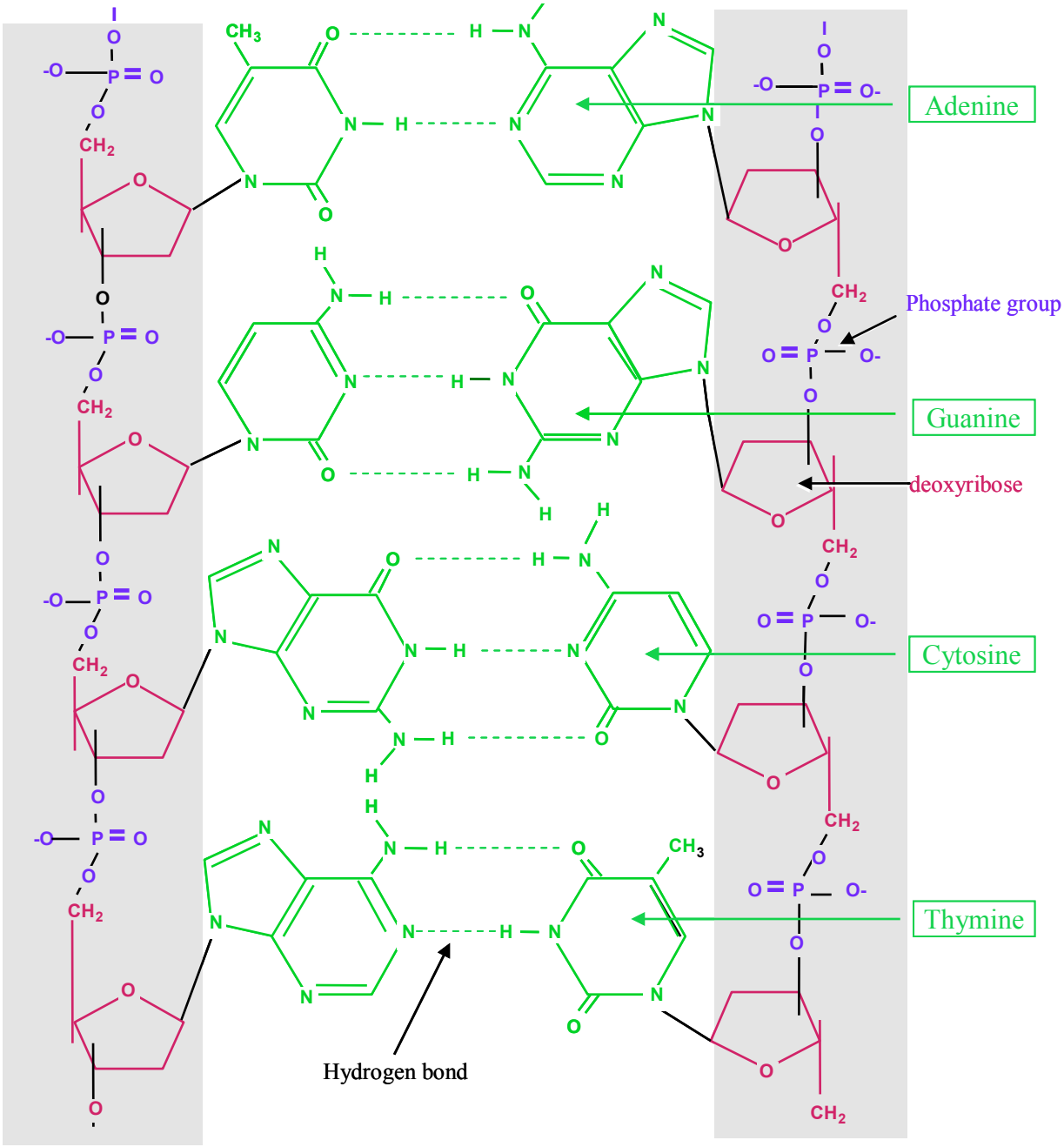


Fig. 2-1. The Watson-Crick model of the DNA molecule in its uncoiled structure. The four bases adenine, thymine, cytosine and guanine form hydrogen-bonded base pairs that hold the two phosphate-deoxyribose backbone strands together.

Mutations –changes in DNA

Changes in the sequence of the bases in DNA can occur in many ways. During replication, a base pair can be deleted (deletion), or it can be added (addition), or it can be replaced by another base pair (substitution). Many different substances in the cell can interfere with the replication leading to such mutations and to incorrect base pairing.

Where to find DNA?

The largest amount of DNA is found in the nucleus of the cell. But DNA can also be found in the cytoplasmic organelles, the chloroplasts and the mitochondria. The least amount of DNA is located in the mitochondria. However, in leaf cells of some plant species, the amount of DNA in the cytoplasm can come up to 15%. One reason for this is that there are many chloroplasts and mitochondria in such

a cell and that each DNA strand is present in many copies in each chloroplast and mitocondrion. There are usually 20-40 chloroplasts per cell, each with 100-150 copies of the chloroplast genome. Similarly, there are 100-3000 mitochondria per cell, each with 2-50 copies of the mitochondrial genome. This presumably reflects the need for high concentrations of photosynthetic and respiratory enzymes. As regards the number of genes in a woody plant, most of them are to be found in the nucleus, about 20 000 - 60 000. A chloroplast in higher plants consists of about 120 genes only and a mitochondrion of still fewer genes. Of importance for evolutionary studies is that chloroplast DNA in conifers shows paternal inheritance, *i.e.* DNA is transmitted with the pollen while it is inherited maternally in angiosperms. Mitrochondria, however, exhibit maternal inheritance in both conifers and angiosperms.

Where is DNA located in the nucleus of the cell and how is DNA organized?

The next questions are where the DNA molecule is located in the nucleus and how it is organized. Already in the 1920s, the German chemist Robert Feulgen showed that DNA was located in the chromosomes. He developed a staining method, called the Feulgen method, where he used a DNA-specific purple dye. This method is still one of the most used for staining chromosomes. It is quite clear that the length of the DNA molecule is much larger than a chromosome in the metaphase stage of mitosis. The problem to solve was how this very long DNA molecule is packed into chromosomes. A further question to be addressed was whether there are many DNA molecules or only one very long molecule. Only the last mentioned alternative is in accordance with the way the DNA replicates – the semiconservative model (see above: the semiconservative replication). In addition, all data from linkage studies point towards the fact that the genes on a chromosome/chromatid are located like pearls on a string. The conclusion drawn was that in a chromosome of higher organisms, DNA exists as only one continuous molecule. In humans, a cell contains about 1 meter of uncoiled DNA in a single, haploid chromosome set. In the largest chromosome, the length of the uncoiled DNA

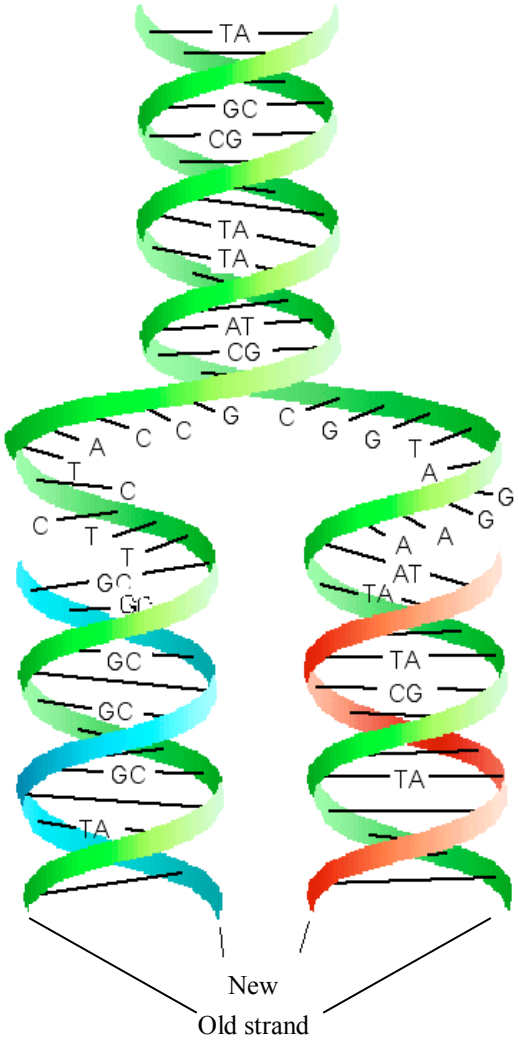


Fig. 2-2. The semiconservative replication of DNA as originally conceived by Watson and Crick. The current model is essentially the same but more complex. The identical daughter double helices consist of one old and one new strand. A, T, G and C denotes the four bases adenine, thymine, guanine and cytosine.

molecule is estimated at 8.5 cm. How can this very long DNA molecule be packed into the chromosome?

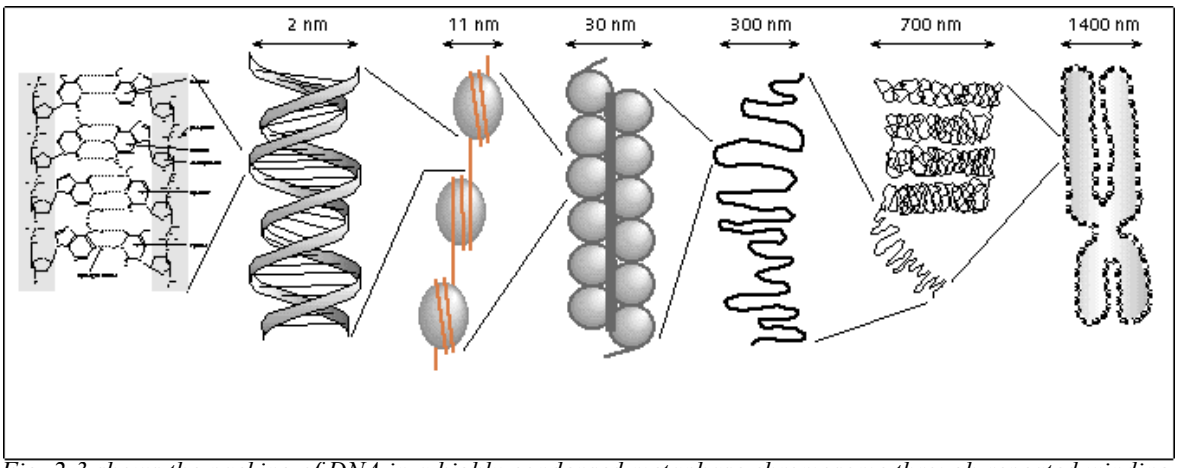


Fig. 2-3 shows the packing of DNA in a highly condensed metaphase chromosome through repeated winding, coiling and folding. (simplified after Alberts et al. 2010).

All the details in this very efficient packing 'system' are not known but the main features are illustrated in Fig. 2-3. Before looking into this figure in more detail, we need to get to know the nucleosome, discovered in the 1970s, because the nucleosome has been shown to be an important packing unit. In electron microscope pictures where the DNA molecule appears in its uncoiled state, the nucleosomes stand out like beads of a necklace. A nucleosome contains eight small chromosomal proteins, called histones, that form an octamer. Two units of each of the histones H2A, H2B, H3 and H4 constitute together an octamer. The DNA is wound in a little less than 2 turns, 146 nucleotide pairs, around the nucleosome (see Fig. 2-3). The DNA molecule is then wound around the next nucleosome until the entire DNA molecule is wound around nucleosomes as is seen in Fig. 2-3. The region of the DNA molecule that links two nucleosomes is called linker DNA and to this region one molecule of a fifth type of histone, H1, is attached. This histone stabilizes the densely packed 30 nm structure during the further coiling into supercoils. In Fig. 2-3, additional stages in the coiling and folding of DNA are illustrated up to the final stage – a metaphase chromosome during mitosis. Conclusion: The DNA molecule exists in a densely packed condition in the chromosomes following progressive windings, coils and foldings.

What is a gene?

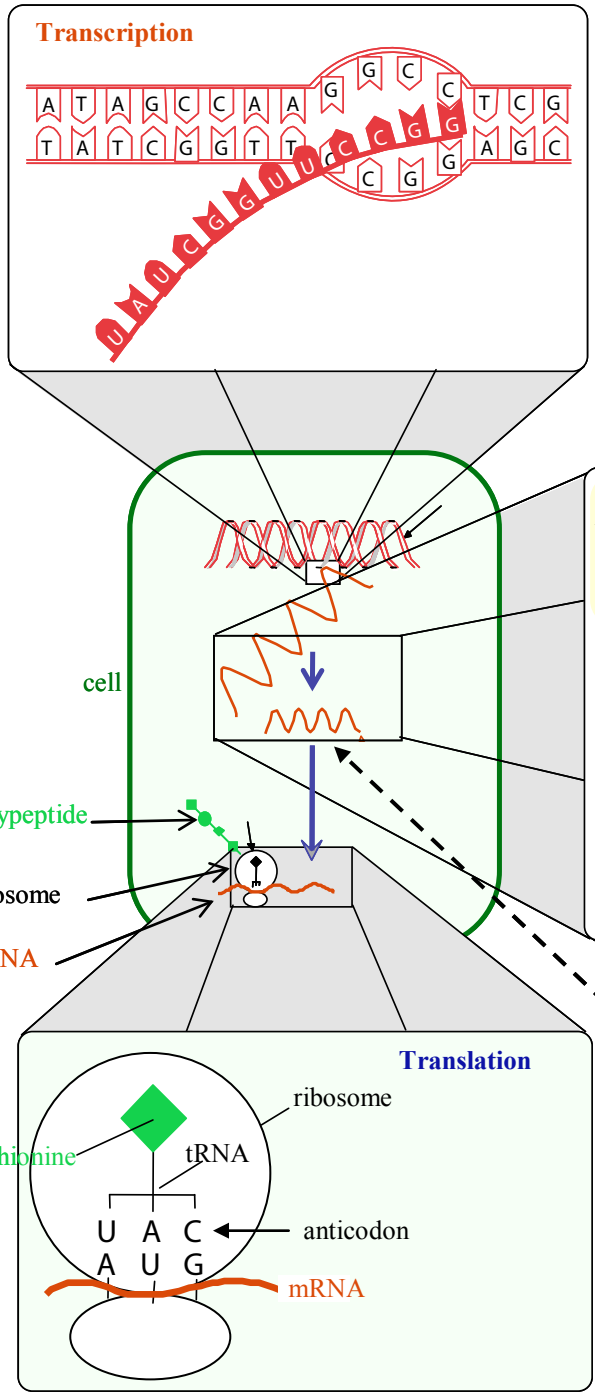
DNA consists of coding, genic DNA and non-coding regions, non-genic DNA. A clearly worded definition of a gene that completely covers the concept is difficult to achieve, but the definition below should be satisfactory:
A gene is a segment of DNA that is essential for a specific function.

Among some viruses, RNA (ribonucleic acid) constitutes the genetic material and in these cases, RNA replaces DNA in the definition above.

During 1940s, informative experiments were made with the fungus *Neurospora*. The two Americans George W. Beadle and Edward T. Tatum used mutants that were unable to synthesize specific amino acids. They could show that biochemical reactions in living cells occur in a series of discrete steps in which each reaction is catalyzed by a single enzyme. Furthermore, the experiments showed that there was a direct relationship between genes and enzymes: the one-gene-one-enzyme hypothesis could be approved. However, one gene often encodes more than one enzyme (protein) via so-called 'alternative splicing'. The concept of the indivisible gene and that the genes were located on the chromosomes like pearls on a necklace were firm convictions for a long time. The gene was the smallest unit of recombination. However, more recent research has shown that the gene, no more than the nucleus of the atom, is indivisible. Instead crossing-over can take place within the gene and the smallest unit consists of a base pair.

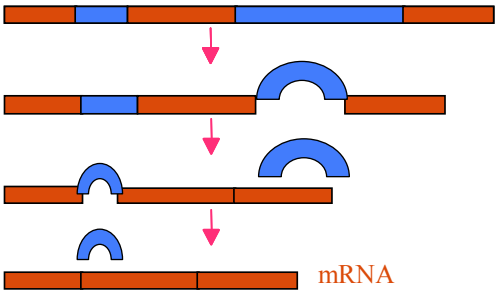
In Box 2-1, the steps from chromosomal DNA to the formation of a polypeptide is illustrated (see also the central dogma in the section The genetic code). This course of events is valid for those genes that are involved in protein synthesis, *i.e.* (1) *genes encoding proteins*. There is an additional type of genes that perform differently. This consists of (2) *genes encoding functional RNA*, for example ribosomal RNA (rRNA) or transfer RNA (tRNA). These genes are only transcribed, not translated.

Box 2-1. Transcription and translation



Transcription
The primary RNA transcript is formed as a complementary strand to the upper single-stranded DNA.

RNA processing
Non-coding regions, **introns** (shown as blue below) are spliced and removed before mRNA leaves the nucleus. The three coding **exon** regions are united in mRNA.



Transport of mRNA to cytoplasm where it binds to a ribosome.

Translation
The polypeptide is synthesized as the ribosomes move along mRNA.
A specific tRNA, transfer RNA, with its anticodon, specific for the amino acid it brings.
The codon AUG on mRNA encodes the amino acid methionine. A = adeninine, U = uracil, G = guanine

Table 2-1. Genome size, estimated number of genes, and per cent genic DNA in various organisms.

Organism	Genome size (bp) haploid cell	Number of genes	Genic DNA %
E. coli	4.7×10^6	4400	100
Yeast	12×10^6	6000	50
Arabidopsis	135×10^6	27 000	50
Populus trichocarpa	485×10^6	40 000	25
Picea abies	20000×10^6	28 000	<3
Fritillaria	85000×10^6	25000?	0.02
Human	3200×10^6	23 000	<2

Eukaryotic DNA can also be classified as below:

* Unique, single and low-copy, functional genes, *i.e.* genes that exist in only one or a few copies per genome

* Repetitive DNA, which includes distinct subclasses:

  Moderately repetitive DNA with functional sequences, for example functional gene families and their closely related nonfunctional so-called pseudogenes which have completely or partly lost their protein-encoding function, and repetitive genes in tandem configuration encoding ribosomal RNA, transfer RNA and certain histones.

  Repetitive sequences with mainly unknown function

  Highly tandemly-repeated DNA located on both sides of the centromere, the satellite DNA, and located at the telomeres, the chromosome ends

  Variable number of tandem repeats, (VNTRs) known as minisatellite DNA used for 'DNA fingerprinting'

  Simple tandem repeats (STRs) or simple sequence repeats, (SSRs) so-called microsatellite DNA, more often used for mapping purposes than minisatellites

  Transposed sequences, for example various types of transposons, so-called 'jumping genes', first found in maize, with the ability to transpose to new places within the genome without any intermediate, and retrotransposons that transpose via an RNA intermediate.

* Spacer DNA, which until now has not been classified in any of the categories above.

Table 2-2. Comparison of genome traits among three parent species, pg = 10^{-12}g.

Trait	***Pinus* spp**	***Eucalyptus* spp**	***Arabidopsis***
Size, pg per haploid cell	24	0.6	0.15
Haploid chromosome number	12	11	5
Repetitive DNA, %	75	75	10
Single-copy DNA, %	25	25	90
Genic DNA, %	0.3	13.3	50

It is a paradox that there is no correlation between the total amount of DNA in plants and animals and their complexity. It is true that the amount of DNA increases from bacteria to human beings, but there are some insects, amphibians and plants with a much larger amount of DNA than humans. For example, *Fritillaria*, belonging to the lily genus, contains about 30 times more DNA than humans (Table 2-1). The explanation for this paradox is that in higher organisms most of the DNA does not code for amino acids in proteins, and the amount of non-coding DNA varies to a great extent among species. Moreover, in most higher organisms and especially in conifers, non-coding DNA comprises a very large part of the total DNA. More than 97 % of DNA in Norway spruce and Scots pine consists of non-genic DNA, not coding for proteins. This is in contrast to both *Eucalyptus* species and *Arabidopsis thaliana* (wall cress) which have a lower proportion of non-genic DNA (Table 2-2). One can ask

why there are such large amounts of seemingly redundant DNA, so-called **junk DNA**, *i.e.* DNA not encoding proteins and often not being transcribed to RNA either.

Repetitive sequences such as transposons make up a large proportion of the genome. Introns also lack any obvious function. Transposons may be regarded as molecular parasites which do not have any specific function in their host. Frances Crick therefore called them 'selfish DNA' because they use their host for propagation only, apparently without being of any use for the host.

On the other hand, transposons have been of great significance for the origin of new genes by generating mutations during evolution:

(1) They have induced rearrangements of DNA, especially gene duplications and deletions

(2) They have given rise to spontaneous mutations by being inserted into a gene and thereby changing the gene product.

The transposons seem to be eliminated very slowly from the eukaryotic genome compared to their induction rate, leading to an accumulation during evolution. In many eukaryotes, the transposons therefore consitute a large part of the genome. It has been calculated that they constitute about 30 % of the total human genome, and more than 50 % of the maize genome.

The same type of transposons (retrotransposons) as in maize has also been found in pine and spruce species. In *Pinus elliottii* these transposons have been shown to be distributed fairly evenly over all 12 chromosomes and they represent a significant part of the genome of this species.

Also the introns (see Box 2-1), DNA sequences located between the coding sequences of the genes expressed, belong to the so-called junk DNA. Their numbers vary greatly among species, from a few as in *Arabidopsis* to a large number in humans and probably also in conifers.

This fragmentation of genes makes them more flexible. In humans, about 60% of the genes are subjected to alternative splicing, that is different protein molecules are generated from the primary DNA transcript by changing the number and order of exons in the final mRNA after splicing out the introns. Alternative splicing seems to be less common in plants.

Conservation of non-genic DNA

Why does the conifer genome contain so much more DNA, mainly non-coding, than the annual plant *Arabidopsis*? The explanations are probably as follows:

(1) Extra DNA is a disadvantage if energy and material are needed for the formation of this DNA during each cell division. This disadvantage, however, seems to be negligible.

(2) Of more importance is that strong correlations exist between a large genome and large cell nuclei, and between large cells and slow mitotic and meiotic cell divisions.

(3) For most tree species, it does not matter whether the genome is large or not, because rapid cell divisions are not critical for survival. Some other factor limits the growth or a very rapid growth is not of ecological importance. Trees and herbs that grow at very northern latitudes do have a fairly low DNA content, probably because they need to pass several developmental stages and go through meiosis during a short growing season.

(4) *Arabidopsis* on the contrary has a very short life cycle of 2-3 weeks from seed to seed. Only very small genomes allow such rapid cell divisions as are needed for such a short life cycle.

(5) Furthermore, a positive selection for large genomes probably occurs in most conifers. With a few exceptions, these species do not have vascular vessels for water transport. Instead they have very long water-conducting cells, so-called tracheids. These tracheids need a large genome as there seems to be a strong correlation between the amount of nuclear DNA and the size of those cells in the cambium that generate tracheids. In a study of 18 North American pines species, it was found that those species adapted to dry areas had a larger genome than those species growing in more moist areas.

In most European plants the amount of non-genic DNA has increased during evolution, for instance, because the ´nuclear parasites' can easily reproduce without a decrease in the competitive capacity of the plants. For some species and in specific environments, natural selection has affected the DNA content in such a way that it is either conserved at a low level as in *Arabidopsis* or at an extremely high level as in most conifers.

Many scientists have been reluctant to accept that non-coding DNA can compose so much of the human and other genomes without having some function in regulating the expression of genes coding for proteins. Interestingly, in the last decade mass sequencing has shown that in humans about four times as much RNA is transcribed from non-genic DNA as from DNA coding for proteins. Much of this RNA is longer than 200 nucleotides and is called long non-coding RNA, or lncRNA, or lincRNA. The sequence of particular lncRNAs is more variable among species than genic DNA, varies among tissues of the same organism, and is associated with diseases such as cancer. Some lncRNAs certainly affect the expression of genic DNA. To date little is known of lncRNA in tree species.

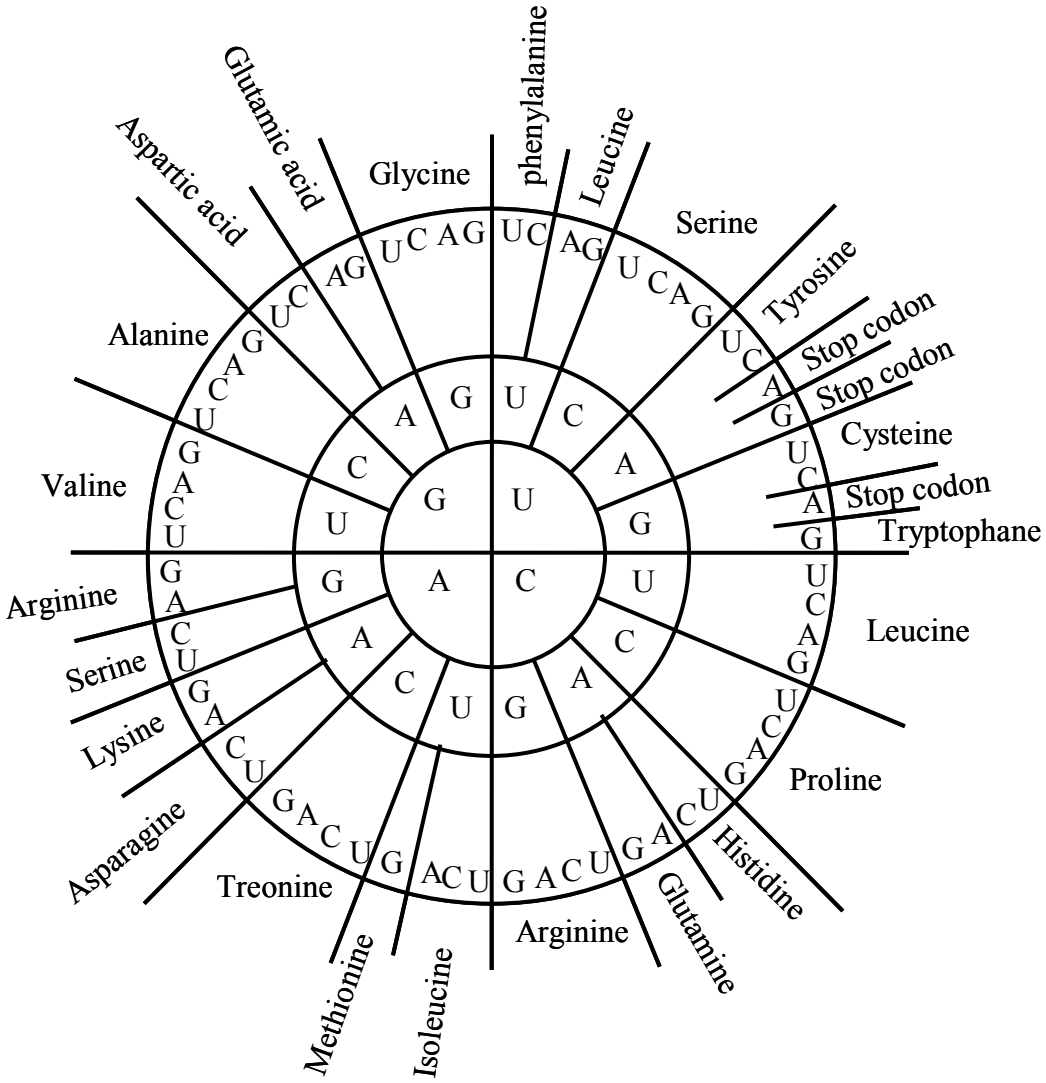


Fig. 2-4. The genetic code. A, U, G and C stand for the four bases adenine, uracil (thymine is exchanged for uracil in mRNA), guanine and cytosine. The innermost cycle indicates position 1 of the codon, the intermediate cycle position 2 and the outer cycle position 3. At position 3 more than one alternative can exist, all resulting in the same amino acid. The code is said to be degenerate. For example, when the order of the bases is CAG or CAA both code for the amino acid glutamine, while AUG only codes for the amino acid methionine. Stop codons are UAA, UAG and UGA which means that the protein synthesis terminates at these codons.

The genetic code

In the early 1950s, it was clear that a linear correlation exists between changes in the base pairs of DNA and changes in a protein. This means that when a change in DNA occurs, this is equivalent to a change at the corresponding site in the protein. This indicates that there is a strong correlation between a gene and a protein. However, the proteins contain 20 different amino acids, but DNA contains only four different bases. Therefore, it was assumed that groups of bases must consitute the code for the order of the individual amino acids in the protein. At that time, it was known that the major part of DNA is located in the chromosomes in the cell nucleus while the synthesis of a protein takes place outside the nucleus. It was also known that there is a large amount of RNA in those cells exhibiting a large protein synthesis. RNA is different from DNA, as the name indicates, as regards the sugar molecule - ribose - to which the bases are linked, and furthermore in that uracil has replaced thymine. Based on these results, it was assumed that RNA could act as an intermediary of information from DNA in the nucleus to the site of the protein synthesis. The relationship between DNA, RNA and a protein is known as Crick's central dogma of molecular genetics:

The flow of genetic information is from nucleic acid to protein, or from DNA to newly replicated DNA. A reverse flow from protein to nucleic acid, which would enable the *inheritance of acquired characters* envisaged

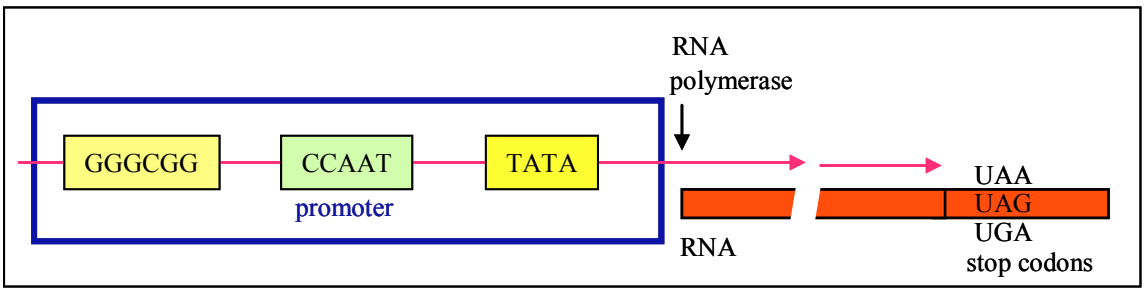


Fig. 2-5. The promoter region in higher eukaryotes. The TATA box and the two upstream elements the CCAAT box and GC-rich box are shown. Elements acting on the promoter at great distances, enhancers and silencers, are not shown.

by Lamarck in the 19th century, is apparently impossible. Some viruses possess an enzyme, reverse transcriptase, that synthesizes a single-stranded DNA molecule using RNA as a template; but this does not contradict the central dogma as formulated by Crick.

Later on it was shown that only one of the DNA strands serves as a template during the RNA synthesis. Thus, RNA consists of the complementary pattern to the strand of DNA that served as a template during RNA synthesis (see Box 2-1). The formation of the RNA strand is called transcription. In eukaryotes, the transcribed DNA contains both coding regions, exons, and non-coding regions, introns. But it is only the coding regions, the exons, that will transfer their information into a protein. This means that the first formed RNA product must get rid of its introns. They are excised in several steps as is illustrated in Box 2-1. After that, the RNA strand leaves the cell nucleus. Since this RNA transfers its message outside the nucleus it was named messenger RNA (mRNA). In the cytoplasm, the mRNA moves to the ribosomes on which the protein sythesis takes place using mRNA as a template, translation. When the ribosomes move along the mRNA molecule the amino acids are linked together forming a polypeptide in the order determined by mRNA. For proteins consisting of more than one polypeptide, the individually synthesized polypeptides are later combined to form the complete protein. Also another type of small RNA molecule, each binding an amino acid, was detected in the cell. These RNA molecules were named transfer RNA (tRNA), because they bring the amino acids to the mRNA molecules on the ribosomes.

In early 1960, a now classical experiment was carried out with mRNA artificially produced and consisting of uracil only. This mRNA construct produced a polypeptide during the polypeptide synthesis that exclusively contained the amino acid phenylalanine, although all other amino acids were available for the polypeptide synthesis. Shortly after this discovery, it was shown that mRNA containing only adenine encoded a polypeptide consisting only of lysine. In the middle of 1960s, by synthesizing mRNA using different bases it could be stated that a sequence of three bases of the mRNA molecule codes for one specific amino acid. This triplet of three bases is called a codon. Thus, the genetic code consists of a series of mRNA codons, each specifying a particular amino acid.

Since the four bases can be combined in 64 different combinations (4^3), it is evident that more than one codon encodes a particular amino acid, *i.e.* the code is what is called degenerate. Out of the 64 possible combinations, 61 encode specific amino acids. The three combinations not encoding any amino acid, UAA, UAG and UGA, code for stop signals that terminate the synthesis of a protein. The codon AUG, which also codes for methionine, is the initiation codon for translation.

In most cases, as is illustrated in Fig. 2-4, it is the two first bases of the triplet that specify a particular amino acid while the third base has no influence on which amino acid will be formed. For example, when the base cytosine is found in the positions 1 and 2 in the triplet, the amino acid proline is always formed irrespective of which base is located in position 3. When a base is replaced by another base without changing the amino acid encoded, the change is referred to as a synonymous substitution.

Each transcribable gene has a region upstream of the start of transcription that regulates the synthesis of mRNA. This region is called the promoter (Fig. 2-5). The synthesis of RNA is carried out with help of an enzyme, called RNA polymerase as it contributes to the formation of an RNA polymer. RNA polymerase binds to the site of transcriptional initiation of the promoter. Eukaryotes have three types of RNA polymerase, each with a different function. The first type mainly transcribes genes for ribosomal RNA, while the second type transcribes protein-coding genes leading to the synthesis of mRNA, and the third type transcribes tRNA genes and some other small nuclear RNA types. As discussed above, stop signals are needed to terminate the synthesis of a protein. Three codons have this function (see Fig. 2-5).

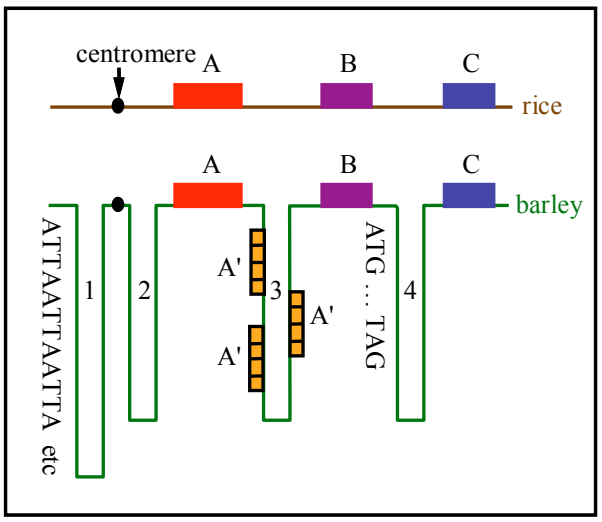


Fig. 2-6. A schematic illustration of a rice chromosome and the corresponding barley chromosome showing (a) the same order of the three hypothetical genes A, B, and C and (b) the occurrence of putative junk DNA and nuclear parasites of various types. Barley contains about 12 times more DNA than rice but the same genes exist in the same order. Only one of the two strands of DNA is indicated.
DNA 1: Highly repetitive DNA; often located in regions around the centromere
DNA 2: Transposon; a transposon is similar to virus and is usually classified as a nuclear parasite
DNA 3: Pseudogenes; a version of gene **A** *that lacks the promoter and can therefore not be expressed, is repeated three times*
DNA 4: A nucleotide sequence that contains a stop codon (TAG) located just behind the start codon (ATG) which inhibits the further protein synthesis.

Regulation of gene activity

Even before the genetic code was deciphered, it was clear that there must be ways of controlling the number and type of proteins formed in the cells. For example in a forest tree, all genes are not active - turned on - at the same time during the whole day and night, or during the annual cycle or during ontogenetic ageing from the juvenile to the adult, flowering-competent phase. A plant of Norway spruce would not be able to survive if genes for active height growth were active even in winter time. Referring to Box 2-1 and the central dogma of molecular genetics, it is easy to see that this control can be exerted at two levels at least: (1) at the time when DNA is transcribed to mRNA, transcriptional control, and (2) when mRNA is translated to a protein, translational control.

In eukaryotes there are many genes that are always constitutively expressed. These so-called house-keeping genes code for essential functions common to all or most cells. Other genes must be turned on or off at appropriate times. The major control of gene expression takes place at the transcriptional level. The essential factor for this control is the promoter. The promoters are classified in three main groups: (1) constitutive; always active more or less, (2) developmentally regulated or tissue specific; active during specific developmental phases or in specific tissues, (3) inducible; activated by various physical and chemical factors. The promoter, often named the core promoter, is the region between the site of transcriptional initiation and the TATA box, an AT-rich sequence. The main controlling elements in most genes are the TATA box and promoter-proximal elements such as the CAAT box and the GC-rich box located upstream of the promoter (see Fig. 2-5). In addition there are elements that can act on the promoter at great distances. These latter elements can either increase the rate of transcription, so-called enhancers, or decrease the rate of transcription, so-called silencers. The enhancers act in such a way that the genes are only transcibed when the proper transcriptional activators are present and bind to the enhancers. The activators, also referred to as transcription factors, are needed for RNA polymerase to initiate transcription to mRNA. In a corresponding way, repressors bind to the silencers to slow transcription.

One example of translational control is provided by so-called masked mRNA. Unfertilized sea urchin eggs contain large amounts of mRNA which are translationally inactive until a few minutes after fertilization when the translation starts. Another example is that translational control can be regulated through factors that increase the lifetime of mRNA molecules during which the mRNA molecules are translated repeatedly. This means that fewer copies of a gene are required to produce a given amount of a particular translational product.

Number of functional genes in plants

Is it possible to estimate the number of genes in the genome of a conifer? What information is available about the number of genes in the genome of a conifer? From comprehensive DNA sequencing, the number of genes is known approximately for *Arabidopsis*, rice, and *Populus trichocarpa*, as 27,000, 50,000, and 40,000, respectively. *Populus trichocarpa* is thought to be a newly formed tetraploid. Recently the *Picea abies* genome was sequenced and estimated to contain 28,000 genes. Examples of genome size and number of genes in various organisms are given in Table 2-1.

Similar gene and gene order over wide taxonomic families

If one compares the genome size of the two cereal species rice and barley, rice has a very small genome (0.45 picogram in a haploid cell) while the barley genome is about 12 times larger (5.5 picogram). Both are diploid species. If we assume that rice and barley have about the same number of genes, we should expect that barley contains much more repetitive DNA. In both these species and in

other cereal species, a large number of genes have been located on genetic maps and the order of the genes has been compared. These studies showed that not only did the same genes exist in both species, but more interestingly, the genes appeared in the same order, *i.e*. the gene order seems to be highly conserved. The picture of a plant chromosome that emerges shows that repetitive DNA is found around the centromere, at the chromosome ends, and in regions of various size between the genes (Fig. 2-6). Also the genes themselves appear in clusters often located near the centromere region or near the chromosome ends. What is remakable is that the genes occupy an insignificant part of the chromosomes as compared to the regions of various types of repetitive DNA. Even in so distantly related species as human and mouse partial conservation of gene order, called synteny, exists. What about conifers? This is being intensively studied, notably in pine species. Preliminary results indicate that synteny is found in pine species as well. This information can be used for mapping purposes. When the gene order in one species is known, this knowledge will facilitate the location of genes in a second species.

The molecular clock

The molecular clock hypothesis is based on the following assumptions:

(1) The substitution of nucleotides in DNA, or amino acids in proteins, occurs at a constant rate.

(2) The degree of difference in DNA (or amino acid) sequence between similar genes (or the corresponding proteins), in two species, gives information about the time when the two species diverged from their common ancestor.

(3) Most spontaneous mutations that persist in the population are neutral, which means that they are insignificant for natural selection; other mutations are often deleterious and will therefore be selected against if natural selection is allowed to act.

(4) A plant can tolerate changes in some proteins but not in others.

With these assumptions we expect that each gene or DNA sequence changes at its own characteristic rate, and that this rate changes little over millions of years, *i.e.* each gene has its own molecular clock that ticks at a near constant rate. Knowledge of changes in DNA sequences is therefore used to calculate relationships between species, genera and higher taxonomic orders. This would correspond to the dating of geological times by measuring the decay of radioactive elements.

At the same time we have to pay attention to the fact that the molecular clocks are different in different species and vary even within a single plant. The difference between species may be due to differences in generation time; species with a long generation time probably accumulate spontaneous mutations due to DNA replication errors at a slower rate than species with a short generation time. This may be one reason why humans have a slower clock than rodents.

Table 2-3. Variation in rates of synonymous and non-synonymous substitution in various mammalian protein-coding genes. Comparisons between human and rodent genes. The time from divergence: 80 million years.

Gene	Substitution rate	
	Non-synonymous ($x10^9$)	Synonymous ($x10^9$)
Histones:		
Histone 3	0.00	6.38
Histone 4	0.00	6.12
Contractile system proteins:		
Actin	0.01	3.68
Actin	0.03	3.13
Insulin	0.13	4.02
Growth hormone	1.23	4.95
Hemoglobin	0.55	5.14
Interferon	2.79	8.59

Rate = substitutions per site per 10^9 years

The variation in molecular clocks within a single individual might be due to the fact that nucleotide changes occur at a slower rate in such codons where a change in the third position causes an amino acid change and synthesis of a different protein. The molecular clock also runs much slower for those genes encoding *e.g*. histones. We have already seen that histones are significant proteins of the nucleosomes in the chromosomes and interact with DNA. Each amino acid along the histone protein is needed at its correct site for the correct formation of a nucleosome. When the histones in humans and in rodents are compared, the histones appear to be identical although the two species have separated about 80 million years ago (Table 2-3). Does this mean that the corresponding DNA also is identical? The answer is definitely no. As we discussed earlier, the genetic code is degenerate, *i.e*. different codons (triplets) can code for the same amino acid. As is seen from Table 2-3, in the histone 3 gene, synonymous substitutions have occurred at a fast rate while no non-

synonymous ones have occurred. Probably, nonsynonymous substitutions induce amino acid exchanges in the histones which are selected against by natural selection, because they are lethal or nearly so. Thus, the histone proteins have been strongly conserved during evolution. It is a general phenomenon that synonymous substitutions occur at a much faster rate than nonsynonymous ones.

At the time when the hypothesis of the molecular clock was introduced in 1965, there was great interest among researchers to use this tool for evolutionary studies. But it also caused much controversy. Notably, the original assumption that changes in DNA and proteins occur at a constant rate has been questioned. Today (in 2013) the constant molecular clock is accepted by most geneticists as an approximation. The molecular clocks have become very valuable tools for calculating the dates of speciation events and for constructing so-called phylogenetic trees. There is often a good correspondence with the expectations from the conventional tree contructions.

Chloroplasts and mitochondria have their own genetic systems resembling those of bacteria

Although only a few proteins are coded by organelle DNA, chloroplasts and mitochondria perform their own replication of DNA, transcription and translation (protein synthesis). Remarkably enough, these processes are more similar to those occurring in bacteria than to those occurring in the cytoplasm of eukaryotic organisms, including plants. For example:
(1) Chloroplast ribosomes resemble ribosomes in *Escherichia coli* both structurally and functionally. For instance, protein synthesis is hampered by the same antibiotic substance. Nucleotide sequences in rRNA in chloroplasts and in *E. coli* are very similar. Chloroplast ribosomes can use bacterial tRNA for synthesis of proteins. In these characteristics, the chloroplast ribosomes differ from the cytoplasmic ribosomes in the same plant cell.
(2) The protein synthesis in the chloroplasts starts with N-formylmethionine, as in bacteria, instead of starting with methionine as in cytoplasmic ribosomes of the plant cell.
(3) In contrast to nuclear DNA, chloroplast DNA can be transcribed by RNA polymerase from *E. coli*, and the mRNA thus formed can be translated by the bacterial machinery to proteins.

The genetic machinery of mitochondria also resembles that of bacteria, but to a lesser extent.

The endosymbiotic hypothesis explains the origin of organelles

How can it be that in some molecular details, chloroplasts and mitochondria are more similar to bacteria than to corresponding characteristics in other organelles of the same cell?

According to the endosymbiotic hypothesis, the eukaryotic cells started as anaerobic, free-living bacteria without any chloroplasts or mitochondria. Later they established a symbiotic relationship with bacteria whose oxidative metabolism they took over and modified for their own use. The result was the progenitor of the mitrochondrion enclosed in an early eukaryotic cell. This event occurred about 1.5×10^9 years ago when oxygen had entered the atmosphere, but before animals and plants were separated evolutionarily. The chloroplasts are assumed to have evolved from a bacterium with photosynthetic capacity. The chloroplasts are strikingly like the modern cyanobacteria, earlier named blue-green algae.

Interplay between the cell nucleus and the organelles

The endosymbiotic theory does not explain satisfactorily why the genomes of chloroplasts and mitochondria are so small, *i.e.* why there are so few coding genes in these organelles. In eukaryotes of today, nuclear DNA encodes about 90 % of the proteins existing in chloroplasts and mitochondria. This means that we have to assume that a large number of genes has been transferred from the original endosymbiotic bacteria to the nucleus. Evidence for such a gene transfer is that nuclear genes coding for mitochondrial proteins are more similar to bacterial genes than to genes coding for corresponding proteins in other organelles. Furthermore, a large number of DNA segments from plant mitochondria can be traced in nuclear DNA, but they contain genes that in most cases have lost their functions. DNA transfer from organelle to nucleus, and even between organelles, still occurs. After partial sequencing of chloroplast genomes, it was observed that the chloroplast genome of some plants contained DNA segments that were copies of segments of the mitochondrial genome. We may ask why this gene transfer has not been completed a long time ago. It must be expensive for the cell to preserve a separate device for the protein synthesis in the organelles, and perhaps unneeded, since there are other organelles that have no DNA of their own. One possibility is that the transfer process was slowed down because the genetic code of proteins was changed in the nucleus while earlier versions of the code were conserved in the chloroplasts and mitochondria. In organisms living today, three or four codons have different meanings for the protein synthesis in the nucleus compared to the organelle protein synthesis.

Genetic linkage maps

Genetic linkage maps are employed for studying genes for quantitative traits (QTL, see Chapter 5) and for comparing genome structure and gene order of different species. If the gene order is conserved among conifer species or hardwoods, only one or a few species have to be investigated more closely. These maps can then be used

Figure 2-7. Examples of restriction enzymes are given. Their recognition sites, where they will cut DNA and give rise to sticky or blunt ends, are indicated. A, T, C, and G stands for the four nucleic acid bases. Pu stands for any of the two purin bases and Py for any of the two pyrimidine bases.

Enzyme	Origin	Recognition site	Sticky or blunt ends
*Eco*RI	*E. coli*	5' G A A T T C / C T T A A G 5'	== G / C T T A =======
*Hin*dII	*Hemophilus influenzae*	5' G T Py Pu A C / C A Pu Py T G 5'	====== G T Py / C A Pu ======
*Hin*dIII	*H. influenzae*	5' A A G C T T / T T C G A A 5'	== A / T T C G A =========
*Hpa*II	*H. parainfluenzae*	5' C C G G / G G C C 5'	== C / G G C ======

for predicting localisation of genes in other species. This knowledge will also be significant for our understanding of the evolution of the conifer and hardwood genomes.

Genetic linkage maps with molecular markers evenly distributed along the chromosomes can significantly contribute to making the analysis of genetic diversity more efficient and to the characterization of gene resources of importance for gene conservation.

Genetic maps may also form the basis of gene cloning and the generation of transgenic trees (see below).

Genetic engineering

Genetic engineering or recombinant DNA technology implies that individual, interesting genes are transferred from one organism to another, often from one species to another species. Thus obtained individuals are called GMO (genetically modified organisms). Genetic engineering relies on molecular genetic methods developed since 1970.

(1) Methods of creating recombinant DNA molecules that include a sequence of DNA in which two non-homologous DNA segments have been combined often from quite different species.
(2) Methods of determining the order of the nucleotide base pairs in a DNA segment, so-called DNA sequencing.
(3) Methods of producing a large amount of specific DNA sequences by the Polymerase Chain Reactions, PCR
(4) Methods of producing synthetic genes or parts of genes.
(5) Methods of revealing gene function

Briefly, what do these methods mean?

(1) Creation of a recombinant DNA molecule is the first step in producing many copies of a gene or part of a gene. This method is called gene cloning. First a DNA fragment including the gene to be cloned is 'cut out' and then inserted into a so-called vector by ligation to produce the recombinant DNA molecule.

The vector is usually a plasmid of bacterial origin and thus often originates from a completely different species than the DNA fragment. A large number of copies of the DNA fragment can be produced in a bacterial cell through the use of such a vector. The plasmids are small, circular DNA molecules that exist in bacterial cells together with their ordinary chromosome. The plasmids contain a few genes only, among them one or more genes for antibiotic resistance. The plasmid can also be equipped with histochemical markers with specific staining properties or nutritional markers that enable cells containing this plasmid to survive on a medium lacking an essential nutrient. The plasmids replicate independently of the chromosome of the host cell and it is this property that is used for multiplication of an introduced DNA fragment. When the host cell, *e.g.* a bacterial cell, is cultivated on a suitable medium it starts to divide and copies of the recombinant DNA molecule are transferred to its daughter cells and a further amplification of the DNA fragment takes place. As the daughter cells originate from one and the same original cell, the colony produced via the cell divisions constitutes a clone (gene cloning).

Using the gene cloning technique, a DNA library can be established including a collection of clones with a nearly complete set of fragments that contains most of the genomic DNA or cDNA of *e.g.* a plant. cDNA is made from mRNA and thus reflects the expressed genes. Hence, the library can be either a genomic library or a cDNA library depending on the purpose of the investigation.

The discovery of a very specific type of enzyme, the so-called restriction enzymes, makes it possible to "cut" the DNA molecule at defined nucleotide sequences. These enzymes act as scissors that cut only at defined nucleotide sequences, recognition sequences, unique for each

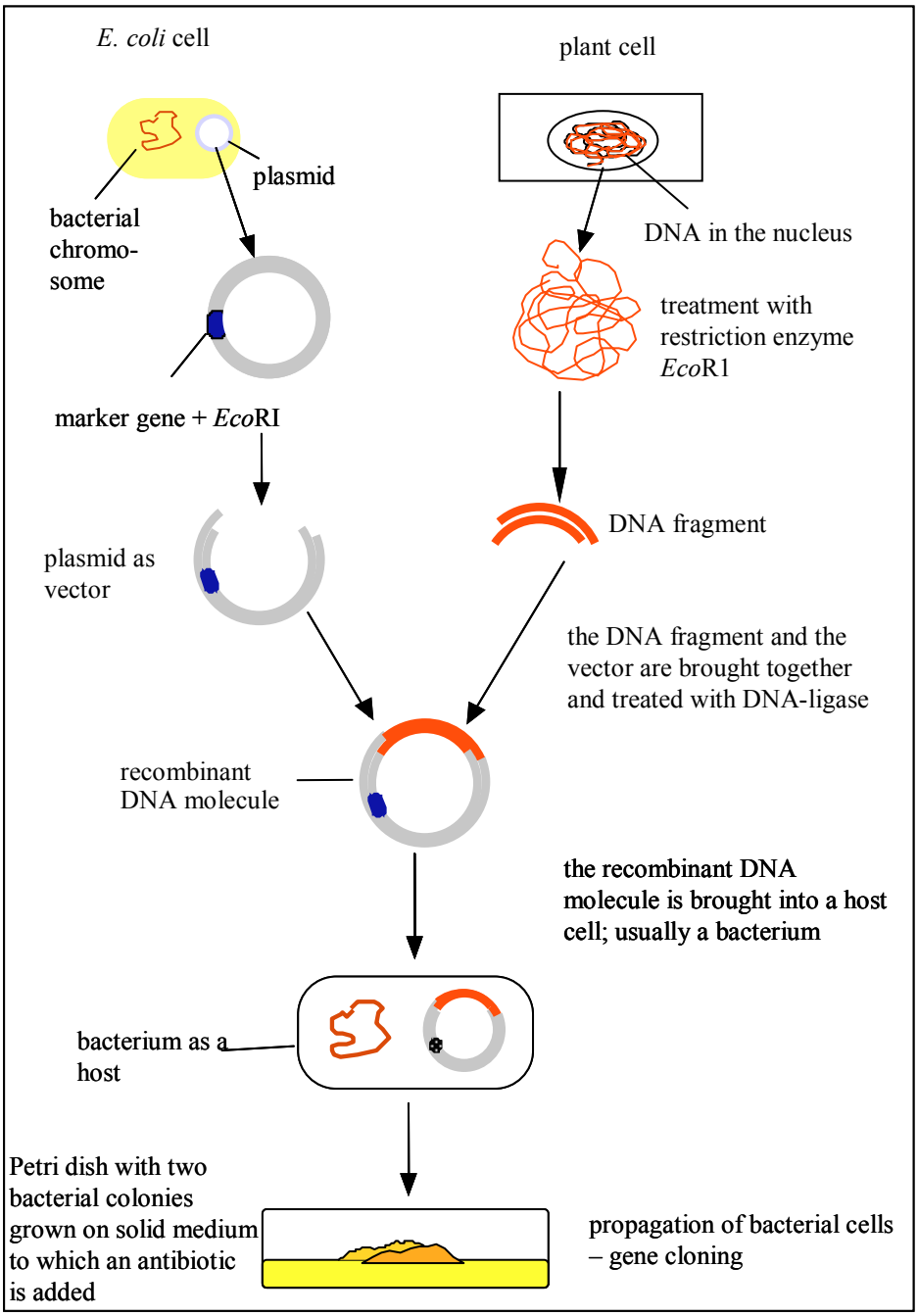


Figure 2-8. Creation of a recombinant DNA molecule and cloning (amplification) of genes. In addition to the important gene, the bacterial plasmid (vector) is carrying a gene for antibiotic resistance which is used for selection so that only those bacterial cells can proliferate that contain the recombinat DNA molecule.

restriction enzyme. Furthermore, the enzymes produce two main types of DNA ends, blunt or sticky ends. In Fig. 2-7, examples of restriction enzymes and their recognition sequences are shown. The recognition sequences consist of between 4 and 8 base pairs. Fig. 2-7 also illustrates that blunt ends appear when the restriction enzyme *Hin*dII is used, because it makes a simple, straight cut, while the other enzymes make a zigzag-like cut that produces sticky ends.

Knowledge of restriction enzymes is essential for the creation of recombinant DNA. In Fig. 2-8, the procedure for making a recombinant DNA molecule is illustrated. In this example, a plasmid isolated from the intestinal bacterium *Escherichia coli* (*E. coli*) is used as vector. This plasmid has one recognition sequence where the restriction enzyme *Eco*RI can cut. When the plasmid is treated with this enzyme, the result is a linear DNA molecule with single-stranded sticky ends. When the same treat-

ment with *Eco*RI is applied to DNA from a plant or an animal cell, we get several DNA fragments all with the same type of single-stranded sticky ends as the vector. In the next step, the DNA fragments and the linear vector molecules are ligated together to form recombinant DNA molecules. The formation of a recombinant DNA molecule is due to the fact that the DNA fragment and the vector contain the same complementary DNA sequences. To ligate the DNA fragment and the linear vector molecule, another type of enzyme, DNA ligase, is used. These enzymes are naturally occurring and have the capacity to repair single-stranded breaks in a DNA molecule. The vector including the DNA fragments is then multiplied using the gene cloning technique discussed above. When a gene in the fragment is cloned, new possibilities open up for studying the gene structure and function, and how it is expressed. For example, the order of the nucleotides can be determined, so-called DNA sequencing (see under 2). A further application is that the gene can be introduced into host cells belonging even to different species, to produce a transgenic organism containing a foreign gene.

An early (1985) and still current industrial application of the recombinant DNA technique was the commercial production of a human growth hormone by the pharmaceutical company, Kabi. The protein hormone synthesized in bacterial is purified and injected into children deficient in the hormone; this converts dwarf to normal growth. Earlier Kabi produced this hormone from deceased persons. Because of the risk of contamination with slow-acting infectious organisms (which could not be completely eliminated) such as prions that cause the Creutzfeld - Jacob's disease (mad cow disease), it was important to be able to introduce an alternative production method of the hormone.

(2) In the mid-1970s, two procedures for DNA sequencing were published. Since then, methodology has developed rapidly, and costs have declined sharply, partly in response to the needs of the HGP/HUGO project for mapping the human genome, 1989-2003; and the sophistication of 'next generation sequencing' has outgrown the scope of individual researchers. DNA for low cost sequencing is usually sent to specialized university service departments or commercial labs. One of the current methods centers on the Illumina Genome Analyzer and the concept of 'sequencing by synthesis'. In brief, short sequence reads, 32-40 bp, are produced from tens of millions of amplified DNA fragments simultaneously, a process taking a few weeks in contrast to the years of the original genome sequencing initiatives. Computer programmes then organize the short sequences into longer reads; not a straightforward process, and requiring at least a year to complete for tree genomes. The model flowering plant *Arabidopsis* was included in the original HGP/HUGO project. Now, the list of plant genomes that have been sequenced includes the tree species *Populus trichocarpa* (2005), *Eucalyptus grandis* (2010), and *Picea abies* (2013). The sequencing of the *Picea abies* genome, which is nearly seven times as large as that of humans, was begun in 2010. About 75% of the spruce genome consists of transposons, with DNA sequences repeated at many places in the genome; this complicates the assembly of the sequencing data.

It is less challenging to sequence a transcriptome, the total of all the mRNAs present in a tissue at a particular time; only 3% of the huge genome of Norway spruce consists of transcribable genes. It is feasible to follow the changes of expression of thousands of genes at different stages of development, or in response to environmental factors such as temperature, daylength and drought stress, or association with disease or symbiotic organisms; a procedure often called expression profiling. In addition to genes coding for enzymes and structural proteins, thousands of genes coding for transcription factors have been characterized in model plant species such as *Arabidopis* and increasingly in forest tree species. In parallel with 'genomics' and 'transcriptomics', other 'omics' are developing: proteomics, the study of the protein complement, and metabolomics, the characterization of the hundreds of small molecule metabolites found in an organism.

Mass sequencing is already greatly influencing forest tree genetics. For example, genomics can clarify the relationship of populations within a species, and the relationships of one species to another; it can indicate the consequences of bottle-necks in population size following a catastrophic event such as an ice-age; and differences in nucleotide sequences between alleles are elucidated.

Genomics combined with expression profiling reveal the physical basis of the polygene concept. Vast quantities of mass sequencing data fill the data banks and the supplementary tables of papers in forest journals. A similar situation applies to the medical field, where a recent paper notes, however, that the deluge of data derived from next-generation sequencing studies might take a relatively long time to be translated into information that is clinically relevant. Can something similar be said of applications of mass sequencing to practical forestry, for example forest tree breeding? The answer is still not available (2013).

Three areas that forest geneticists and particularly tree breeders must take into account are epistatic interactions (expression of a gene varying with genetic background), genotype-environment interactions and uncertain juvenile-mature correlations (a character that is conveniently measured in a seedling usually shows little or no correlation with the character in the mature tree). Furthermore, a particular allelic variant rarely accounts for as much as 5% of the total variation, even for a family derived from a single cross between selected parents of interest for breeding. These considerations interact with and complicate early selection of individuals based on DNA sequencing (to be discussed in Chapter 9 under marker-aided selection).

Here is an appropriate place to take up epigenetics. An epigenetic change is an alteration in genetic information where the sequence of bases in the DNA remains the same. The two main epigenetic mechanisms are methylation of specific cytosine bases in the DNA, and modification of histones, leading to changes in chromatin structure. In a third mechanism, non-coding RNAs induce covalent modifications of chromatin structure. The epigenetic change affects gene expression, and is inherited by the daughter cells after mitosis. Most often, the epigenetic change is reversed at meiosis, in important contrast to true mutation, where the sequence of bases is altered and can revert only following a rare reverse mutation. But several epigenetic changes, also in plants, are transmitted to the next sexual generation. Even so, epigenetic inheritance does not 'prove that Lamarck was right after all', since the changes are not usually inherited sexually. We can generally observe the phenomena of Mendelian and polygenic inheritance, and we are not constantly confronted with the inheritance of environmentally induced phenotypic variation. Furthermore, even when an epigenetic change survives meiosis and appears in the next sexual generation, it may not continue into subsequent generations.

Epigenetic changes transmitted mitotically are probably important in long-lived organisms such as trees, though they have been little characterized to date; trees are virtuosi at coping with environmental change in the course of their lifetime. Of interest here are comparisons of allelic and epiallelic variation, *i.e*. changes in the methylation of cytosine bases at the same loci. Next generation sequencing is enabling such studies. It is feasible to identify epialleles arising during tree development, to compare developmental histories in contrasting populations, and relate epigenetic changes to stress and more generally to phenotypic plasticity, *i.e.* variation in the phenotype. Small RNAs, see following, are important in epigenetic gene silencing.

Small RNAs,which have been intensively characterized recently as sequencing methods have developed, include small interfering RNA (siRNA), small nuclear RNA (snRNA), small nucleolar RNA and microRNA (miRNA).

Plants can often slow the replication of many plant viruses by a process called RNA silencing, so that an infected plant recovers from the viral disease. A family of plant enzymes called 'Dicer-like proteins' (DCL), or 'Dicer', cleaves viral dsRNA (double-stranded DNA) into fragments 21-24 nucleotides long, called small interfering RNA (siRNA). The two strands of the siRNA are separated, and one binds to the multisubunit structure called the RNA- induced silencing complex (RISC). The siRNA binds to complementary nucleotide sequences of the viral RNA molecules, after which an RNA nuclease, part of the RISC complex, degrades the viral RNA and prevents the accumulation of virus in the plant (Fig. 2-9). siRNAs are also part of the plant's defence against the spread of transposons, and are thought to have evolved in this connection as well as in defence against viruses. Protection against the spread of transposons is of particular importance for conifers, with their huge genomes and high content of transposon DNA. Furthermore, siRNAs act as mobile signals for epigenetic gene silencing.

A variant of RNA silencing called RNA interference (RNAi) has been used extensively in *Populus* and other species to inactivate specific genes in transgenic plants and help identify gene function. An artificial gene is constructed with two copies of a DNA sequence from the target gene, about 500 bp, arranged head-to-head. The transcript containing the repeat folds back into dsRNA as a hairpin. In the plant cell this is cleaved into siRNA and silences the target gene (Figure 2-9).

A first step in gene expression is the production of RNA transcripts. Transcription factors and chromatin modification control the rate at which they are produced. After transcription, gene function is further regulated by RNA splicing, by the length of life of the mRNA before it is broken down, and by the efficiency of its translation into protein.

As outlined above under regulation of gene activity, the primary transcript of a gene coding for a protein includes exons – the coding regions – as well as non-coding regions known as introns. The introns are removed from the primary transcript by RNA splicing (Box 2-1). The

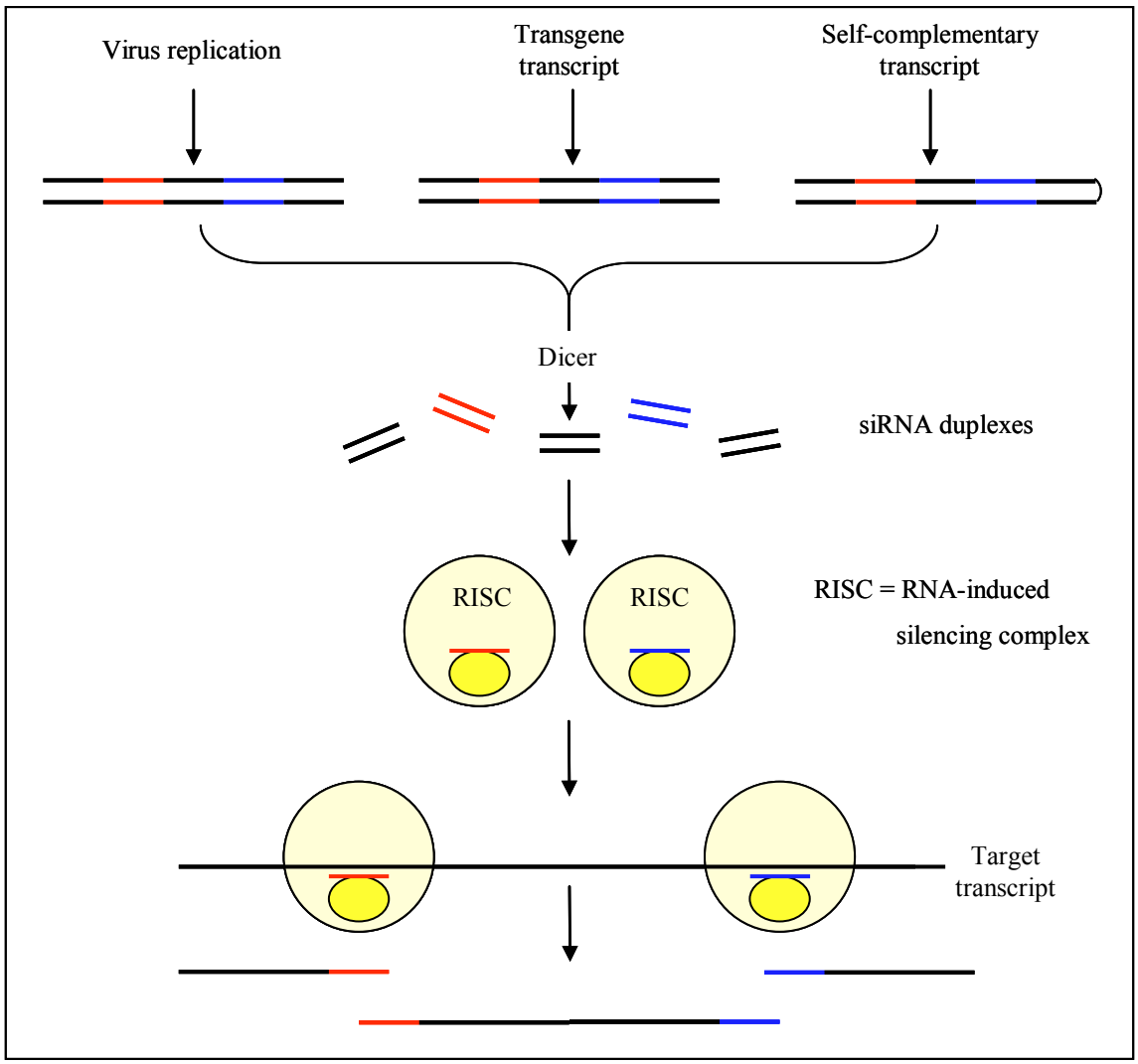


Figure 2-9. Silencing by RNA interference. Long double-stranded RNA (dsRNA) formed during viral replication can trigger RNA interference. Dicer enzyme cleaves the dsRNA into duplexes of small interfering RNAs (siRNA). The single-stranded mature siRNA is incorporated into a RISC silencing complex (see text and Fig. 2-11) where it directs cleavage of the matching sequence in the viral RNA. Transgene transcripts of self-complementary transcripts can also trigger silencing by RNA interference. After Smith et al. 2010.

primary transcript binds to an assembly of protein molecules and small RNA molecules, less than 200 nucleotides long, called the spliceosome, where the introns are cut out (excised) from the transcript.

The small RNAs called miRNA are 21-24 nucleotides long and regulate the life length or translatability of mRNA molecules. Different miRNA molecules are produced from longer precursor RNAs, which are transcribed from miRNA genes. Their origin therefore differs fundamentally from that of siRNAs.

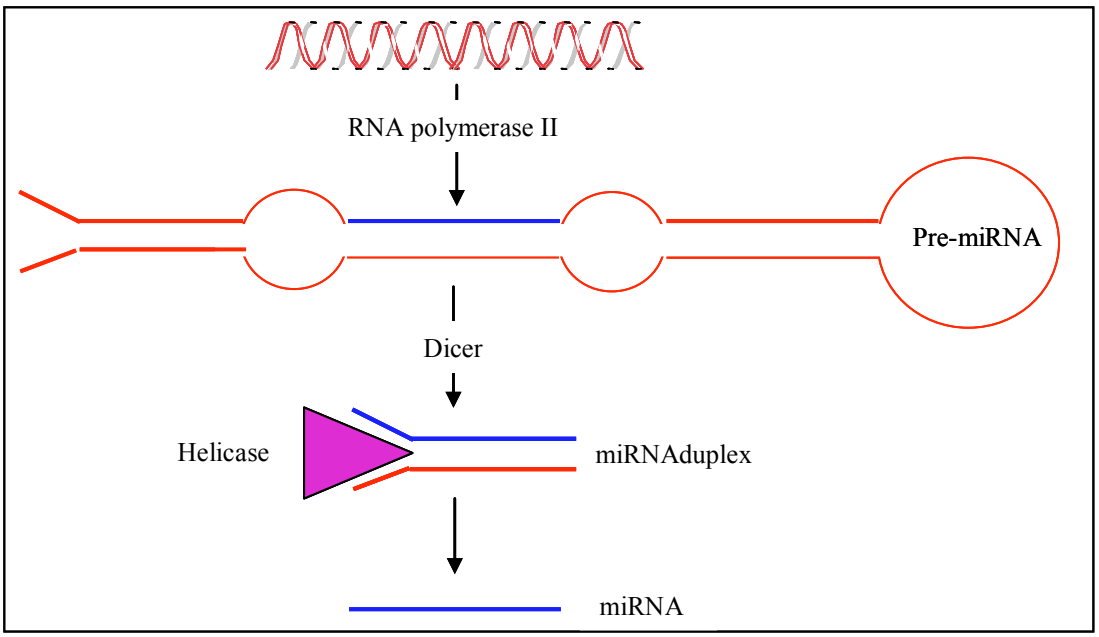


Figure 2-10. Origin of microRNAs (miRNAs). Specific genes (miR) are transcribed by the enzyme RNA polymerase II to give a transcript called pre-miRNA for each gene. This has self-complementary sequences and folds back on itself to form a region of double-stranded RNA that includes the miRNA sequence (blue), The enzyme dicer cleaves out this region of pre-miRNA, and a helicase enzyme separates the strands to yield the mature miRNA. After Smith et al. 2010.

The primary transcripts contain inverted sequence repeats so that they fold to form double-stranded RNA (dsRNA). A Dicer RNase recognizes the double-stranded regions and cleaves the transcript into 21-24 nucleotide fragments (Fig. 2-10). These, associated with the protein ARGONAUTE, can recognize specific target mRNA molecules. The target mRNA is cleaved, or if the match is imperfect, translation is inhibited (Figure 2-11).

In *Arabidopsis*, about 100 miRNA have been identified. Family members are encoded at different loci but are believed to target the same mRNAs. In poplar miRNAs, some not known from *Arabidopsis*, are believed to correct growth against tension and compression stresses. The miRNAs in conifers are 21 rather than 21-24 nucleotides long and are produced by a dicer enzyme that differs from that of angiosperms. In Norway spruce they have been implicated in epigenetic aspects of climatic adaption.

(3) By means of the polymerase chain reaction (PCR), a gene or a DNA sequence can be amplified in a more efficient way than by gene cloning. The method was very quickly accepted after the first reports in mid 1980s. The arrival of this method is a great breakthrough in molecular biology. Automated PCR machines can now be found in every well-equipped molecular laboratory. A DNA sequence can easily be amplified 1 million times. This technique provides a number of applications. As only a very small amount of DNA is required initially, this technique can assist very efficiently in for example, criminal cases in which the potential perpetrator only has left minor traces. Examples of application in human medicine, are within cancer therapy to reveal cancer cells among a large population of normal cells; and fetal diagnosis to determine the sex or for diagnosis of heritable diseases. PCR has also opened up possibilities for studies of evolution at the molecular level. Fossil DNA, often occurring in minute amounts, can be amplified and becomes amenable for analysis. Furthermore, for gene mapping and studies of the structure and function of genes, PCR technique is an indispensable tool.

(4) In the search for more efficient methods of gene sequencing, techniques were developed for producing short synthetic DNA, so-called oligonucleotides. Nowadays, also this technique is automated, thanks to the development of programmable machines. The oligonucleotides are mainly used as probes, or for production of synthetic genes. A probe with a known DNA sequence can be employed for investigating whether a gene of interest is expressed in your material or whether an unknown DNA sequence contains the same sequences as the probe. If so the unknown DNA sequence or gene can usually be identified independently of its origin. Examples of genes that were among the first to be synthesised are the interferons.

(5) The next great challenge will be to reveal the function of the located and sequenced genes. An unexpected result from completely sequenced organisms was that the number of genes is much higher than conventional genetic analysis had indicated. For instance, in yeast, *S. cerevisiae*, it was found that only 30 % of genes had been previously identified. There are three major methods

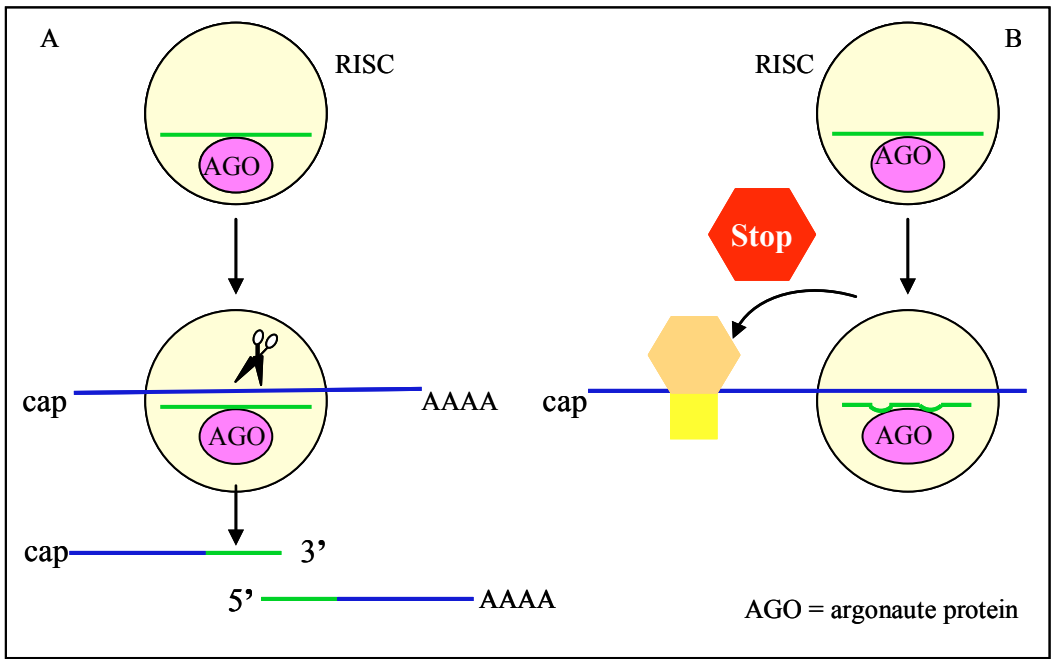


Figure 2-11. Interaction between miRNA and ARGONAUTE (AGO) protein. The miRNA binds to the AGO protein, which is part of the RNA-induced silencing complex (RISC). The miRNA in RISC probes for matching mRNAs. (A) If the match is perfectly complementary, the mRNA is usually cleaved. (B) If the match is partial, the mRNA remains intact but translation is often inhibited. After Smith et al. 2010.

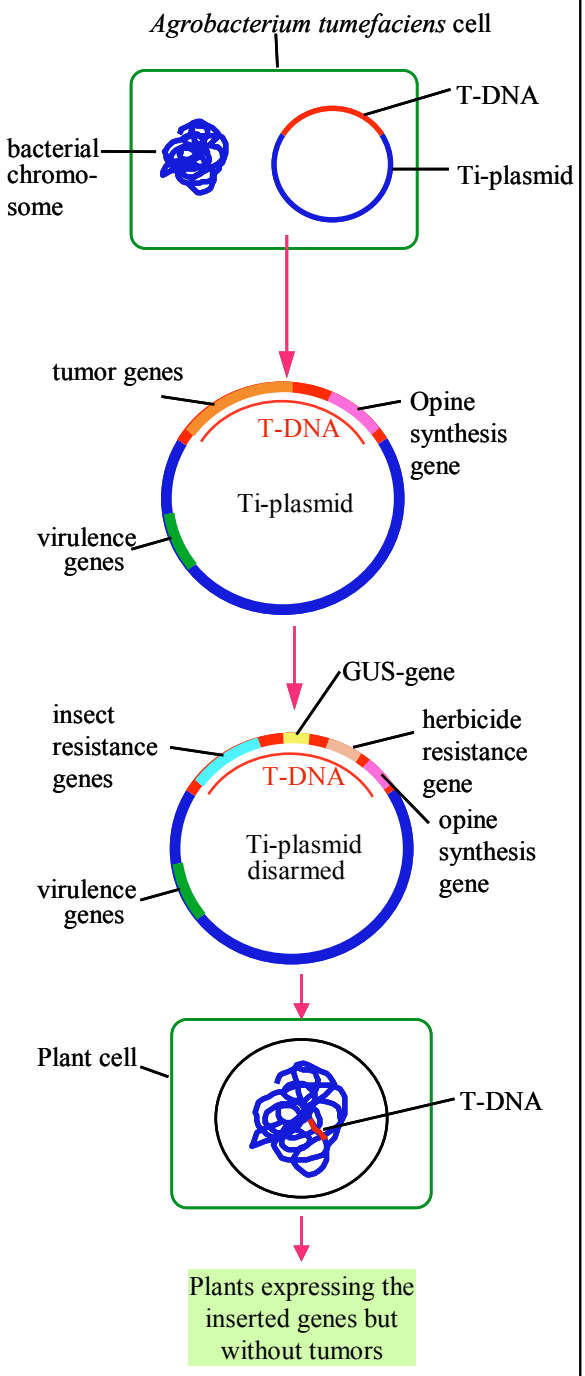
most often used for determining the function of unknown genes. (1) Database searching with the purpose of finding homologous genes in other organisms for which the function is known. The premise for this approach is that there is a good correspondence between genes with similar DNA sequences and their functions even in distantly related organisms. (2) Inactivation of a gene and then search for loss of function. (3) In transgenic plants or plant tissues the gene can be overexpressed and the effect on the phenotype assessed. (4) One promising device for the functional analysis of genes consists of tiny droplets each containing a cloned and sequenced gene and placed in a microarray, on a microscope slide. This can be used to monitor the expression of thousands of genes simultaneously in a particular tissue, by hybridization with mRNA extracted from the tissue (more precisely, with a fluorescently labelled reverse-transcribed copy of the mRNA). A UV microscope is used to detect the fluorescing spots in the microarray representing genes in the microarray that have hybridized to the labelled cDNA and were therefore active in the tissue. This is a very powerful technique that among other things can potentially answer many of the questions raised by traditional quantitative genetics, by precisely defining how the individual members of a population vary genetically.

Here we should mention 'real-time PCR.' A real-time PCR machine follows the amplification of the DNA sequence in real time, using fluorescent markers. The method, 'real-time reverse transcriptase PCR', allows accurate quantification of the activity (mRNA abundance) of a specific gene relative to that of a reference gene, or group of reference genes. It is sensitive enough to need only a small sample of tissue, such as a few needles. This enables proper replication and statistical analysis, so that the measurements of gene activity bear scrutiny by traditional geneticists. It is probably feasible to detect significant differences in gene activity as small as 20% between samples. Knowledge obtained from measurements of this kind can refine the results from microarray analysis and should be relatively easy to integrate into traditional forestry.

How can genetic engineering be applied to forest trees?

One major advantage of genetic transformation via genetic engineering is that only one important gene or a group of genes will be transferred at a time into a variety improved by conventional breeding methods and the remaining set of genes will be preserved more or less unaffected. For our forest trees and particularly those with a long breeding cycle, it is impossible to transfer one single gene by crossing experiments because the first cross must be followed by repeated backcrosses (see Fig. 9-3 for explanation) to recover most of the original set of genes. Therefore, in the future, genetic engineering can play an important role when integrated into conventional breeding programmes. In the near future, practical applications of transgenic plants will probably first appear in broadleaved species, particularly *Populus* and *Eucalyptus,* with rapid growth and short rotation cycles.

Box 2-2. Gene transfer by *Agrobacterium tumefaciens*



In basic research, genetic transformation has been intensively studied in those species where application of this technique is possible. In particular, it is a powerful tool for studying gene function and regulation of gene activity in forest trees. For example, it makes it possible to overexpress a gene if a strong promoter is added or to downregulate the expression of a naturally occurring gene. This technique has been commercially exploited, for instance in tomato; the expression of the gene coding for ethylene production has been reduced resulting in delayed maturation and improved storage capacity.

Genetic transformation means the transfer of recombinant gene constructs into plant cells, selection of transgenic cells and regeneration of these cells into transgenic plants. For achieving this, research is required within three major areas: (1) isolation and identification of genes of importance for tree breeding, whether of major or minor effects, and finding promoters that enable a suitable degree of gene expression in the appropriate cell type; (2) development of reliable means of gene transfer; (3) development of an efficient regeneration system for production and propagation of transgenic plants.

(1) Methods of isolation and identification of genes have been touched upon in previous sections. The promoters are derived from genes that are highly expressed such as the widely used 35S promoter originating from cauliflower mosaic virus. The most common selectable marker gene confers antibiotic or herbicide resistance to the cells. When grown on selective medium containing an antibiotic or a herbicide, only the transformed cell will survive. For example, in *Picea abies*, a reporter gene (GUS, to demonstrate transgene expression) and a gene conferring herbicide resistance (Basta) were fused to a ubiquitin promoter from maize. This promoter construct has successfully been involved in the production of hundreds of transgenic plantlets of *Picea abies*.

(2) To date, the two main methods used for gene transfer are:

(a) Biological vectors, most often gained from bacteria that belong to the genus *Agrobacterium*

(b) Direct gene transfer using biolistic methods

The first method can exclusively be used in plants as animals cannot be infected by *Agrobacterium*. The second method has proved to be successful both in plants and in animals.

(a) Biological vectors. The most widely used vectors in plants are those isolated from the two bacterial species *Agrobacterium tumefaciens* and *A. rhizogenes*. Both are common soil bacteria. *Agrobacterium* infects injured plants and causes gall formation on the stem (*A. tumefaciens*, crown gall disease) or hairy roots (*A. rhizogenes*). When a bacterial cell infects a plant cell, a plasmid, the Ti plasmid, is transferred into the plant cell (Box 2-2). A part of the Ti plasmid, the T-DNA region, is integrated into the chromosomes of the plant cell. The plant cell starts to produce hormones in excess which induces uncontrolled cell divisions and a gall (tumor) develops (*A. tumefaciens*). *A. tumefaciens* mainly infects dicotyledons including woody plants like *Populus* and *Salix* while monocotyledons are usually resistant in field conditions. Both *Agrobacterium*

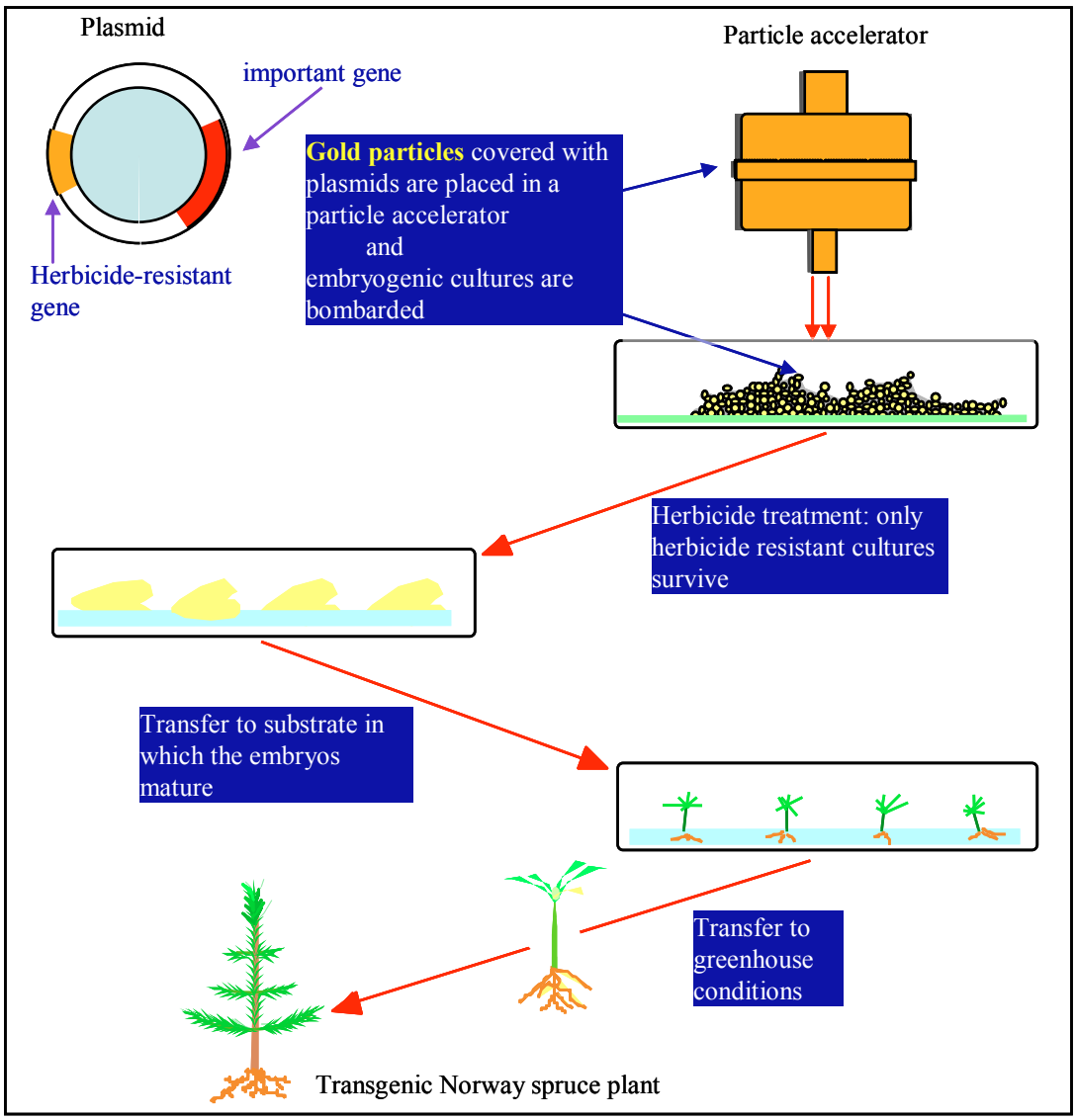


Figure 2-12 shows a method for gene transfer using a particle accelerator and the subsequent regeneration of transgenic spruce plants.

species can accomplish this type of gene tranfer also in conifers. In natural conditions, conifers are seldom infected by *Agrobacterium*, but infection can be induced, for instance, if the bacterial cells are inoculated in the stem. The genes that regulate the synthesis of homones can be excluded from the bacterial plasmid and replaced by genes *e.g.* important for tree breeding. When this disarmed Ti plasmid is allowed to infect conifer cells growing in artificial culture, they no longer produce an excess of hormones, and can therefore be stimulated to proliferate, mature and finally grow on to a new plant. A premise for this vector method and also for the direct transfer method is access to an efficient regeneration system *in vitro* for production and propagation of transgenic plants.

Agrobacterium is routinely used for the production of transgenic plants in several herbaceous species including monocots nowadays. This is also the case for *Populus* and *Betula*. Among conifers, European larch (*Larix decidua*) was the first species in which transgenic plants were produced using *A. rhizogenes*. In *Picea abies*, as a contrast, to date this bacterium can only develop transgenic roots but no transgenic plants. However, *A. tumefaciens* can transform embryogenic suspension cultures of *Picea abies* at high efficiency resulting in many transgenic sublines.

(b) Direct gene transfer using biolistic methods. Successful transformation methods of this type are: electro- and chemical poration and microprojectile bombardement. The latter method makes use of a particle accelerator to deliver high-velocity microprojectiles into plant cells (Fig. 2-12). The microprojectiles, usually gold or tungsten particles, have been coated with *e.g.* plasmid DNA. Successful production of transgenic plants has been achieved in *Picea glauca*. Also in other *Picea* species, including *Picea abies*, hundreds of transgenic cell lines and plants have been produced.

(3) An efficient regeneration system for proliferation of transgenic cells up to propagation of transgenic plants is a bottleneck in many forest tree species, in particular conifers. However, such regeneration systems are available in a number of woody species, including hybrid aspen, poplars, *Picea glauca, Picea abies, P. mariana, Pinus radiata, Pinus elliotii* and *Pseudotsuga menziesii.*

Which traits are most amenable to genetic engineering?

The economic benefits of transgenic tree crops can be great, both for society and for forest industry. But also the environmental benefits can be substantial, reducing the use of herbicides and pesticides through the introduction of transgenic plants with herbicide tolerance or resistance to insects and pathogens. Furthermore, an increased wood fiber production via genetic engineering will reduce the need to harvest native forests. Of crucial importance is of course the public and legal acceptance of transgenic plants (See also Postscript in Chapter 9).

There seems to be a general consensus about the major categories of traits amenable to genetic engineering including:

* Herbicide tolerance
* Resistance to insects, pathogens, and abiotic stress
* Reproductive capacity
* Lignin modification

In the near future also additional traits such as those affecting wood formation and fiber quality will be included.

Historically, herbicide tolerance (glyphosate) was the first trait introduced in *Populus* via genetic transformation. Herbicide tolerance has also been introduced in transgenic crop plants such as maize and soybean.

Plants have evolved efficient strategies for resistance to various insects and pathogenes. For instance, plants can activate a biochemical defence when exposed to stress conditions. Therefore, one breeding goal can be to increase this defence reaction (defence enzymes such as proteinase inhibitors) via genetic engineering. Another option is the transfer of genes coding for insect toxins obtained from the bacterium *Bacillus thuringensis* (*Bt*). This bacterium contains a large number of genes coding for delta-endotoxins that punch holes in the guts of insect larvae. Transgenic *Populus* carrying a *Bt* toxic gene controlled by the constitutive 35S promoter showed endotoxin activity against insect larvae. As regards crop plants, large areas of maize, cotton and potatoes carrying *Bt* toxic genes are currently under cultivation.

Reproductive capacity can be changed in two ways: (1) accelerated flowering and (2) induction of male or female sterility. A desirable breeding goal would be to reduce the extended juvenile phase and breeding cycles in forest trees and in this way increase the genetic gain per time unit. Potential genes for this approach are floral meristem identity genes isolated from *Arabidopsis*. For example, early flowering was induced in transgenic hybrid aspen (*Populus tremula* x *P. alba*), by the transfer of a floral meristem identity gene. *Populus* also, as well as *Pinus radiata*, is being engineered for male sterility. A gene required for the development of pollen, or of the entire flower or male strobilus, can be blocked by *e.g.* the antisense RNA technique, or a gene encoding a toxic product can be fused to a promoter conferring expression only in male tissue. The benefits of male sterility in conifers are (i) to avoid unwanted spread of genes via pollen to native populations; (ii) to facilitate full-sib matings in indoor seed orchards, where no isolation of the female flowers will be needed; (iii) an opportunity to increase the biomass production via reduction of abundant male flowering which demands carbon resources.

The present state of research in flowering may be taken as a case study of the potentials and problems of the application of molecular genetics to forestry. The control of flowering is a prioritized area of traditional forest research. As discussed elsewhere in this book, shortening the time to flowering from the normal 20-30 years in conifers to nearer 10 years implies a prospect of two to three times faster progress in genetic improvement by traditional selective breeding.

Mainly because of its central importance for agricultural crops such as the cereals, flowering is being intensively studied in *Arabidopsis*. This 'model' plant species has many advantages for modern genetic research; in particular, a short generation time of about three weeks, a fully sequenced genome, easy genetic transformation, and a set of lines in each of which the expression of a known gene has been 'knocked out' or 'knocked down' by mutation or transformation so that its function can be studied in detail. Many genes from *Arabidopsis* have been shown to regulate critical aspects of flowering. This knowledge has led to the identification of genes of more or less closely related nucleotide sequence in other species. For woody plants, work of this kind has proceeded furthest with *Populus*, the first forest tree for which the genome has been sequenced. It is important to remember, however, that a gene of closely similar sequence to a well characterized flowering gene in *Arabidopsis* may have a different function in *Populus* or other species. One talks of a 'candidate gene' for the regulation of flowering in the other, less well characterized, species. A candidate gene is believed to influence the character of interest that requires further study.

An interesting example is the *FT* gene (*Flowering Locus T*). Mutants of *Arabidopsis* defective in the *FT* gene flower late in long days, leading to its identification as a gene regulating flowering. The protein encoded by *FT*

is probably a main component of 'florigen'. This is the previously unidentified substance, or substances, that in the 1930s was shown to be induced in leaves exposed to a floral inductive treatment, such as long days in *Arabidopsis*. Florigen is transported in the phloem to the site of action, a bud that is induced to flower. An *FT* homologue is a key regulator of both flowering and growth cessation in *Populus* and probably other angiosperm trees. *FT* belongs to a family of related genes called *PEBP*. In conifers another gene from the *PEBP*. family, *Terminal Flower I-like (TFL1-like*), regulates growth cessation and terminal budset. Furthermore, expression of conifer *TFL-1-like* in *Arabidopsis* delays flowering, as does overexpression of the *Arabidopsis TFL* gene. It seems that the *FT*/*FTL* ancestral branch of the gene family was present in conifers before the evolution of the angiosperms, and that a duplication occurring only in the angiosperm branch resulted in two types, *FT-like* and *TFL-like*, one promoting and the other inhibiting flowering.

Another interesting gene apparently related to flowering is *DAL1*, isolated from Norway spruce. Its sequence is partly similar to a class of flowering genes in *Arabidopsis*. Plants of *Arabidopsis* transformed with *DAL1* from Norway spruce flower early. Such experiments are, however, difficult to interpret because of often occurring changes of function of genes during evolution, as mentioned above. What makes *DAL1* interesting from a practical forestry point of view is that it is unexpressed until Norway spruce trees are about 4-6 years old. Then it is expressed increasingly in the flanks of the meristem, bud-scale primordia, and vascular strands of needles and stems needles of post-dormant trees, reaching maximum expression after 15-20 years. *DAL1* expression is therefore believed to be a marker of the tree's progress through the juvenile phase and young adult phase after which it is competent to flower. Later the gene is expressed in entire female and male cones at early stages of their development.

As a result of mass sequencing, a large number of genes or gene products, including small RNAs, are known to interact with each other and with the biological clock in the photoperiodic regulation of budset and flowering. Knowledge is extensive both for the angiosperm model tree *Populus* and increasingly for conifers such as the spruces. The anonymous polygenes influencing quantitative traits, see Chapter 5, are acquiring a name and personality as they are sequenced and their function is explored; see Fig. 2-13 for genes active in growth cessation and budset.

Because lignin removal during pulp and paper production is costly, modification of the lignin content and composition is a breeding goal amenable to genetic engineering.

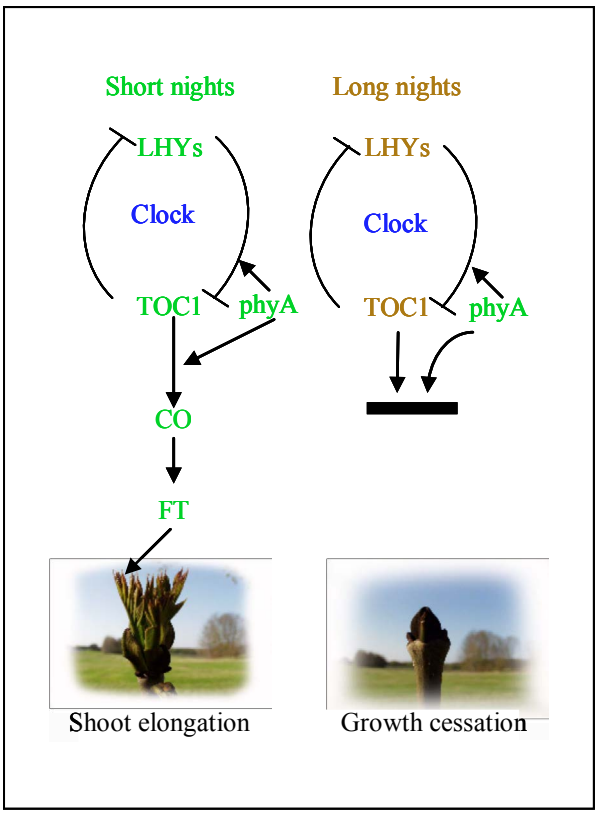


Figure 2-13. Genes that interact in the regulation of budset in Populus. *The plants cease growth and set terminal buds as the nights lengthen in autumn. phyA, a member of the phytochrome family of protein light receptors, is important in detecting, from the leaves, the seasonal change in nightlength. It interacts with the genes and proteins of the biological clock, represented here by LATE ELONGATED HYPOCOTYL1 and 2 (LHYs) and TIMING OF CAB EXPRESSION 1 (TOC1). In short nights the signal from the biological clock stabilizes the level of the CONSTANS (CO) protein and promotes expression of the* FT *gene; the* FT *protein is transmitted from the leaf to the meristem where it signals continued growth. When the night reaches a critical length that varies genetically and epigenetically with the provenance, the altered signal from the biological clock blocks expression of the* CO *and* FT *genes, leading to growth cessation and budset. The genes play similar roles in the photoperiodic control of flowering. (After Cooke et al. 2012.)*

The lignin composition differs between angiosperms and gymnosperms. The lignin in angiosperms is relatively easier to extract by chemicals than lignin from gymnosperms. Therefore, besides reducing the total amount of lignin, a change of the lignin composition so that it will be more like the angiosperm lignin would be a desirable goal of genetic engineering in conifers.

The risks associated with transgenic trees are (1) an unwanted spread of transgenes to native populations, as indicated above, and (2) instability of gene expression. To mitigate the impact of these risks, two main options exist, to obtain reproductive sterility and to screen for stable gene expression. Both options pose great scientific challenges to genetic engineering in forest trees. However, a great incentive to take up these challenges is that forest biotechnology has the potential to offer significant economic and environmental benefits in the future.

It should be added that fears have been expressed that the diversity of livelihoods in the Third World will be eroded if the local varieties are driven out of the market and superseded by a few genetically engineered products. However, this applies equally to traditional breeding, if only a few commercial varieties are grown. In this context the function of different populations ought to be considered. This is discussed further in Chapters 9 and 11.

Summary

DNA is the molecule that carries the genetic information in most organisms. The three-dimensional structure of DNA was proposed by Watson and Crick in 1953. DNA, deoxyribonucleic acid, is a double-stranded polymer consisting of polynucleotides twisted around one another to form a double helix. A nucleotide is composed of a base, purine or pyrimidine, a deoxyribose sugar and a phosphate group. The 'backbone' of the polynucleotide consists of alternating sugars and phosphates. The bases adenine (A) and guanine (G) are purines, whereas cytosine (C) and thymine (T) are pyrimidines. Equal proportions of purines and pyrimidines are found in DNA because the bases are paired, adenine with thymine (A-T) and cytosine with guanine (C-G). The base pairing holds the two complementory polynucleotide chains together. This structure of the DNA molecule also indicates that the genetic information lies in the sequence of the bases, unique for each gene.

The replication of DNA follows the semiconservative model, in which each parental strand serves as a template for the synthesis of a new strand and thus the two daughter helices will consist of one old parental and one new strand. Although the DNA molecule is characterized by great stability, mutations can occur during replication that change the sequence of the bases.

DNA is located in the chromosomes, and exists as only one continuous molecule in each chromosome. It is densely packed following progressive windings, coils, and foldings.

DNA consists of coding, genic DNA and non-coding regions, non-genic DNA. Eukaryotic DNA can exist as unique, single and low-copy, functional genes, *i.e.* genes that are found in only one or few copies per haploid genome, and as different types of repetitive DNA, or as spacer DNA.

In most higher organisms and especially in conifers, non-genic DNA, so called junk DNA, comprises a very large part of the total DNA. More than 97% of DNA in Norway spruce and Scots pine is probably of minor importance for their environmental adaptation.

The central dogma of molecular genetics says that DNA transcribes its information to an RNA molecule called messanger RNA (mRNA) that moves out of the cell and to the ribosomes on which the information is translated into proteins. RNA, ribonucleic acid, is a single-stranded polynucleotide in which deoxyribose is replaced by ribose and the base thymine with uracil. Small RNA molecules, the transfer RNAs (tRNA), bring the amino acids to the mRNA on the ribosomes in the sequence determined by the the order of the nucleic-acid bases on mRNA. Thus, a sequence of three bases of the mRNA, a codon, codes for a specific amino acid. The genetic code is the set of rules specifying the correspondence between the codons in DNA or RNA and the amino acids in the proteins. Special codons serve as start and stop signals for protein synthesis. Each transcribable gene has a region at which the transcription of the gene is regulated, *i.e.* that regulates the synthesis of mRNA. This region is called the promoter. The synthesis of RNA is carried out with help of an enzyme called RNA polymerase that binds to the site of transcriptional initiation of the promoter. Before the RNA molecule is released from the nucleus, it is processed in several steps. The main feature of the processing is to excise the so-called introns, short sequences of DNA that interrupt the coding regions, the exons. The gene expression is controlled both at the transcriptional and the translational level.

The gene order seems to be highly conserved both within genera, *e.g.* among pine species, and over wide taxonomic families, independently of genome size. This facilitates gene mapping.

The molecular clock hypothesis assumes that each gene has its molecular clock that ticks at an approximately constant rate. The molecular clocks have become very valuable for calculating the dates of speciation events and for constructing so-called phylogenetic (evolutionary) trees.

The largest amount of DNA is found in the nucleus of the cell. Additional DNA can be found in the cytoplasmic organelles, the chloroplasts and the mitochondria. The DNA in these organelles directs its own replication, as well as transcription and translation. These processes are very similar to those occurring in bacteria, which according to the endosymbiotic hypothesis, indicates that these organelles are of bacterial origin.

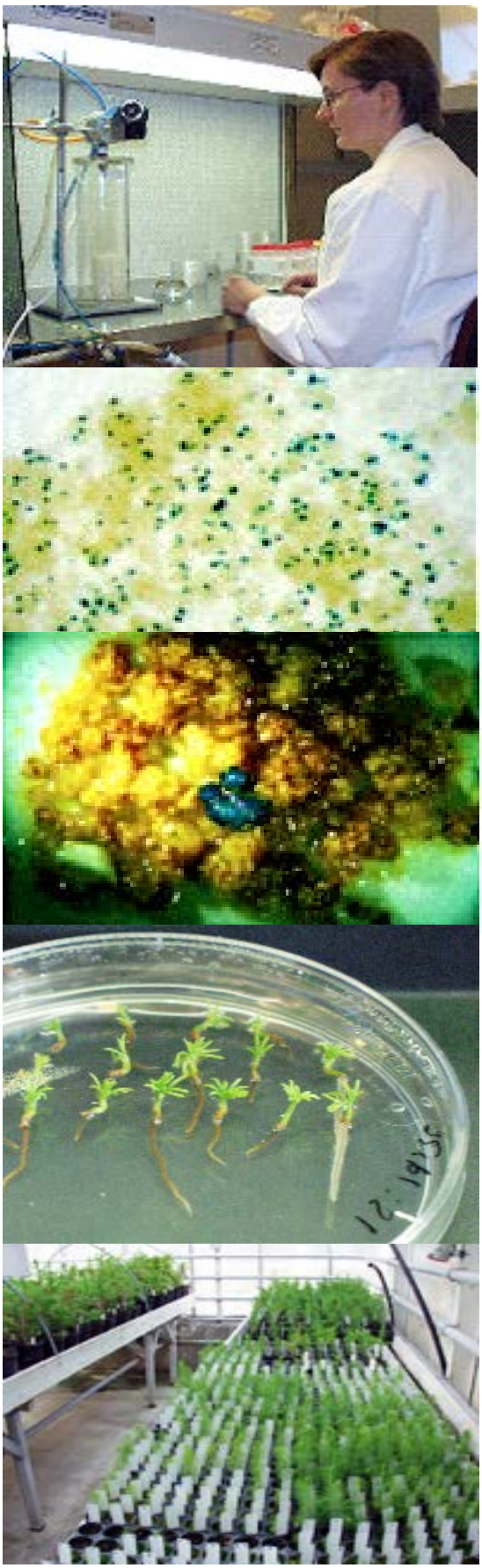
Genetic linkage maps were developed for a number of tree species. The main purposes are to identify genes for quantitative traits (QTL) for use in tree breeding, and to form the basis of gene cloning and production of genetically engineered trees.

Another application of molecular genetic methods is DNA sequencing to determine the order of bases. To date (2013) complete or nearly complete sequences have been published for the tree species *Populus trichocarpa*, *Eucalyptus grandis*, and *Picea abies,* and is well advanced for *Pinus taeda*.

The next great challenge will be to reveal the function of the sequenced genes. One promising method is the microarray technique, which reveals the genes that are active in a particular tissue at a particular moment. Another approach is mass sequencing of the cDNAs corresponding to the mRNAs active in the tissue. This has become especially attractive with the development of the rapid and relatively inexpensive methods known as 'next generation sequencing'. The importance for gene regulation of small RNAs 21-24 nucleotides long such as siRNA and miRNA has emerged following advances in sequencing.

By means of the polymerase chain reaction (PCR), a gene or a DNA sequence can easily be amplified one million times in a very efficient way. The arrival of this method in mid-1980s was a great breakthrough in molecular genetics and and is now a routine in every well-equipped molecular laboratory for gene mapping and studies of the structure and function of genes. This technique provides a number of additional applications, *e.g*. in forensic medicine, as only a small amount of DNA is required initially.

Genetic engineering or recombinant DNA technology are synonyms for transferring genes between organisms, often from one species to another species using molecular genetic methods. The first step, after isolation of a DNA fragment including a desired gene, is to insert the DNA fragment into a so-called vector that can be a plasmid, a small circular chromosome from a bacterium. This technique relies heavily on the discovery of the restriction enzymes which cleave the DNA at defined nucleotide sequences, characteristic of each enzyme. The plasmid, now a recombinant DNA molecule, is then introduced into a host cell in which it can replicate and produce many copies. Also these host cells can proliferate if cultivated on a selective medium allowing only those cells with recombinant DNA molecule to survive and propagate. This multiplication of a gene is an example of gene cloning. The next step is to introduce the gene into a plant cell or an animal cell. For this purpose, two main methods exist: gene transfer via biological vectors and direct gene transfer via biolistic methods.



Picture 2-1. Steps involved in production of transgenic Picea abies *plants. Transformed cells carry the* GUS *gene which encodes an enzyme producing the blue stain. Photograph Hartmut Weichelt.*

Finally, to produce a transgenic plant, an efficient regeneration system is needed. This step is a bottleneck in many forest tree species, in particular conifers. But the number of species in which transgenic plants can be produced is increasing.

A general consensus exists about traits amenable to genetic engineering in forest trees. herbicide tolerance, resitance to biotic and abiotic stress, reproductive capacity and lignin modification. In the near future also traits such as those affecting wood formation and fiber quality will probably be included.

However, there are risks associated with transgenic trees such as an unwanted spread of transgenes to native populations, and instability of gene expression. The mitigation of these risks poses great scientific challenges to genetic engineering in forest trees.

Further reading

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. 2010. Essential Cell Biology. 3rd Ed. Garland Publ. Inc. NY.

Ashley, N. E, J Schlueter, J., Spooner, D.M. 2012. Applications of next-generation sequencing in plant biology. Am. J. Bot. 99: 175-185.

Bonawitz, N.D. and Chapple, C. The genetics of lignin biosynthesis: Connecting genotype to phenotype. Ann. Rev. Genet. 44: 337-363.

Bräutigam, K., Campbell, M., Cervara, M.-T., Diaz-Sala, C., Fernandez Fraga, M., Fluch, S., Fossdal, C.G., Guevara, A., Gutierrez Marcos, J., Johnsen, O., Lafon-Placette, C., Maury, S., Mirouze, M., Rhode, A., Strauss, S.D.H., Vining, K.J. 2010. White paper on 'Epigenetic regulation in forest tree species', workshop on 'Epigenetic response to climatic change', 29th September - 1st October 2010, Aranjuez, Spain.

Cooke, J.E.K., Eriksson, M.E., and Junttila, O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ. 35: 1707-1728

Dolgosheina, E.V., Morin, R.D., Aksay, G., Sahinalp, S.C., Magrini, V., Mardis, E.R., Mattson, J., and Unrau, P.J. 2008. Conifers have a unique small RNA silencing signature. RNA 14: 1508-1515.

Grennan, A.K. 2008. Arabidopsis microRNAs. Plant Physiol. 146: 3-4

Lu, S., Sun, Y.-H., Shi, R., Clark, C., Li, L., and Chiang, V. 2005. Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*. Plant Cell 17: 2186-2203.

Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., and Baulcombe, D.C. 2010 Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328: 872-875.

Nystedt, B. et al. 2013. The Norway spruce genome sequence and conifer genome evolution. Nature doc:10.1038/Nature 12211.

Richards E.J.. 2011. Natural epigenetic variation in plants species: a view from the field. Current Opinion in Plant Biology 14: 204-209.

Smith, A.A., Coupland, G., Dolan, L., Harberd, N., Jones, J. Martin, C., Sablowski, R, and Amey, A. 2010. Plant Biology. Garland Science, Taylor and Francis Group. New York.USA and Abington, UK. 664pp.

Yakovlev, I.A., Fossdal, C.G., and Johnsen, Ø. 2010. MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. New Phytologist 187: 1154-1169.

3 Qualitative inheritance

In this chapter we shall first discuss how to distinguish between genetic and nongenetic variation. After that we shall explain the different types of inheritance, and how they depend on the number of genes involved. Discrepancies from these basic types of inheritance are also discussed.

Even before the Czech monk Gregor Mendel, in 1865, demonstrated the laws of heredity for certain characteristics in plants, it was commonly known that characteristics are transmitted from one generation to the next. Children resemble their parents, and sisters and brothers resemble each other. Before we consider the details of heredity, we shall first discuss the difference between variation due to genetic effects and variation without any genetic influence.

Genetic variation and non-genetic variation

All variation in nature need not be due to heredity; part of the variation can be due to environmental effects. Plants, in particular, have the ability to modify their growth and morphology so that two plants may become identical in appearance even if they carry different genes. Alternatively, morphologically different plants may be identical genetically, owing to differences in development or physiology.

In 1909, the Danish botanist Wilhelm Johannsen published his classical selection experiments with beans, Phaseolus vulgaris, from pure lines. A pure line means a progeny from a homozygous mother plant and is usually produced by repeated self-pollination. When he made selections within a pure line, irrespective of whether he selected for big beans or small beans the resulting mean bean weight was the same; neither an increase after selection for big beans nor a decrease after selection for small beans. Since he obtained no response to selection if the material was genetically homogeneous, the observed variation within the material must be due to environmental influences. This is a good proof of the fact that variation must be due to genetic differences for selection to cause a change. This experiment also shows that it is important not to take for granted that differences observed in nature are genetically determined.

Mendelian inheritance

Mendel made a series of crosses between pea plants from different pure lines with contrasting phenotypic characteristics. The progeny or hybrids, which in genetic crossing experiments are conventionally designated $\mathbf{F_1}$ (the first filial generation), was often identical with one of the parents. Then, individual plants from the $\mathbf{F_1}$ progeny were crossed and Mendel observed that there was a segregation of the different parental traits in this second filial generation, $\mathbf{F_2}$. This was a fundamental discovery, since the result could not be explained as being caused by a mixture of parental traits, as was commonly believed in the middle of the 19th century when Mendel was active. Mendel observed a certain conformity of segregation in the $\mathbf{F_2}$ generation. In most cases he observed that 3/4 of the plants carried one of the traits while 1/4 carried the other trait. The trait observed in highest frequency in the progeny was called dominant while the other was called recessive. Following studies on an extensive crossing material, Mendel could state that the segregation ratio 3:1 was generally found. Based on these results, he could provide a theoretical explanation of the inheritance of certain traits. Mendel denoted genes by letters and he let capital letters stand for dominant traits and small letters for recessive traits. Even this must be considered a stroke of genius. If we have two traits and let the dominant be **A** and the recessive be **a**, and make a cross between two individuals both with the constitution **Aa**, the female and the male parent will produce the same number of sex cells with **a** as with **A**. The sex cells are often called gametes in genetic contexts. The constitutions **AA**, **Aa** and **aa** are called genotypes as was discussed in Chapter 1. A Punnett square, or checkerboard, is often used by geneticists for studying the segregation in which we assume that the gametes are formed in equal frequency in both the female and the male.

♀ \ ♂	A	a
A	AA	Aa
a	Aa	aa

The result from such a square shows that an egg cell with **A** is just as likely to be combined with **A** pollen as with **a** pollen. Similarly, it is just as likely that an **a** egg cell combines with **A** pollen as with **a** pollen. Therefore, the ratio of the genotypes in the progeny will be 1 **AA**: 2**Aa**: 1**aa**. Because **A** is dominant the phenotypic ratio will be 3 dominant: 1 recessive. It is not possible to determine which plants are **AA** and which are **Aa**. To identify which plants are **AA** or **Aa**, the plants are self-pollinated, if the species allows self-pollination. The homozygotes **AA** produce only **AA** progeny, while the heterozygotes **Aa** segregate in a ratio of 3:1. Another alternative for identifying the

AA and **Aa**, is by crossing the **AA** and **Aa** with plants that are homozygous recessive **aa**. This is called a test cross. If the recessive **aa** is crossed with the homozygote **AA**, the result is that all plants in the $\mathbf{F_1}$ progeny will be **Aa** and show the phenotype of the dominant **A** allele. A segregation in the $\mathbf{F_1}$ progeny will only occur if the recessive **aa** is crossed with the heterozygote **Aa** and the ratio will be 1:1. This can easily be verified by a Punnett square as described above. In this case the four squares are replaced by two squares as the homozygotes **aa** only produce one type of gamete, the **a** gamete.

In his book "Genetics: Basic and Applied", the Swedish geneticist Arne Müntzing has made an exellent summary of the great significance of Gregor Mendel's discovery as follows:

Mendel realized that his results could only be explained by the assumption that hereditary differences between the intercrossed parents depended on individual, constant units of heredity – later on called genes, which in an unchanged condition are transmitted by the sex cells from one generation to the next.

This concept of constant units of heredity was something quite new for people at that time and it would take some additional decades until the discoveries of Mendel were more widely spread in biological research. This occurred in 1900, when three researchers independently rediscovered Mendel's results.

In genetics, the genotype of the female is, by convention, written first in a cross. Thus, in the cross
aa x **Aa** the female is homozygous recessive **aa** and the male is heterozygous **Aa.**

Mendel also studied what happened when more than one pair of traits appeared in the parents. For example, he crossed pea plants with yellow and round seeds with other pea plants with green and wrinkled seeds. All plants in the F_1 progeny were yellow and round. Then Mendel self-fertilized the F_1 progeny. In the F_2 progeny he observed four seed phenotypes and their segregation was in good accordance with

9 yellow and round
3 yellow and wrinkled (brown frame in the Punnett square in next column)
3 green and round
1 green and wrinkled (furthest down to the right in the Punnett square in next column)

We immediately observe that two of the trait combinations were not present in the parents at all, i.e. yellow and wrinkled as well as green and round. How to explain this segregation? Because the $\mathbf{F_1}$ progeny showed yellow and round seeds we must assume that these two traits are dominant and we let **Y** stand for yellow seeds and **R** for

♀ \ ♂	YR	Yr	yR	yr
YR	YYRR	YYRr	YyRR	YyRr
Yr	YYRr	YYrr	YyRr	Yyrr
yR	YyRR	YyRr	yyRr	yyRr
yr	YyRr	Yyrr	yyRr	yyrr

round seeds. Green and wrinkled seeds must be recessive traits and their genes are designated as **y** and **r**, repectively. If we assume that the segregation of the two seed traits, seed colour and seed shape, is dependent on two pairs of alleles in two loci and that these alleles undergo independent assortment, the $\mathbf{F_1}$ progeny will produce four types of gamete in equal frequency, **YR, Yr, yR** and **yr**. This is illustrated in the 4 x 4 Punnett square above.

In this grid, 9 of the 16 squares include both **Y** and **R**, which means that these squares contain genotypes that will show yellow and round seeds. It should be observed that there are four different genotypes behind this phenotypic trait combination. Behind each of the two new trait combinations there are two genotypes. Finally, the recessive trait combination green and wrinkled seeds is represented by one genotype, the double homozygote **yyrr**. Crosses between parents differing at two loci display a dihybrid segregation while crosses between parents differing at one locus display monohybrid segregation.

If we have three pairs of traits, a plant heterozygous at all three loci will produce eight different gametes. If the alleles at the third locus is designated **T** and **t**, each of the four gametes produced in the dihybrid cross will combine with **T** or **t**, and eight different gametes will appear. The Punnett square for this segregation will thus contain 64 squares. The segregation ratio of phenotypes will be
27: 9: 9: 9: 3: 3: 3: 1.
The phenotypes have different colors in the large Punnett square at the top of the next page. From this trihybrid square it is clear that plants homozygous at all three loci are very rare. The more loci involved in segregation the lower the frequency of plants being homozygous at all loci.

Based on these Punnett squares, a couple of general formulae can be framed concerning number of different gamete types and genotypes produced in the progeny when the parents are heterozygous at all loci.

	Gametes 2^n	Genotypes 3^n
Monohybrid	2	3
Dihybrid	4	9
Trihybrid	8	27

♀ / ♂	*YRT*	*YRt*	*YrT*	*yRT*	*Yrt*	*yRt*	*yrT*	*yrt*
YRT	*YYRRTT*	*YYRRTt*	*YYRrTT*	*YyRRTT*	*YYRrTt*	*YyRRTt*	*YyRrTT*	*YyRrTt*
YRt	*YYRRTt*	*YYRRtt*	*YYRrTt*	*YyRRTt*	*YYRrtt*	*YyRRtt*	*YyRrTt*	*YyRrtt*
YrT	*YYRrTT*	*YYRrTt*	*YYrrTT*	*YyRrTT*	*YYrrTt*	*YyRrTt*	*YyrrTT*	*YyrrTt*
yRT	*YyRRTT*	*YyRRTt*	*YyRrTT*	*yyRRTT*	*YyRrTt*	*yyRRTt*	*yyRrTT*	*yyRrTt*
Yrt	*YYRrTt*	*YYRrtt*	*YYrrTt*	*YyRrTt*	*YYrrtt*	*YyRrtt*	*YyrrTt*	*Yyrrtt*
yRt	*YyRRTt*	*YyRRtt*	*YyRrTt*	*yyRRTt*	*YyRrtt*	*yyRRtt*	*yyRrTt*	*yyRrtt*
yrT	*YyRrTT*	*YyRrTt*	*YyrrTT*	*yyRrTT*	*YyrrTt*	*yyRrTt*	*yyrrTT*	*yyrrTt*
yrt	*YyRrTt*	*YyRrtt*	*YyrrTt*	*yyRrTt*	*Yyrrtt*	*yyRrtt*	*yyrrTt*	*yyrrtt*

The number of different gametes is 2^n, where n stands for the number of heterozygous loci. The number of different genotypes is still larger = 3^n. For Norway spruce and Scots pine with 12 chromosome pairs, heterozygosity at one locus on each chromosome pair for the two parents crossed, should theoretically generate 3^{12} different genotypes in their progeny. This is a number somewhat larger than 500 000. For a lime tree with its 41 chromosome pairs, heterozygosity at one locus on each chromosome pair will result in 3^{41} different genotypes in the progeny. The number is unbelievably large, = 3.6 x 10^{19}, probably greater than the total number of lime trees in Sweden. In Box 3-1, the general formula is given, which also includes those situations when a locus has more than two alleles, multiple alleles. With this knowledge about the immense possibilities of variation in mind, it is easy to see that all living individuals in cross-fertilized organisms are unique genetically, except for those individuals regenerated via cleavage of a fertilized egg, as is the case for identical twins.

If you are uncertain over how different traits are inherited you can carry out the same type of crosses as Mendel did. What is important to remember is that the number of plants in the progeny should be large enough so that the segregation can be verified with statistical significance. By chance, we will seldom or never expect to get exactly the segregation ratios 3:1 or 9:3:3:1 and so forth. The statistical method used to estimate the probability of one type or the other type of segregation is called the chi-square (χ^2) method. One example will illustrate the procedure for estimating the goodness-of-fit between the observed numbers and and the expected numbers. In an $\mathbf{F_2}$ progeny obtained from the original cross between two parents, one with yellow and round seeds and the other with green and wrinkled seeds, the segregation in the progeny was as follows:

yellow and round	100
yellow and wrinkled	27
green and round	25
green and wrinkled	8

Based on these figures, we can suspect that the segregation is 9:3:3:1, which is the expected frequency of phenotypes. This expected, ideal frequency should be compared with the observed frequency. If we start with our 160 plants, we will get the following expected numbers:

yellow and round	90
yellow and wrinkled	30
green and round	30
green and wrinkled	10

To estimate the goodness-of-fit, we must first calculate the difference between the observed and the expected values, thus, 100-90; 27-30; 25-30 and 8-10. χ^2 value is the sum of the squared deviations divided by the expected values, respectively. In general, this is written $\chi^2 = \sum(d^2/m)$, where d is the deviation and m the expected number. In our case we get:

$\chi^2 = 10^2/90 + (-3)^2/30 + (-5)^2/30 + (-2)^2/10 = 2.63$

Box 3-1

Number of genotypes – N - formed during recombination of heterozygous loci on the assumption of no linkage between loci:

$$N = \left[\frac{r(r+1)}{2}\right]^n$$

r = number of alleles in each locus
n= number of segregating loci
N = number of genotypes resulting from recombinations

Theoretically, *Picea abies* and *Pinus sylvestris,* both having 12 pairs of chromosomes, can produce 531441 different genotypes if only one locus per chromosome pair is heterozygous. For practical purposes the resulting number of gernotypes is infinite.

Using tables in textbooks of statistics, we can find the probability for the observed segregation being caused by chance under the hypothesis put forward for the expected frequences. By this probability, called the p value, we can infer whether the observed segregation deviates from the expected segregation by chance, or because it does not agree with the hypothesis. In our material we have four classes and therefore three degrees of freedom. In a χ^2 table for three degrees of freedom we find that the probability value is between 0.3 and 0.5. This means that it is a fairly high probability that we are dealing with a 9:3:3:1 segregation. The larger the χ^2 value the less is the probability that the observed segregation ratio agrees with the expected segregation ratio. Mendel reported an extremely good agreement with the expected ratios. This may be because he stopped the experiments when they reached a close fit to what he was expecting; a procedure that statisticians forbid.

A premise of attaining segregation ratios such as 3:1 and 9:3:3:1, is the independent assortment of alleles *i.e.* that genes in different pairs of alleles are inherited independently of each other. In some cases, it has been observed that the traits of the parents appear in a higher frequency than expected. The reason for this is probably that loci for these traits are sited on the same chromosome. The loci belong to the same linkage group. If the two loci are located very near each other on the same chromosome, perhaps only the parental combinations will be found in the $\mathbf{F}_2$ progeny. The only way of breaking up the parental combinations is if crossing-over between the two loci takes place during meiosis. Let us assume that we make the following type of cross, **AAbb x aaBB**, where the **a** locus is linked to the **b** locus, and that the $\mathbf{F}_1$ progeny is selfed. We further assume that one out of 10 gametes is a crossover gamete. This will give us the following gamete ratio: 9 **Ab**: 9 **aB**: 1 **AB**: 1 **ab**.

The two last gametes are results of crossing-over between the **a** and **b** loci. To derive the genotypes formed in the next generation we have to consider that the gametes do not occur in equal frequencies. In population genetics this is usually done by introducing the fractions of the gametes in the Punnett square. These fractions we obtain by dividing each figure (9, 9, 1, 1) by 20, which results in two gamete frequencies 0.45 (**Ab** and **aB**) and 0.05 (**AB** and **ab**). However, to simplify the calculations in the Punnett square we use the whole numbers 9, 9, 1, 1. In the Punnett square we introduce these for the gamete frequencies: Phenotypically, we get the following segregation:

201	**AB**
99	**Ab**
99	**aB**
1	**ab**

Using the χ^2 method this segregation can be compared with the expected segregation after independent assortment: 225:75:75:25. This will result in a very large χ^2 value, indicating that the observed deviations from expected are not caused by chance but have other causes, *i.e.* the **a** locus and the **b** locus are closely linked.

Also other deviations from the expected 9:3:3:1 ratio can be found in the progeny from crosses between parents that are heterozygous at two loci. One deviation is caused by the **B** allele. If this allele is only expressed in the presence of the **A** allele, the phenotypic segregation ratio will be **9:3:4**. In other cases, **A** and **B** can be mutually dependent on each other, which results in a **9:7** ratio that can be difficult to separate from a **1:1** ratio. A large progeny is needed to be able to statistically separate these two ratios. If a trait is expressed only when certain alleles at other loci are present, as in the two cases discussed above, this is called gene interaction or epistasis.

♀ \ ♂	9Ab	9aB	1AB	1ab
9Ab	81AAbb	81AaBb	9AABb	9Aabb
9aB	81AaBb	81aaBB	9AaBB	9aaBb
1AB	9AABb	9AaBB	1AABB	1AaBb
1ab	9Aa bb	9aaBb	1AaBb	1aabb

Gene effects at the biochemical level

As the relationship between genes and proteins has been demonstrated, we can more easily understand such phenomena as dominance and recessiveness. If the enzyme E, that the gene **A** is encoding, is needed for a precurser D to be transformed to substance F, we can easily realize that this transformation can take place in the homozygote $\mathbf{A}_1\mathbf{A}_1$ as well as in the heterozygote $\mathbf{A}_1\mathbf{A}_2$. In contrast, the homozygote $\mathbf{A}_2\mathbf{A}_2$ results in a plant/tree with the phenotype D. The picture will be more complicated if we assume that the phenotype F can only be produced when the enzyme exists above a certain threshhold value. If we further assume that $\mathbf{A}_1\mathbf{A}_1$ produces more enzyme E than $\mathbf{A}_1\mathbf{A}_2$, the expression of the phenotype of $\mathbf{A}_1\mathbf{A}_2$ depends on whether the amount of enzyme E is above or below the threshhold value. If it is above the threshhold, $\mathbf{A}_1\mathbf{A}_1$ and $\mathbf{A}_1\mathbf{A}_2$ will again show the phenotype F, while if it is below the threshhold, the heterozygote $\mathbf{A}_1\mathbf{A}_2$ will have the D phenotype. In some instances, the phenotype of the heterozygote will be completely intermediate between the two homozygotes. In such cases, we must assume that development of the different phenotypes is limited by the enzyme produced by the genes, so that two genes of $\mathbf{A}_1$ produce double the amount of enzyme compared to the production of single genes. In Norway spruce and

Scots pine, there are several chlorophyll mutants with decreased amounts of chlorophyll. In a heterozygote of such a mutant, Björn Walles observed that the amount of chlorophyll was half that in the homozygous dominant plants.

Summary

Gregor Mendel elucidated the hereditary transmission of the so-called qualitative traits. He realized that the hereditary determinants, later called genes, were transmitted unchanged from one generation to the other and that no unspecific mixture of the contribution of the two parents occurred. He also realized that some genes are dominant, others are recessive. Depending on how many traits are involved in the experiment with crosses between two heterozygote parents we shall get:

for one gene pair, a segregation ratio of 3:1 for dominant:recessive

for two gene pairs, a ratio of 9:3:3:1, dominance for both traits : dominance for one trait : dominance for the other trait : recessive for both traits.

In general, the number of gametes produced in a plant heterozygous at n loci = 2^n. The number of different genotypes produced after a cross between two parents both being heterozygous at the same n loci = 3^n. Linkage exists if loci that control two traits are located close to each other on the same chromosome arm. If this is the case, the two traits do not segregate independently. The closer the two loci are located, the larger are the deviations from the expected segregation when the two loci are located on different chromosomes.

To determine whether the observed segregation ratio is in accordance with a certain expected segregation ratio, a χ^2 test is required.

Further reading

Griffith, A.J.F., Gelbart, W.M., Miller, J.H., and Lewontin, R.C. 1999. Modern genetic analysis. W.H. Freeman and Company, New York, USA, Basingstoke, England.

Hartl, D.L. and Jones, E.W. 1998. Genetics. Principles and Analysis. 4th ed. Jones and Bartlet Publishers, Sudbury, Massachusetts, Boston, London, Singapore.

Population genetics - Hardy-Weinberg law

In this chapter we focus on the Hardy-Weinberg law. The concept of effective population size is presented. Estimates of population differentiation and inbreeding are introduced. A brief introduction to F statistics is also given. Issues on population genetics are also presented in Chapter 6.

Population genetics deals with studies of allele frequencies in populations and their changes. Such changes may be caused by mutations, genetic drift, gene flow, and natural selection. Therefore, population genetics is of great importance for evolutionary issues, which are treated in more detail in Chapter 6. The meaning of mutations has been outlined earlier. Genetic drift is a random process that is of greatest significance in small populations. Various types of migration among populations are called gene flow. Natural selection has occurred when certain individuals in a population have been more successful in passing their alleles to the progeny generation than other individuals of the same population. This ability must be attributed to a better vitality of the successful individuals under the environmental conditions where the population grows.

In the simple Punnett squares used for deriving monohybrid, dihybrid, and trihybrid segregations in diploid organisms, all alleles occur at frequencies of 50%. Mostly we express the frequency in fractions of 1 and in this case the frequency is written as 0.5. Populations in nature can have all kinds of allele frequencies between 0 and 1. In populations the allele frequencies of two alleles at one locus are usually designated as **p** and **q,** where **p** + **q** = 1. When there are multiple alleles in one locus the alleles are designated as **p, q, r,** etc. Note that the sum **p** + **q** + **r**... also in this case is 1.

To analyse the changes in allele frequency of a population from one generation to the next we benefit from Hardy-Weinberg law. The name emanates from the two men who independently of each other presented their findings. The law is most simply described by an example. In a very large population the genotype frequencies are assumed to be the following:

$\mathbf{a_1a_1} = 0.60$
$\mathbf{a_1a_2} = 0.20$
$\mathbf{a_2a_2} = 0.20$

To enable us to derive the genotypic composition after complete random mating we have to assume that the genotypes contribute gametes in the frequency with which they occur. Thus $\mathbf{a_1a_1}$ contributes 60 % of the gametes while $\mathbf{a_1a_2}$ and $\mathbf{a_2a_2}$ each contributes 20 % of the gametes. For the allele $\mathbf{a_1}$ the genotype $\mathbf{a_1a_1}$ will give rise only to $\mathbf{a_1}$ gametes while half of the gametes from the heterozygote $\mathbf{a_1a_2}$ will carry $\mathbf{a_1}$ alleles:

Female \ Male	0.7 a_1	0.3 a_2
0.7 a_1	0.49 a_1a_1	0.21 a_1a_2
0.3 a_2	0.21 a_1a_2	0.09 a_2a_2

$$0.6 + ½ \times 0.2 = 0.7.$$

In an analogous way we shall find the frequency of $\mathbf{a_2}$ alleles to be:

$$0.2 + ½ \times 0.2 = 0.3.$$

The probability that an $\mathbf{a_1}$ allele will participate in the fertilization in this large population is = 0.7 while the corresponding probability for an $\mathbf{a_2}$ allele is 0.3. These frequencies are valid if the matings are random, there are no new mutations in this **a** locus, that there is no gene flow, and that genetic drift or natural selection does not occur. Since the frequencies of the two alleles differ we have to introduce these frequencies in the Punnett square above to obtain the frequencies of the three genotypes in the progeny. The probability that an $\mathbf{a_1}$ pollen will fertilize an $\mathbf{a_1}$ egg cell is in our case 0.7 x 0.7 = 0.49.

The genotype frequencies are equal to their probabilities and by summarising the probabilities for the heterozygotes in the Punnett square we obtain the following genotype frequencies:

$\mathbf{a_1a_1} = 0.49$
$\mathbf{a_1a_2} = 0.42$
$\mathbf{a_2a_2} = 0.09$

Which gamete frequencies do we get from this population? For the $\mathbf{a_1}$ allele we get the following frequency: 0.49 + ½ x 0.42 = 0.70; in an analogous way we get for $\mathbf{a_2}$: 0.09 + ½ x 0.42 = 0.30. In other words the allele frequencies remain unchanged and we can use the same Punnett square as above to derive the genotypic frequencies in the second generation progeny. The Hardy-Weinberg law says that the allele and genotype frequencies remain constant from generation to generation if none of the factors listed above, mutations, genetic drift, gene flow, natural selection, exert any influence on the population.

The Hardy-Weinberg law might be generalised by expressing the allele frequencies as **p** and **q** for the two alleles $\mathbf{a}_1$ and $\mathbf{a}_2$. The genotype frequencies become $\mathbf{p^2}$ $\mathbf{a_1a_1}$, $\mathbf{2pq}$ $\mathbf{a_1a_2}$, and $\mathbf{q^2}$ $\mathbf{a_2a_2}$ by expanding $\mathbf{(p + q)^2}$. With these genotype frequencies the population is in equilibrium according to the Hardy-Weinberg law. For a population in Hardy-Weinberg equilibrium it is easy to derive the genotypic frequencies when the allele frequencies are known for two alleles in a locus.

Another characteristic of the Hardy-Weinberg law is that the equilibrium in one locus is obtained immediately after random mating. If we consider two or more loci the equilibrium is reached somewhat more slowly. This law also shows that genetic variation remains from generation to generation under the conditions given above. Deviations from the expected genotypic frequencies according to the Hardy-Weinberg law suggest that mating is not random, that there is gene flow into the population or that natural selection is in operation.

Other important information from the Hardy-Weinberg law is that rare alleles mainly occur in heterozygotes. An example will shed some light on this. If the rare allele occurs at a frequency of 0.01 we shall have the following genotypic frequencies:

$\mathbf{a_1a_1} = 0.0001$
$\mathbf{a_1a_2} = 0.0198$
$\mathbf{a_2a_2} = 0.9801$

Generally the rarer an allele, the wider the gap between the frequencies of the homozygous and heterozygous carriers of the rare allele. This means that it is hardly possible to clean the population from a rare recessive vitality-reducing allele since most of the recessive alleles occur in the heterozygotes, which cannot be distinguished from the dominant homozygote.

Hardy-Weinberg law helps to explain why the frequency of homozygotes decreases when isolates are broken, which has been called the Wahlund principle (see Box 4-1). Breaking of isolates is of positive significance for recessively conditioned human diseases such as cystic fibrosis and sickle-cell anemia.

One reason for deviations from random mating may be that all individuals do not participate in flowering or fruiting in a population. The sum of those individuals that contribute are referred to as the effective population size and is designated $\mathbf{N_e}$. It should be noted that $\mathbf{N_e}$ is usually estimated in a more complex way than presented here. It is a general biological phenomenon that all individuals in a population do not contribute to the production of a progeny. This is of great significance for decisions about how many trees should be included in a gene resource population.

Box 4-1 Wahlund's principle

A simple example makes the meaning of this principle clear. Two populations, both large enough for random mating according to the Hardy-Weinberg law, have the following genotype and gamete frequencies:

Genotype frequency		
$\mathbf{a_1a_1}$	0.64	0.16
$\mathbf{a_2a_2}$	0.04	0.36
$\mathbf{a_1a_2}$	0.32	0.48
Gene frequency		
$\mathbf{a_1}$	0.80	0.40
$\mathbf{a_2}$	0.20	0.60

Total frequency of homozygotes $\dfrac{0.64 + 0.04 + 0.16 + 0.36}{2} = 0.6$

After the fusion of these two populations to a single population with random mating, according to the Hardy-Weinberg law, the following gamete and genotype frequencies will be obtained:

$\mathbf{a_1} = \dfrac{0.8 + 0.4}{2} = 0.6$ $\quad$ $\mathbf{a_2} = \dfrac{0.2 + 0.6}{2} = 0.4$

$\mathbf{a_1a_1} = 0.36$ $\quad$ $\mathbf{a_1a_2} = 0.48$ $\quad$ $\mathbf{a_2a_2} = 0.16$

The frequency of homozygotes has decreased from 0.60 to 0.52, which is what Wahlund's principle says. By breaking an isolate the frequency of homozygotes will be lower if the merging populations have different gene frequencies

In nature the effective population size may vary from one generation to the next. The effect of a strong reduction of $\mathbf{N_e}$ means that it becomes much less than the arithmetic mean over generations. To estimate the $\mathbf{N_e}$ the following equation is used:

$$1/\mathbf{N_e} = 1/t \times \Sigma 1/\mathbf{N_i}$$

in which t stands for the number of generations, $\mathbf{N_i}$ stands for $\mathbf{N_e}$ in a certain generation. If $\mathbf{N_e}$ over five generations is 20, 80, 100, 125, and 175, respectively, we obtain from the above equation $\mathbf{N_e} = 58$, which is considerably less than the arithmetic mean of 100.

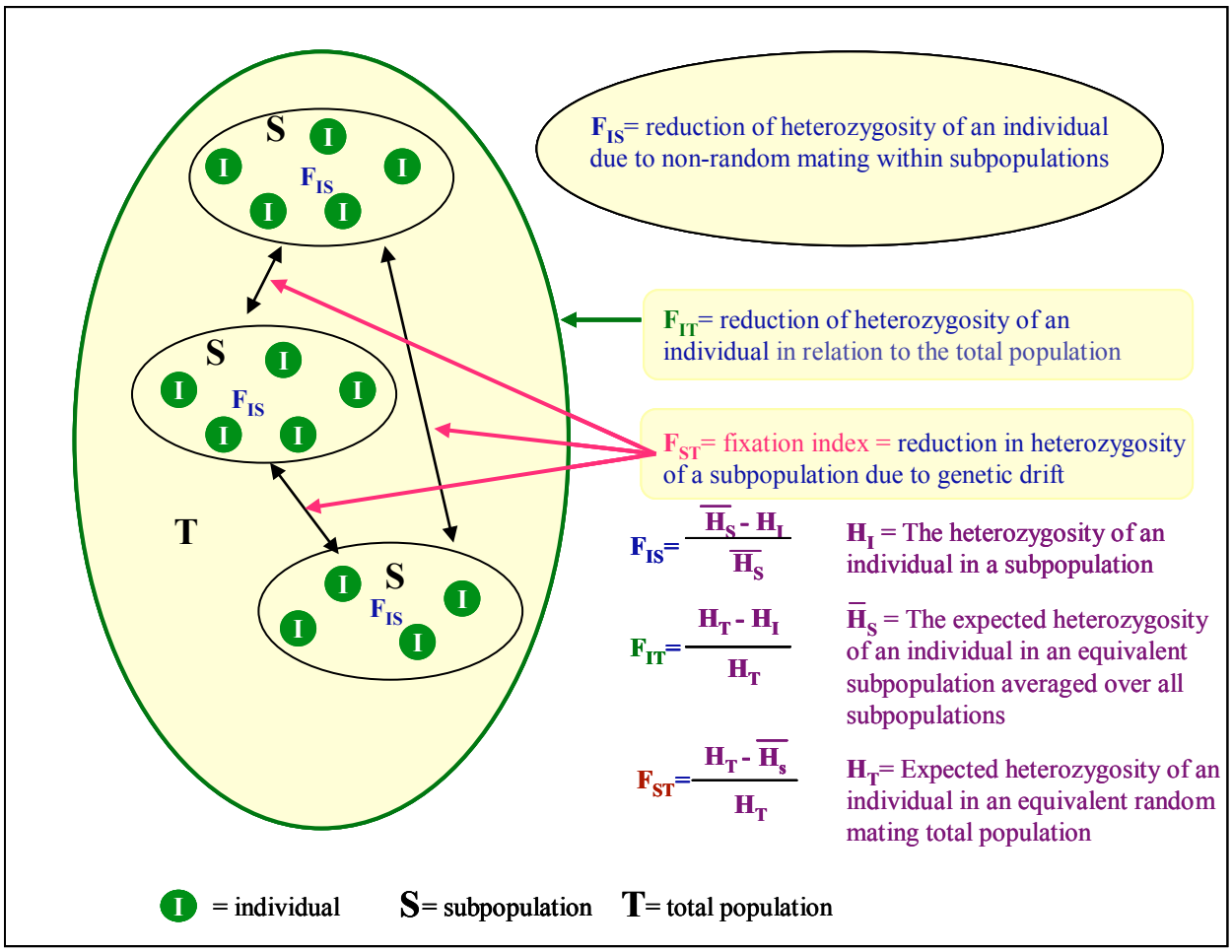


Figure 4-1. A schematic illustration of the concepts FST, FIS, *and* FIT *and their relationship*

For species with different genders, the number of females and males play a role for $\mathbf{N_e}$. For such a situation the following equation is valid:

$$\mathbf{N_e} = 4\,\mathbf{N_m}\mathbf{N_f}/(\mathbf{N_m} + \mathbf{N_f})$$

in which $\mathbf{N_f}$ stands for the number of females and $\mathbf{N_m}$ for the number of males. If the number of females is 50 and the number of males is 200, $\mathbf{N_e}$ becomes 160, which is considerably less than the total number of individuals.

F statistics

Before describing **F** statistics it should be noted that it is beyond the scope of this book to carry out derivations of the concepts introduced in this section.

As will be discussed in more detail in chapter 11 it is of interest to encompass existing genetic variation when sampling gene resource populations. It is also of interest to avoid a high degree of inbreeding in the gene resource population. **F** statistics are useful means to get information on population differentiation and amount of inbreeding. An attempt to visualise **F** statistics parameters is made in Fig. 4-1. All three parameters, $\mathbf{F_{IS}}$, $\mathbf{F_{IT}}$, and $\mathbf{F_{ST}}$, are kinds of inbreeding coefficients. The concept of inbreeding coefficient will be introduced in next chapter. $\mathbf{F_{ST}}$ estimates the reduction in heterozygosity in a subpopulation due to genetic drift and thus is a measure of the relative differentiation in allele frequencies between subpopulations. Therefore, estimates of $\mathbf{F_{ST}}$ are frequently presented in reports on population differentiation studied with isozymes or other markers. The inbreeding within subpopulations is estimated by $\mathbf{F_{IS}}$, which is a measure of the reduction of heterozygosity of individuals within subpopulations. $\mathbf{F_{IT}}$ is an estimate of the reduction in heterozygosity of an individual in relation to the total population. Expressed in another way, $\mathbf{F_{IT}}$ is the total inbreeding in all subpopulations. It is thus a combined effect of non-random mating within subpopulations ($\mathbf{F_{IS}}$) and the effect of population subdivision ($\mathbf{F_{ST}}$).

It should be noted that it is not always straight-forward to compare $\mathbf{F_{ST}}$s from different studies since these estimates depend on the loci analysed and whether non-polymorphic loci are included in the estimate or not. The selection of populations influences the estimates of $\mathbf{F_{ST}}$ too.

$\mathbf{G_{ST}}$ is another parameter frequently used for estimation of population differentiation by markers. $\mathbf{F_{ST}}$ and $\mathbf{G_{ST}}$ are identical if there are only two alleles at a locus.

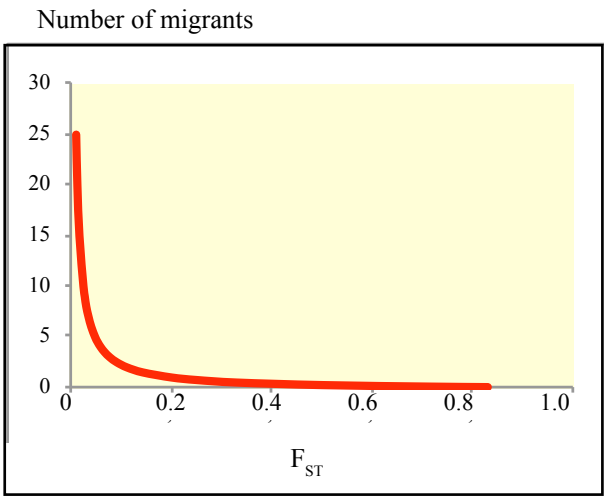


Figure 4-2. The relationship between F_{ST} and number of migrants. At absolute differentiation, F_{ST}= 1 there is no migration.

$\mathbf{F_{ST}}$ estimates have been used to estimate the number of migrants per generation (Fig. 4-2). The formula for this relationship is:

$F_{ST} = 1/(4nm+1)$

in which nm = number of migrants. As seen from this figure $\mathbf{F_{ST}}$ is inversely related to the number of migrants. This means that migration strongly prevents population differentiation.

The latter is intuitively understood from the definition of gene flow, which is *migration to a recipient population from another population with a different allele frequency*. The stronger the migration into a population the smaller the difference between these two populations. Gene flow into a small population has a stronger impact than into a large population. This has bearing on the attempt to restore the vitality of the Swedish wolf population. A combination of a reduction of the existing wolf population and a simultaneous introduction of non-related wolves from other countries is a better approach than keeping as many Swedish wolves as possible.

Most alleles involved in the regulation of a quantitative trait cannot be identified. To enable a comparison of population differentiation between marker traits and quantitative traits, estimates of the latter, designated $\mathbf{Q_{ST}}$, were derived based on different variance components, $\mathbf{V_p}$, $\mathbf{V_{Pb}}$, and $\mathbf{V_e}$. (Variance is a statistical estimate of the variation in a specific trait in a population.) $\mathbf{V_p}$ is the population variance component, $\mathbf{V_{Pb}}$ is the population x block interaction variance component, and $\mathbf{V_e}$ is the variance of individuals (phenotypic value of a tree within a population). Assuming Hardy-Weinberg equilibrium the following formula is used for estimates of $\mathbf{Q_{ST}}$:

$$\mathbf{Q_{ST}} = \mathbf{V_P} / [\mathbf{V_P} + 2\mathbf{h^2}(\mathbf{V_{Pb}} + \mathbf{V_e})].$$

The denominator contains $\mathbf{h^2}$, which is the heritability of the trait. Heritability is presented in Chapter 5, *Quantitative genetics*. The heritability for a trait is an estimate of the resemblance between related individuals for that trait. As can be seen from the equation $\mathbf{Q_{ST}}$ decreases by increasing heritability.

In the early part of this chapter it was stated that population genetics is of great importance for the understanding of evolution. Before evolution is discussed (Chapter 6) it is necessary to introduce different quantitative genetics concepts, which is done in the next chapter. Observed population differences for markers, $\mathbf{F_{ST}}$, and quantitative traits, $\mathbf{Q_{ST}}$, are presented in Chapter 7.

Summary.

Hardy-Weinberg law says that one generation of random mating causes equilibrium of the gene frequencies at one locus. This equilibrium is kept as long as the mating is random. This requirement for random mating is hardly ever fulfilled owing to mutations, natural selection, genetic drift, and gene flow. **F** statistics, with its parameters $\mathbf{F_{ST}}$, $\mathbf{F_{IS}}$, and $\mathbf{F_{IT}}$, is frequently used in population genetics research. These three parameters are a kind of inbreeding coefficients. Inbreeding will be treated in next chapter. In many studies on population differentiation $\mathbf{F_{ST}}$ estimates based on isozyme variation are reported. The formula for estimates of population differentiation of quantitative traits is also presented.

Further reading

Hartle, D.L. and Clark, G. 1989. Principles of population genetics. 2nd ed. Sinnauer Ass, Inc, Sunderland MA 01375 USA.

Quantitative genetics

The characteristics of quantitative traits are presented. The molecular genetics technique for detection and localization of quantitative trait locus (QTL) is outlined. Important concepts in quantitative genetics such as heritability, breeding value, combining ability, genotype x environment interaction, inbreeding, selection differential, selection intensity, genetic gain and genetic correlation are presented.

Characteristics of quantitative traits

In many plants, traits of value for the adaptation to certain environmental conditions such as frequency of flowering, seed production, growth rhythm and tolerance against diseases show continuous variation and are said to be quantitatively varying or quantitative traits. This means that there is no possibility to distiguish a distinct segregation in the progeny in contrast to the traits studied by Gregor Mendel.

After random mating from the crosses **Aa** x **Aa, AaBb** x **AaBb,** and **AaBbCc** x **AaBbCc**, we obtain the genotypes shown in Chapter 3. If we prefer to study the segregation of genotypes only we shall get the following frequencies for the three simplest types of inheritance:

	monohybrid	dihybrid	trihybrid
No capital letter	1	1	1
1 capital letter	2	4	6
2 capital letters	1	6	15
3 capital letters		4	20
4 capital letters		1	15
5 capital letters			6
6 capital letters			1

A mathematically skilled person sees that these figures are equal to the coefficients after expanding the binominal expression $(a + b)^n$, in which n = 2, 4, and 6 respectively. This exercise in figures is meaningful for an understanding of the inheritance of quantitative traits, which may be affected by alleles at many more loci than the three discussed. For simplicity let us assume that there are alleles at four loci that influence height growth in such a way that the homozygote **aabbccdd** has a height of 20 meters at an age of 100 years. Let us also assume that each allele with a capital letter gives an additional height of 0.1 meter. If we know the coefficients for the binominal expression with n = 8 the frequency of the phenotypes are easily derived for this purely hypothetical case. The possibilities for us to distinguish the different classes 20.0, 20.1, 20.2,.....20.8 are evidently small owing to the slight differences among the different genotypes with different numbers of captital letters. In addition, the environmental conditions might blur the picture to make the distribution continuous rather than stepwise. Fig. 5-1 illustrates that the distribution is close to a normal distribution. In most cases we assume that quantitative traits have a normal distribution.

The quantitative traits do not give distinct segregation in the progeny in contrast to what Mendel obtained in his crosses between yellow and green peas or wrinkled and round peas. The absence of distinct classes is a characteristic of quantitative traits. Frequently quantitative traits are affected by alleles at a large number of loci and the influence of each allele on the trait is minor. Modern molecular genetics has revealed that alleles at a certain segment of a chromosome exert a much larger influence than others. The technique is not detailed enough to say whether it is one locus that is involved or whether several linked loci are in action.

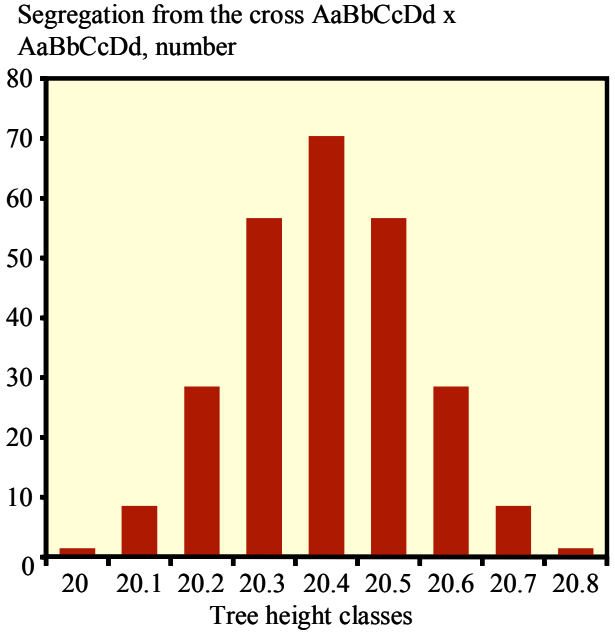


*Figure 5-1. The distribution of tree height among different classes in the progeny from the cross **AaBbCcDd** x **AaBbCcDd** on the assumption that tree height is 20 metres for the recessive homozygote at all four loci and that each capital letter contributes 0.1 metre to the tree height.*

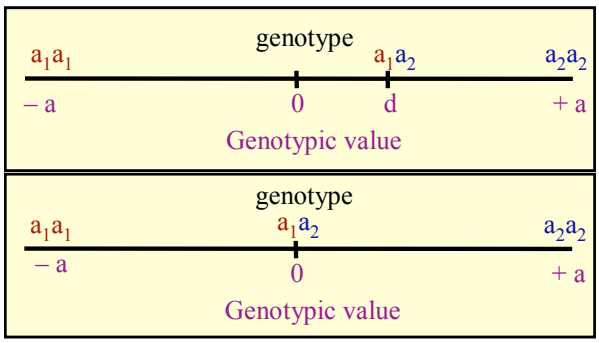


Figure 5.2. The genotypic values for three genotypes at one locus, in which the mean values of the homozygotes is 0. The value of the heterozygote is usually denoted as dominance deviation, d, and may take any value on the scale, -a to +a, as well as outside this scale. When the heterozygote is intermediate to the two homozygotes (below) d is zero and the gene action is completely additive.

Since the alleles at a locus do not always influence a trait in such a simplistic way as assumed for the tree height in the example above, the scale in Fig. 5-2 is used to describe the allelic effect.

The use of capital letters and small letters to designate alleles may be misleading for quantitative traits. Therefore, the alleles are given indices to separate different alleles from each other. In Fig. 5-2 the genotypic value of the heterozygote, $\mathbf{a_1a_2}$ is closer to the homozygote, $\mathbf{a_2a_2}$, than the other homozygote. The value d may be positive or negative. The heterozygote may even have its genotypic value outside the scale shown in Fig. 5-2. At complete dominance, as was the case for the traits studies by Mendel, d is equal to a. The heterozygote $\mathbf{a_1a_2}$ is therefore phenotypically equal to $\mathbf{a_2a_2}$.

The phenotype of a quantitative trait is the joint action of alleles at many loci as well as the effect of the environment at the site where the plant or tree is growing. Also for such traits as survival, for which there are only two classes, alive or dead, there is an underlying quantitative genetic variation. When the pooled genetic and environmental effects are below a certain value the plant will survive, while it dies above this value. This value is usually called the threshold value. Healthy or diseased is another example of a trait that has only two classes, but with an underlying genetic variation.

When the effect of the alleles is simply added to each other, as in the above hypothetical example of tree height, the gene action is completely additive. This was also the case in the first example of quantitative gene action, described for kernel colour in wheat by the Swedish wheat breeder, Herman Nilsson-Ehle. His experiment revealed that there were three loci involved in the kernel colour of wheat, which is a hexaploid species. This is not surprising since there is one locus affecting the kernal colour in each of the three genomes of wheat.

Quantitative trait locus - QTL

QTL stands for Quantitative Trait Locus, *i.e.* those loci on the chromosomes that contain genes for quantitative traits. By making use of a large number of DNA segments it is sometimes possible to find associations with loci regulating a quantitative trait (QTL). To date, traditional biometrical models have not been able to study individual genes for quantitative traits. Furthermore, in these models many simplified assumptions had to be made, for example, that a trait is affected by a very large number of genes, each with a small effect on the trait, that genes act additively and that they segregate independently. However, the molecular marker techniques developed for studying genes may significantly increase our knowledge about genes for quantitative traits. These techniques can provide information about the number of genes affecting the phenotypic variation of a trait, whether the variation is due to a few genes with large effects or a large number of genes each with small effects or whether a combination of both alternatives prevails. Information about interactions between genes at different loci and between genes and environment can also be gained. Construction of genetic linkage maps for QTL and gene markers will inform about the chromosomal positions of the genes for quantitative traits. Its application in breeding will be briefly presented in Chapter 9.

A prerequisite to make progress with QTL is that some of the quantitative alleles have a relatively strong influence on the development of the trait. Another prerequisite is that the parents have pairs of loci in linkage disequilibrium. This means that the alleles $\mathbf{a_1}$ and $\mathbf{b_1}$ always occur in the gametes of one parent and that $\mathbf{a_2}$ and $\mathbf{b_2}$ always occur together in the gametes of the other parent. The alternative linkage disequilibrium with $\mathbf{a_1} + \mathbf{b_2}$ and $\mathbf{a_2} + \mathbf{b_1}$, respectively, is equally useful. Constructing genetic linkage maps for QTL demands that a large number of plants/trees per family is analysed. Moreover, it is assumed that linkage disequilibrium is rare in wind-pollinated tree species with a large and continuous distribution since there is a large gene flow among populations. Therefore, it is essential that the population designed for QTL detection is segregating (heterozygous) for as many of the QTLs as possible.

Methods for constructing genetic linkage maps for QTL

It should be observed that QTL is a segment of a chromosome that may contain not only one but in some cases more than one locus affecting the quantitative trait of interest. The mapping of QTLs means that they are localized to their sites, respectively, on the chromosomes. At the same time, their number and the proportion of the total phenotypic variation that they can explain are estimated.

Construction of genetic maps involves several steps:

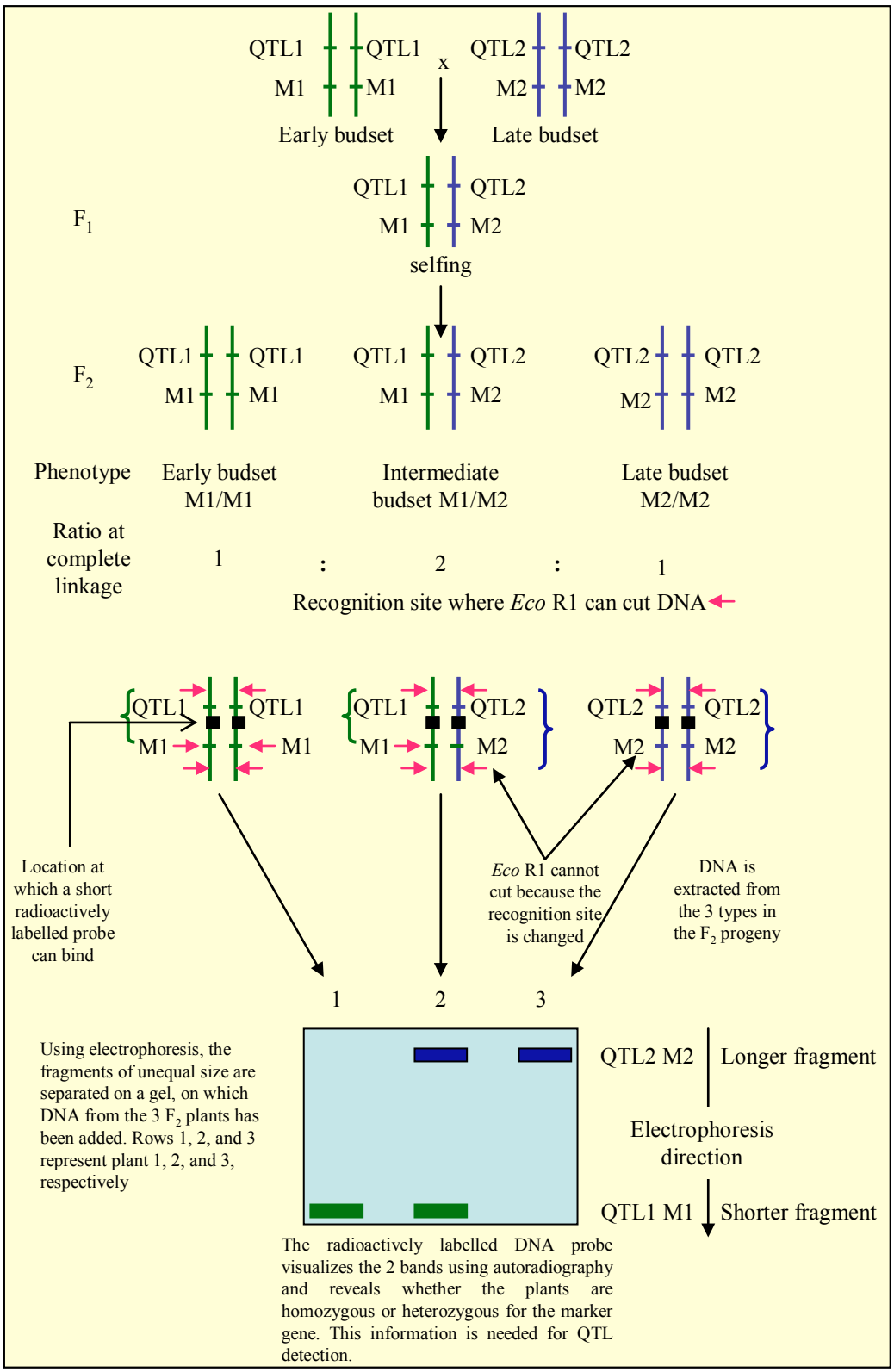


Figure 5-3. Linkage determination between a marker gene locus with two alleles M1 *and* M2 *and a locus for a quantitative trait, the timing of budset, with two alleles* QTL1 *and* QTL2*. Parent 1 shows an early budset and is homozygous for the allele* M1 *and for* QTL1*, while parent 2 shows a late budset and is homozygous for allele* M2 *and for* QTL2*. After mating of the two parents an F_1 progeny is obtained that is intermediate in timing of budset, heterozygous for* QTL1/QTL2*, and heterozygous for marker loci* M1/M2*. We assume that the budset timing is a completely additive trait. After selfing of F_1 an F_2 is obtained. In case of complete linkage between marker and* QTL *locus (no crossovers), F_2 will consist of three types of plants, early* M1/M1*, intermediate* M1/M2*, and late* M2/M2*. If significant differences in budset timing are found between the three classes of markers, linkage is established and a* QTL *locus has been detected and mapped. In the lower part of the figure, an example is given showing how to proceed in order to determine whether the plants are homo- or heterozygous for the marker gene. The example given holds for marker genes of* RFLP *type, for which both alleles in the* RFLP *locus can be identified, here denoted* M1 *and* M2*. We assume that this locus has a recognition site for the enzyme EcoRI (see below) but that a change has occurred in allele* M2 *so that the enzyme cannot cut. This causes unequal RFLP-fragments during the separation of the fragments on a gel using electrophoresis. The plant with late budset has a long fragment (indicated by the blue brace) that contains* QTL2 *and* M2*, and can therefore only move a short distance on the gel, while the plant with early budset has a short fragment (indicated by the green brace) with* QTL1 *and* M1 *and therefore moves a longer distance. The plant showing intermediate budset is heterozygous for both* QTL *and* M *loci and can be identified because both fragment sizes occur on the gel.*

If the marker locus and the QTL *locus are at long distance from each other, or on different chromosomes (in different linkage groups), all three marker gene categories will be intermediate in timing of budset.*

(1) Generation of maps based on genetic markers where the markers should cover the whole genome, the denser the sites of the markers the better is the chances to detect linkage between marker loci and QTLs; the genetic markers should show a high degree of polymorphism, which means that it is highly probable that two individuals carry different alleles at each locus; the markers should also be neutral so that they do not affect the trait of interest or affect the regeneration capacity.
(2) Establish linkage between marker locus and QTL. This presupposes that there exists a sufficiently large phenotypic variation in the quantitative trait in populations used for mapping purposes and that the QTL segregates in the population (Fig. 5-3). Therefore, the selection of suitable mapping populations is very crucial. The mapping populations employed to map QTLs in forest trees consist of various full-sib or half-sib mating designs. In addition, the adequate marker, whether dominant or co-dominant, has to match the type of mapping population used.
(3) Advanced statistical methods of analysis have to be elaborated to detect significant associations between markers and QTL. An array of methods is now available for different mapping populations. However, the methods have their limitations and need to be developed further.

Results from detection and mapping of QTL

In forest trees, detection of QTLs has been published for both conifers and hardwoods. Interspecific mating schemes have been used mainly in broadleaved trees. The quantitative traits involved are economically important traits such as growth, wood quality, adaptive traits to abiotic and biotic stress, and reproduction capacity.

Examples from studies of growth traits in a few species are given in Fig. 5-4, in which the range of phenotypic variance explained by individual QTLs is given. Thus, in *Pinus taeda*, three QTLs for diameter and four QTLs for height were detected. The range of phenotypic variance explained by individual QTL for these growth traits was 15 percentage units. A few examples from other traits are given in next paragraph.

Five QTLs were individually responsible for at most 5% of the phenotypic variance of wood density in *Pinus taeda*. For the same trait, the five QTLs detected in an interspecific hybrid, *Eucalyptus grandis* x *E. urophylla*, individually explained up to 10% of the phenotypic variance. A similar magnitude of explained phenotypic variance was obtained for frost tolerance in *Eucalyptus nitens*, but only two QTLs were detected. For the interspecific hybrid *Populus trichocarpa* x *P. deltoides*, significantly larger phenotypic variances were explained. For example for the bud phenology trait, budburst, five QTLs were detected and one of these were responsible for 52% of the phenotypic variance. In an interspecific cross between

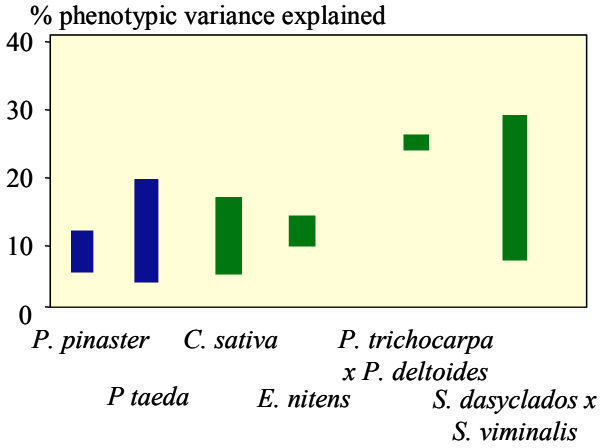


Figure 5-4. The range of phenotypic variance explained by single QTL in Pinus pinaster (6), Pinus taeda (3-4) Castanea sativa (28), Eucalyptus nitans (2-3), *and the species hybrids* Populus trichocarpa x P. deltoides (1-2) *and* Salix dasyclados x S. viminalis (1-8). *Figures in brackets give the number of QTL for each species or species hybrid.*

the male hybrid clone *Salix viminalis* x *S. schwerinii* and a female *S. viminalis* clone, six QTLs were detected for the timing of budburst. Individual QTL explained 12% to 24% of the phenotypic variance.

According to the expectation QTLs in species hybrids usually explain a larger part of the variation than in pure species, mainly due to linkage disequilibrium. Additional information gained from QTL mapping, is that QTL for different traits seem to appear in clusters, suggesting tightly linked QTLs or pleiotropy, *i.e.* that a single QTL affects more than one trait. The latter is what you should expect if there is high genetic correlation between two traits. This was the case in a study of *Salix* hybrids. Sometimes QTLs are co-localized to the same chromosome arm.

As regards the results presented, one has to be somewhat cautious, because many of these estimations probably underestimate the number of QTLs involved in each trait and overestimate the percentage phenotypic variance explained by each trait. The main reason for this is that the size of the family used for mapping was too small. This was indicated by simulation experiments in which it was shown that at a family size of less than 250 a few QTLs were erroneously identified, each with an exaggerated effect on that trait.

Genetic marker maps were developed not only for markers like RFLP and RAPD but also for AFLPs, microsatellites (SSRs), ESTs (For explanation see Chapter 7), and SNPs.

Following mapping of QTLs, the next task will be to identify the genes associated with QTLs. A procedure

suggested for this purpose is the so-called ”candidate gene” analysis. The assumption behind this analysis is that candidate genes, sequenced genes probably affecting the trait expression, occupy a large portion of the QTLs affecting the trait. One way to go is to search in available genetic databasis to obtain their sequences when the genes are isolated.

An additional task will be to study whether the QTL expression is stable over years, various environments and genetic backgrounds. Even if the family size is satisfactorily large in a QTL study it ought to be stressed that the QTLs identified are valid for that particular family. Therefore, it is urgent to test more than one family to identify QTL that are of general importance and not limited to just one family. Such knowledge will be of importance for their potential use in marker assisted selection in breeding programmes (See Chapter 9).

Heritability

Heritability is a concept of great significance in breeding and evolution. The meaning of heritability is visualised in Fig. 5-5. The phenotypes of the progenies are plotted against the phenotypes of their parents. The upper part of Fig. 5-5 shows a fairly good agreement between the parental and progeny phenotypes. This is a case of high heritability. If there is a high heritability for a trait there are possibilities to improve this trait in breeding since a tree with a good phenotype will give rise to a progeny with good phenotypes, too. Such a trait has the potential to become changed by natural selection. In the lower part of Fig. 5-5 a case is illustrated in which there is poor agreement between parents and offspring, which means that the heritability is low. For traits with low heritability it is impossible to identify the good genotypes via their phenotypes. The only way to reveal the good parents is to test their progeny.

To estimate heritability, statistical methods are applied to analyze data from progeny trials. Mathematically the heritability = the additive variance (σ^2_a) divided by the phenotypic variance (σ^2_{ph}). Somewhat later the meaning of additive variance will be presented. In formal terminology heritability of a trait = an estimate of the degree of resemblance between relatives for this trait.

Hertitability is a relative concept that depends on the individuals tested as well as the environmental conditions during the test. An example will illustrate this. If frost hardiness is tested a few hundred meters above the timber line, the probability is high that all plants will die. We shall not be able to reveal any genetic variation in frost hardiness, which is a condition for obtaining a value of the heritability departing from zero. If the progeny trial is located a few hundred meters below the timber line, the probability is high that we shall be able to reveal genetic variation in the survival of the local population. In consequence a heritability differing from zero may be estimated. In certain experiments with Scots pine in northern Sweden the open-pollinated progenies from individual trees in a population had an amplitude of 50 percentage units or more (Fig. 5-6). As will be discussed in Chapter 7, phenological traits, such as budburst and growth cessation, are of great significance for survival and good performance. The size of heritabilities for this kind of trait is much dependent on the point of time for the assessment. If the assessment is too early or too late during the process of development, limited variation will

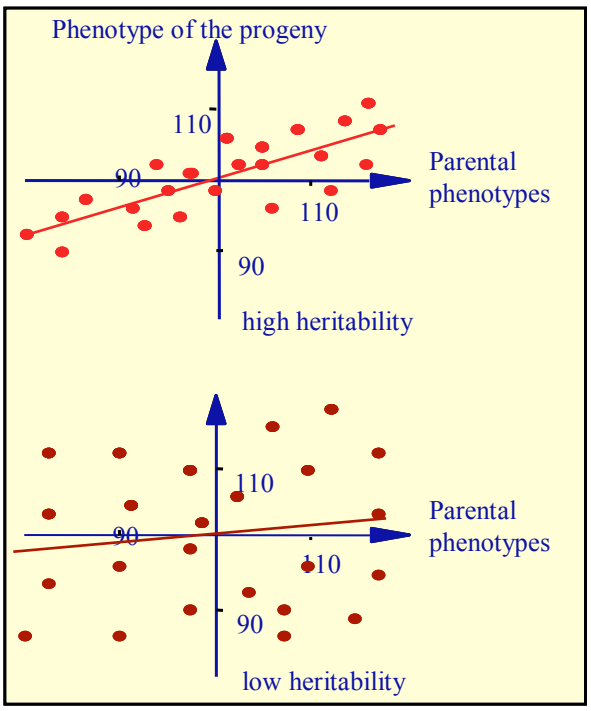


Figure 5-5. The relationships between the performance in parents and offspring, in which the mean values are 100 for parents and offspring. At a good agreement between parents and offspring the heritability is high.

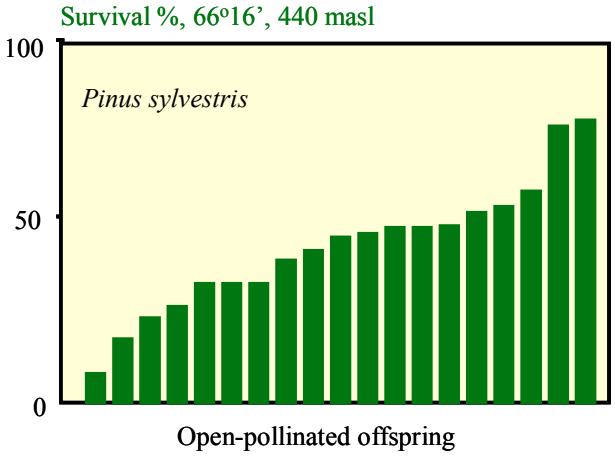
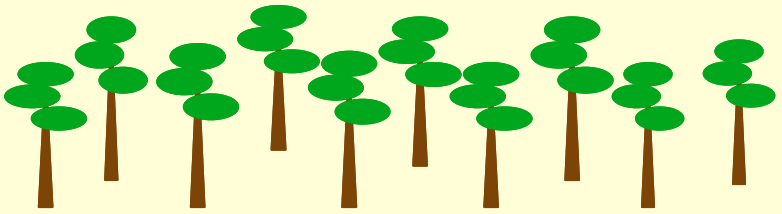
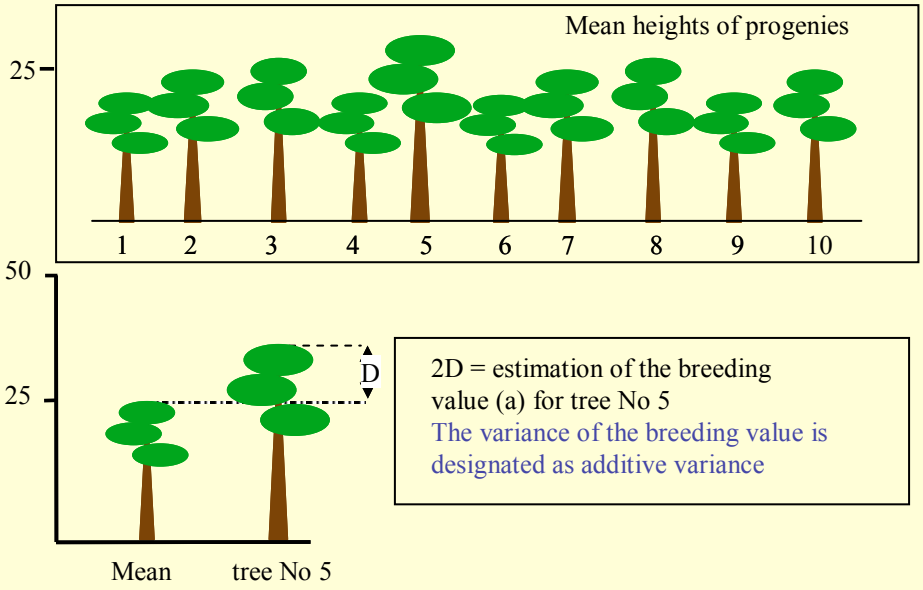


Figure 5-6. The survival of open-pollinated progenies from a population in a 20 year old field trial of Pinus sylvestris *in northern Sweden.*

Box 5-1. Derivation of breeding value and additive variance



Ten Scots pine trees are crossed with each other in all combinations.
The resulting seeds are used for establishing a progeny trial



2D = estimation of the breeding value (a) for tree No 5
The variance of the breeding value is designated as additive variance

be revealed and heritability will be misjudged. The ideal date of assessment for such traits is when the grand mean has reached 50 % of the development. The unfortunate situation is that we do not know this beforehand.

The relationship between parental and progeny phenotypes is just one of several options for estimations of the heritability for a trait. In destructive tests, as often with freezing tests or after inoculation with pathogens, we are forced to estimate the heritability with the aid of siblings.

With well designed experiments we can estimate both the additive variance and the phenotypic variance, the ratio of which is the heritability. It is worth mentioning that variance is a statistical concept which estimates the variation in a plant material. A prerequisite is that there are replications in the experiment. The meaning of additive variance is explained in Box 5-1. Ten Scots pine trees are mated in all possible combinations with all other trees. The seed harvested from these crosses is used for establishment of well designed progeny trials. The mean tree height for all progenies in which tree 1 is one of the parents is shown in the center part of Box 5-1. In a similar way all mean values of the other nine trees are calculated and illustrated. In order to facilitate the understanding it should be noted that the differences are exaggerated compared to a situation in nature. Tree number 5 has the highest progeny and its deviation (D) from the mean value of all trees is illustrated. The breeding value is defined as 2D and the variance of the breeding values is the additive variance which we are interested in. Of great interest for breeders is to estimate the coefficient of additive variance (frequently abbreviated CV_A) which is the square root of the additive variance divided by the phenotypic mean of the trait under consideration. The CV_A gives us a possibility to evaluate the potentials for improvement

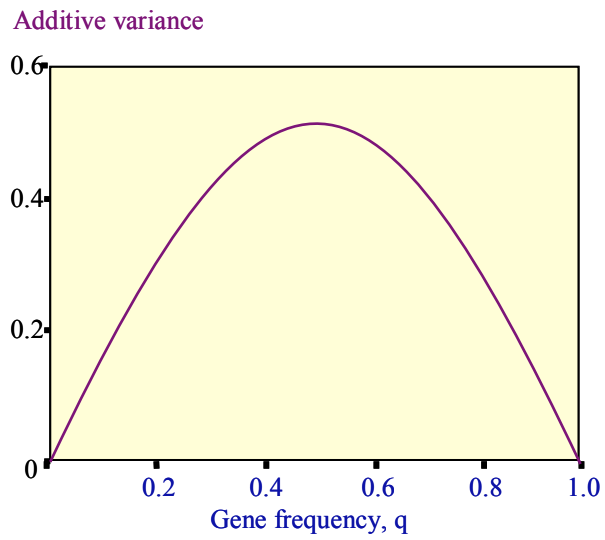


Figure 5-7. The relationship between gene frequency and additive variance with completely additive gene action; a is the value a illustrated in Figure 5-2.

by breeding for this trait. Theoretically we can find all 4 combinations of high and low heritability with high and low CV_A. The possibilities for genetic improvement may be as good for a trait combining low heritability with high CV_A as for a trait with a high heritability and low CV_A.

The reason for defining the breeding value as 2D is that only half of the genetic material comes from one of the parents, the other half coming from other parents. As is evident from Box 5-1 the breeding value is dependent on the other parents tested as well as the environmental conditions under which the testing took place. This means that breeding value, additive variance, and heritability are relative estimates. They are valid for the population under test and the conditions under which they are tested. This in turn means that our tree No 5 might be inferior to these trees in another test with other parents than the ones in Box 5-1.

To link the statistically derived heritability with the quantitative genetics the heritability might be expressed with genotypic values as was done in Fig. 5-2 in the following way:

$$h^2 = \sum 2pq[a + d(q - p)]2/\sigma^2_{ph} \qquad (1)$$

in which q and p are allele frequencies and a and d are the values explained in Fig. 5-2. The effects of all the alleles at all loci that affect the trait must be summed. In the equation the factor [a + d(q - p)] is an expression for the effect on the genotypic value of an exchange of $\mathbf{a}_1$ for $\mathbf{a}_2$ or $\mathbf{a}_2$ for $\mathbf{a}_1$. It might seem surprising that such changes of one or the other allele and vice versa do not have the same effect. This is a consequence of the exchange of the allele frequencies p and q as is evident from the equation. In the equation **a** stands for the proportion of the genotypic effects that are added to each other and that are easiest to exploit in breeding.

It should be noted that the allele frequencies p and q may vary from locus to locus and that the heritability has a maximum at p = q = 0.5 since the product pq which is part of the numerator has its maximum at 0.25. This is reflected in Fig. 5-7 which illustrates the relationship between allele frequency and additive variance for complete additive gene action. In cases with dominance the curve takes another shape but the alleles at low frequencies do not contribute much to the additive variance in that case, either. In summary, alleles at very low or very high frequencies do not contribute much to the additive variance. A consequence of this is that alleles at such frequencies are hard to change by breeding. Similarly such alleles are hardly changed by natural selection.

The effects of **a** and **d** can vary from locus to locus. All these conditions make it impossible to distinguish the effects of the alleles at a particular locus. We have to be satisfied with the knowledge of the joint effect of alleles at several loci that affect the trait. Equation 1 shows that it is possible to connect the statistically estimated heritability with known genetic concepts such as allele frequencies and the effects of exchanges of alleles $\mathbf{a}_1$ for $\mathbf{a}_2$ and vice versa.

Table 5-1. Hypothetical values for all families after crosses between all parents.

	1	2	3	4	5	6	$\overline{X}$
1	-	**32**	24	25	26	21	25.6
2	**33**	-	**31**	**31**	**29**	**30**	**30.8**
3	23	**30**	-	25	23	23	24.8
4	25	**32**	24	-	21	27	25.8
5	24	**28**	24	22	-	**34**	26.4
6	20	**31**	22	26	**32**	-	26.2
$\overline{X}$	25.0	**30.6**	25.0	25.8	26.2	27.0	26.6

Table 5-2. Deviations from the mean value for all families in Table 5-1, 26.6, and the values for the general combining abilities, GCA.

	1	2	3	4	5	6	$\overline{X}$
1	-	**5.4**	-2.6	-1.6	-0.6	-5.6	-1.0
2	**6.4**	-	**4.4**	**4.4**	**2.4**	**3.4**	**4.2**
3	-3.6	**3.4**	-	-1.6	-3.6	-3.6	-1.8
4	-1.6	**5.4**	-2.6	-	-5.6	0.4	-0.8
5	-2.6	**1.4**	-2.6	-4.6	-	**7.4**	-0.2
6	-6.6	**4.4**	-4.6	-0.6	**5.4**	-	-0.4
$\overline{X}$	-1.6	**4.0**	-1.6	-0.8	-0.4	0.4	

There is a close relationship between heritability and another concept, the **G**eneral **C**ombining **A**bility (the abbreviation GCA is frequently used in texts). As with heritability, it can be estimated in progeny trials having material raised from systematic matings. When data from such trials are analysed, one often finds that one parent, independently of mating partner, gives rise to well performing progenies. This is an example of a parent with good GCA. More precisely expressed, the average deviation of this parent´s progeny from the grand mean of the trial is an estimate of the GCA of that parent. To illustrate this, hypothetical values are given in Table 5-1 for a trial in which all possible matings between 6 parents are involved except for selfing. When parents serve both as females and males, the mating design is designated as diallel. (A more detailed description of mating designs is carried out in Chapter 9 in connection with tree breeding, since mating designs are of great importance in breeding.) From Table 5-1 it is evident that parent No 2 has high values in its progenies. Parent No 2 is thus an example of a parent with good general combining ability. The progenies 5 x 6 and 6 x 5 have values that deviate in a conspicuous way from the mean values of these two parents, which are close to the grand mean of this trial. Such a deviation is designated as **S**pecific **C**ombining **A**bility (SCA).

The grand mean for all crosses in Table 5-1 is 26.6. The family deviations and the parental deviations from the grand mean are given in Table 5-2. These latter deviations are estimates of the parental GCAs on the assumption that the experimental error = 0. A comparison of the information in Box 5-1 and Table 5-2 reveals that the GCA of a parent = half the breeding value of this parent. Under the same assumption it is possible to estimate the specific combing abilities with the following general equation:

$y_{ij} = m + GCA_i + GCA_j + SCA_{ij}$

where

$\mathbf{y_{ij}}$ is the value for the cross i x j
m is the overall mean value
$\mathbf{GCA_i}$ is the general combining ability of parent **i**
$\mathbf{GCA_j}$ is the general combining ability of the parent j
$\mathbf{SCA_{ij}}$ is the specific combining ability of the cross **i x j**

For the cross 5 x 6 we can approximately estimate the SCA in the following way:

$$y_{5x6} = 33 = 26.6 + (-0.3) + 0 + SCA_{5x6}$$

$$SCA_{5x6} = 33 - 26.3 = 6.7 \qquad (2)$$

Table 5-3. Effects that can be distinguished in an experimental series planted at more than one test site (or alternatively exposed to more than one treatment) and containing more than one population with full-sib families or open-pollinated families.

Full-sib progeny trial	Open-pollination progeny trial
Effects	Effects
grand mean	grand mean
population	population
population x site/treatment	population x site/treatment
Female (population)	family (population)
male (population)	
female x male (population)	
female x site/treatment	family x site/treatment
male x site/treatment	
(female x male) x site/treatment	
residual	residual

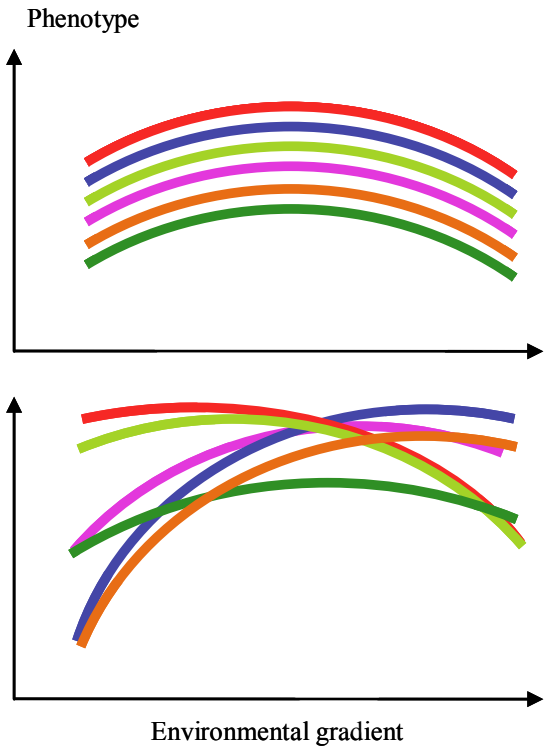


Figure 5-8. . The curves show the phenotypic performance of different genetic entries (provenance, population, family, clone) along an environmental gradient. In the part below there is a great change in ranking among the entries. This means that a genotype x environment interaction exists. This is not the case in the upper part of the figure.

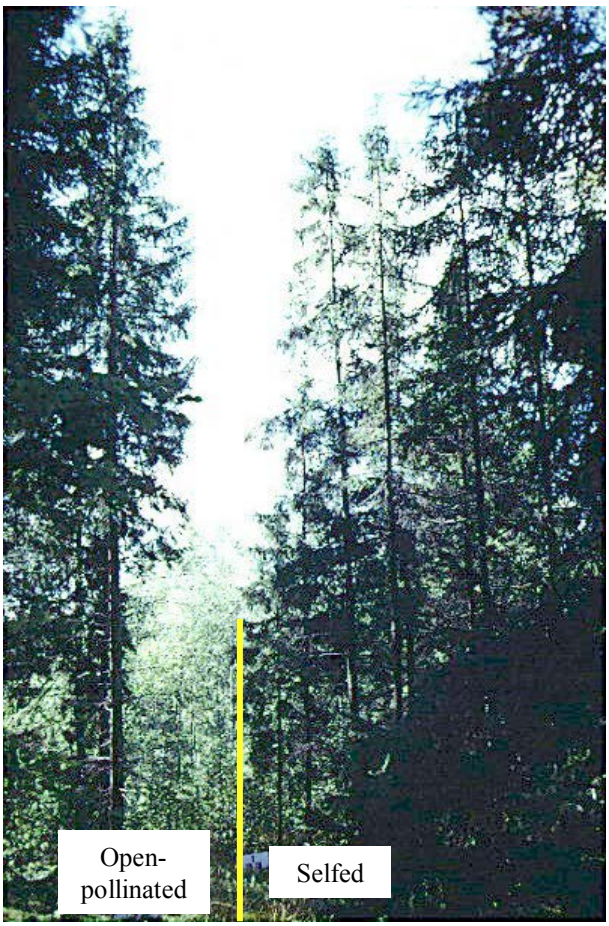
The estimations of the two combining abilities under real conditions takes place by using a more complex statistical model in which mating design and experimental design are important components. This enables an estimation of the significances of the two combining abilities. An example of the effects that can be distinguished in an experimental series planted at more than one test site and containing more than one population is given in Table 5-3. As seen from this table, experiments with full-sibs increases our possibilities to identify different effects compared to the situation for open-pollinated families. All experimental trials containing the same crosses are designated as an experimental series.

The estimation of GCA is one of the main objectives in forest tree breeding and enables an identification of the genetically most valuable trees. It should be noted that the general combining ability of a tree is a relative estimate and depends on which parents are tested and the environment of the testing.

Genotype x environment interaction

Another objective of progeny testing is to estimate how stable the performance of the progenies is when tested under different environmental conditions. In Fig. 5-8 two situations are illustrated. Above is shown that the ranking is totally stable over the environmental gradient tested. In the part below there are several ranking changes. Such changes in ranking are called genotype x environmental interactions. To verify such an interaction we need at least two experimental plantations which differ with respect to the environmental conditions. A study of the genotype x environment interaction at two sites with similar environmental conditions is of no value for estimates of genotype x environment interaction.

Knowledge of genotype x environment interaction is of value both for breeding and for studies of evolution. Forest genetic progeny trials belonging to one experimental series are therefore frequently located to shifting site conditions. According to which objective is of greatest importance we can calculate the heritability on data from all trials or heritabilities from individual trials. With a large genotype x environment interaction the heritability based on data from all experiments will be low. To evaluate the importance of the genotype x environment interaction for breeding, forest geneticists relate the variance component for the interaction to the parental variance component. As a rule of thumb, with a value above 1.0 there is a need for delineation of different breeding zones with separate breeding in each zone.



Picture 5-1. The oldest progeny trial with selfed Picea abies *trees at age 60. To the left of the yellow line there are open-pollinated trees to the right there are selfed trees.*

Inbreeding and heterosis

It is well known that different types of inbreeding in cross-fertilising organisms cause a decrease of the vigour of the affected individuals. This is called inbreeding depression. Thanks to the Swedish tree breeder Nils Sylvén's pioneering effort, the oldest progeny trial with selfed Norway spruce was established in 1916 (Picture 5-1). This trial was established before statistics were considered and it has no replications. Despite this, the results are spectacular with a stem volume of the selfed trees amounting to less than 50 % of the stem volume of the outbred trees. Still poorer performances of selfed Douglas fir, noble fir, ponderosa pine, and Scots pine have been observed in experiments with replications.

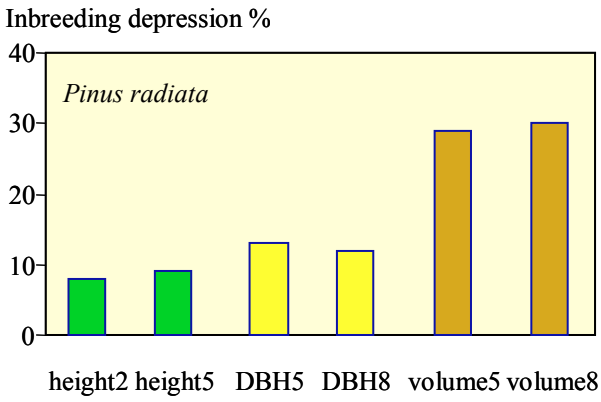
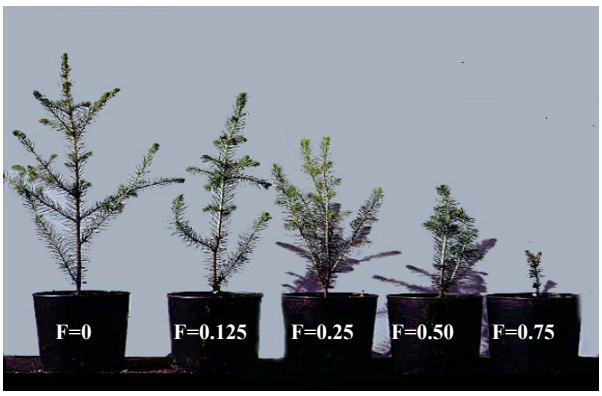


Figure 5-9. The percentage inbreeding depression at different ages of Pinus radiata *studied in New Zealand. The field test was carried out at a locality with good site conditions.*



Picture 5-2. Seedlings of Picea abies *with different inbreeding coefficients.*

Somewhat lower inbreeding was noted in an experiment with *Pinus radiata* (Fig. 5-9). The good site conditions at the test site is a possible explanation for this somewhat lower depression. Several studies with selfed forest tree species in nurseries have resulted in an inbreeding depression of approximately 20%. These results, as well as results from other plant species, suggest that the inbreeding depression is less pronounced under good conditions than under severe conditions. Is there any solid genetic explanation for this dramatic inbreeding depression of the selfed material?

In quantitative genetics, an equation that describes the relationship between the size of the inbreeding depression and the degree of inbreeding has been derived. To enable an understanding of this equation the concept, inbreeding coefficient, must be clarified. In trees with both female and male flowers a high degree of inbreeding can be obtained via repeated selfings. A prerequisite is of course that there is no prevention of fertilization with the pollen of the same tree. Fullsib, halfsib, and first cousin matings are other types of inbreeding with decreasing degree of relatedness in that order. In quantitative genetics, the inbreeding coefficient, F, is an estimate of identity by descent of alleles. Identity by descent means that copies of one and the same allele at an ancestor have been brought together in an offspring. It is important to observe that it is not enough with homozygosity but the alleles at a homozygote must originate from one common allele at an ancestor. This is further explained in Box 5-2. It is certainly true that the degree of homozygosity also increases following inbreeding. The inbreeding depression for various types of inbreeding are illustrated in Fig. 5-10 and

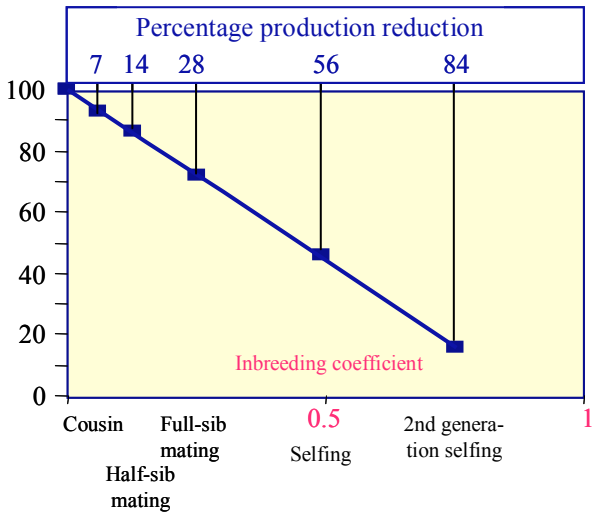


Figure 5-10. The relationship between inbreeding coefficient, F, and the relative stem volume in Picea abies. *The figures give the inbreeding depression as percentage of the stem volume obtained without any inbreeding.*

Picture 5-2. The relationship between the magnitude of the inbreeding depression and the inbreeding coefficient is evident from the formula:

$$m_F = m_0 - 2F\sum dpq \qquad (3)$$

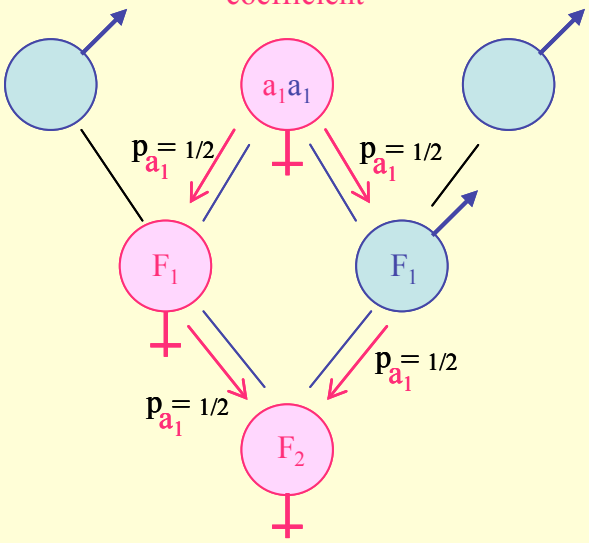
in which m_F designates the value of a trait such as tree height or stem volume in a population with the inbreeding coefficient F while m_0 is the value for the trait studied before any inbreeding took place, p and q represent the average gene frequencies in loci affecting the trait, and d is the dominance deviation (cf Fig. 5-2). The sign $\sum$ stands for summation of the effects from all loci involved.

From equation 3 we can extract the following information:

1. The equation shows that there is a linear relationship between the size of the inbreeding depression and the inbreeding coefficient, F.
2. If the dominance deviation for all loci is equal to zero, there will be no inbreeding depression. When d = 0 the gene action is totally additive. This was the assumption we had in the example with tree heights to derive the quantitative inheritance. Since inbreeding depression occurs in most cross breeding organisms one can conclude that d is different from zero and mostly on the plus side according to Fig. 5-2.
3. The allele frequencies have great impact on the size of the inbreeding depression in agreement with the situation for heritability.

This interpretations is correct if all alleles involved operate in an additive way. For Norway spruce and Scots pine there are data suggesting that this may be the case. In spite of the additive gene action we have, as mentioned before, a large inbreeding depression in these species, which is contrary to the predictions according to equation 3. One possible explanation is that the inbreeding depression depends on vitality-decreasing alleles at very low frequencies. Homozygotes should mainly arise in such cases after crosses among related individuals.

Box 5-2 Derivation of inbreeding coefficient



An example of the derivation of inbreeding coefficients. We have a mating between a homozygous a_1a_1 with two non-related males to obtain two individuals (F_1) in the progeny, which in turn are mated and give rise to the individual designated F_2, which is the individual we should derive the inbreeding coefficient for. The two a_1 alleles are shown in red and blue, respectively. The probability that the red a_1 allele is transferred from the a_1a_1 female to its daughter is ½. The probability for transfer of the a_1 allele from F_1 to F_2 is also ½. The probability of transfer from a_1a_1 to F_2 is obtained by multiplying these two probabilities, which gives ¼. Similarly the transfer on the right-hand path from a_1a_1 to F_2 is also ¼. These two probabilities have to be multiplied, ¼ x ¼, which gives the probability = 1/16 for obtaining a homozygous F_2 individual. Another way to estimate this probability is to start from the F_2 individual and follow the red lines back to the starting point and for each step that is taken multiply by ½. The latter procedure may be simpler in more complex pedigrees than in the box. The other homozygote that can have copies of the same allele is a_1a_1. The probability that this homozygote will arise is equally large as the probability for a_1a_1. To calculate the total probability for identity by descent the two probabilities have to be summed. After summation we obtain 1/8, which is the inbreeding coefficient for half-sib mating. Note that the males in the top line cannot give rise to identity by descent. Shown in an analogous way the inbreeding coefficient for full-sib mating is ¼.

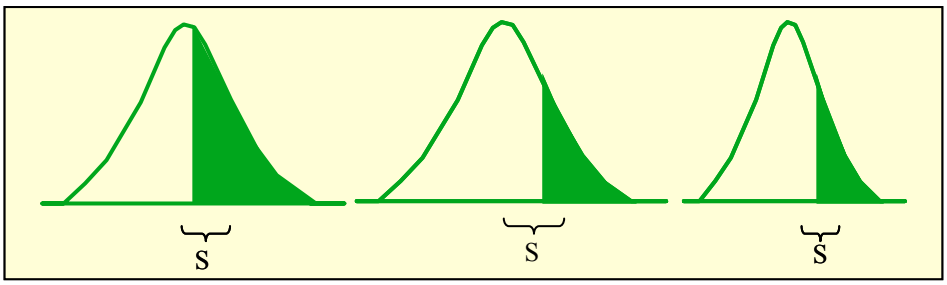


Figure 5-11. The figure illustrates that the selection differential (S) – the difference between the mean values of the selected part of the population and the entire population depends on the proportion of individuals selected as well as the distribution of the trait in the population.

Heterosis is the opposite to inbreeding depression and is thus an increase of vitality after mating between inbred individuals. The best known example is hybrid breeding in maize. In maize several generations of inbreeding were carried out before mating took place between individuals from different inbred lines. Through this method one has achieved very spectacular results but the value of the technique has been challenged in recent decades. Also in this case there is a quantitative genetic equation that describes the value, H_{F1}, that we expect in the progeny from mating between two inbred lines:

$$H_{F1} = \sum d(p_1 - p_2) \quad (4)$$

in which p_1 is the frequency of one allele in one of the inbred lines and p_2 is the frequency of the same allele at another inbred line. Summation of the effects over loci affecting the trait must take place in this case too. An analysis of the equation reveals that the larger the difference in gene frequencies between lines, the larger H_{F1} will be. The largest effect is obtained when the allele frequency is 0 in one line and 1 in the other line, *i.e.* one line is homozygous $\mathbf{a_1a_1}$ and the other homozygous $\mathbf{a_2a_2}$. Also in this case d is involved and in analogy with the inbreeding depression there will be no heterosis if d at all loci involved is 0. Another condition for heterosis is that d at most loci is positive.

Matings between individuals from different inbred lines immediately restore the vitality lost by inbreeding, which is important for conservation genetics. Parenthetically it might be mentioned that this equation had a large impact on the early breeding of Norway spruce in Norway and Sweden.

Selection differential, selection intensity, and genetic gain

In this section we shall discuss the effects of different strength of artificial selection, while the effects of natural selection will be discussed in the next chapter.

In Fig. 5-11 the meaning of selection differential (S) is illustrated. The selection differential is equal to the difference between the mean of the selected part of the population and the mean of the total population. As may be seen from this figure the selection differential depends on the distribution of the trait. If the same proportion of individuals is selected, the selection differential is larger if the distribution is larger.

To enable a comparison of different cases of selection the selection intensity (**i**) has been introduced. The selection intensity is obtained by dividing the selection differential by the standard deviation. The selection intensity is non-linearly related to the proportion selected. To increase the selection intensity from 2 to 3 requires a much larger population than the increase from 1 to 2.

To make it possible to calculate the result of a certain selection for a particular trait it is necessary to know the genetic proportion of variation in this trait. If we aim at a mass selection, *i.e.* to select several individuals as parents for a new generation, the genetic effects that are added to each other are of importance. Thus, it is the heritability that is of interest and the improvement is equal to the heritability multiplied by the selection differential. The result of this product is usually referred to as genetic gain, ΔG. As equation we get:

$$\Delta G = h^2 \times S \quad (5)$$

or if we use selection intensity instead of selection differential:

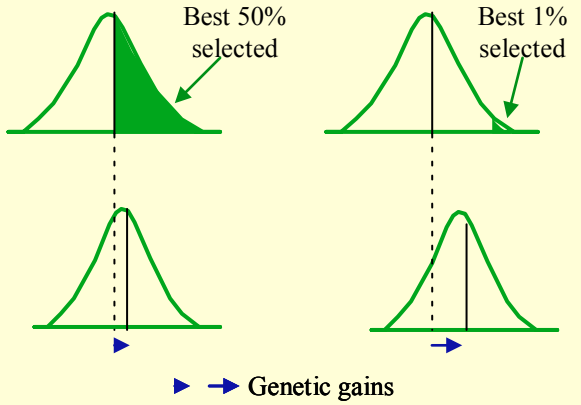
$$\Delta G = h^2 \times i \times \sigma_{ph} \quad (6)$$

In order to visualise the great impact of the additive variance for making progress by artificial or natural selection, the heritability can be expressed as the ratio σ_a^2/σ_{ph}^2. If this ratio is included in equation 6 we get the genetic gain in the following way:

$$\mathbf{\Delta G = i \times \sigma_a^2/\sigma_{ph}} \quad (7)$$

in which **i** expresses the selection intensity for the selected trees, σ_a^2 is the additive variance, and σ_{ph} is the standard deviation in the entire population.

Box 5-3 Genetic gain and selected proportion



The filled green part is the selected part of the parental population. Even with such a high heritability as 0.4 the genetic gain is rather limited when half of the population is selected. When only 1% is selected the gain becomes pronounced.

On the assumption of a heritability of 0.4, the genetic gain that is obtained after selection of 1 % or 50 % of the best individuals is illustrated in Box 5-3. The effects of the selection as illustrated in this box are a confirmation of what we said before, that the larger the selection differential the larger the gain by selection.

Besides the selection of parents there are other types of selection. We can select all individuals in a progeny, which geneticists frequently refer to as family selection. We can select the best individual in the best family. When destructive tests are used such as at freeze testing of whole plants or inoculation with pathogens we can select siblings to the plants tested, which is designated as sibling selection.

Genetic correlation

From classical genetics several cases are known in which the same allele influences two different traits. Therefore, it is quite logical that loci affecting quantitative traits may also affect more than one quantitative trait. For tree breeding it is particularly important to be able to disclose how other traits are affected when selecting for one specific trait. The understanding of evolution is also simplified if we know the genetic relationship between different traits. It would not be surprising if two consecutive stages during budburst in Norwy spruce are affected by the same alleles. On the other hand it is less certain that the point of time for budburst during spring and growth cessation during late summer or autumn are affected by the same alleles. To disclose whether this is the case genetic correlations are calculated. The genetic correlation is in most introductory texts referred to as a correlation of breeding values of two traits. In advanced texts it is disclosed that genetic correlations are not that simple but it is beyond the scope of this book to go into further detail. In agreement with estimates of breeding values, the genetic correlations are valid for the population tested and the conditions under which it is tested. In the equation for the genetic correlation, the covariance between traits x and y is one part; this covariance estimates the covariation between the two traits. The genetic correlation is frequently designated as $\mathbf{r}_a$ and it is equal to:

$$r_a = cov_{xy}/(var_x \times var_y)^{1/2}$$

To enable high precision in the estimates of genetic correlations it is required that the experiments contain progenies of numerous parents, at least 100 being desirable. Owing to the original design of many forest tree breeding programmes there are frequently no more than 40 parents in each experimental series. This means that the precision in the estimates is not as good as desired.

Summary

Quantitative traits are affected by a large number of alleles, each with a small effect on the trait. This means that we cannot observe any discrete segregation in the progeny population, rather we frequently note a normal distribution of quantitative traits. The environment influences the traits as well. Finally, the gene action is rarely fully dominant or recessive; instead we have a certain degree of dominance.

Heritability, general combining ability and genotype x environment interaction are parameters that are estimated by statistical methods in well designed experiments. All three are important to enable predictions of the effect selection has on a certain trait. Inbreeding depression and heterosis are explained by quantitative genetics equations. The inbreeding depression after selfing is substantial in Norway spruce and Scots pine as well as in many other cross-fertilizing species. With decreasing degree of relatedness of the parents inbreeding depression becomes less pronounced.

The selection differential is the difference between the mean values of the selected part of the population and the whole population. The selection differential divided by the standard deviation gives the selection intensity, which is independent of the distribution of the trait. The genetic gain is equal to the heritability multiplied by the selection differential for the trait under study. The genetic correlation is an estimate of the strength of the relationship between the breeding values of two traits.

Further reading

Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th ed. Longman group Ltd., Essex, UK.

Lynch, M and Walsh, B. 1998. Genetics and analysis of quantitative traits. Sinnauer Ass. Inc., Sunderland, MA 01375 USA.

Sewell, M.M., Neale, D.B. 2000. Mapping quantitative traits in forest trees. In Jain, S.M. and Minotcha, S.C. (eds) Molecular Biology of Woody Plants, Vol. 1. Kluwer Acad. Publ., Dordrecht, The Netherlands.

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

6 Evolution

In this chapter we mainly focus on the principles of evolution and we will present a limited number of empirical data. Population differentiation observed for many traits is shown in the next chapter. First we give a short presentation of natural selection, random genetic drift, mutations, and gene flow. The phenotypic plasticity of a trait and its role in evolution is also discussed. Later on we present in more detail the evolutionary factors. We raise the question whether or not any perfect form could be reached in nature. Finally, evolutionary aspects of global warming are discussed

Evolution is a continuously ongoing process, in which species arise, flourish, and become extinct. From the fossil record, some scientists have come to the conclusion that 99.9 % of all species that once existed have become extinct. The most probable prediction we can reach for the biological world is that all presently existing species will become extinct in the long-run. Certain times during the millions of years in the past were characterized by mass extinction. Besides the extinction that is unavoidable in a world of evolution, man is causing even more extinction by various activities. Above all, the activities that cause unnecessary erosion of species have to be identified. There is no question that the human population explosion is the greatest threat against the majority of the vulnerable species.

In passing it might be mentioned that the definition of the species concept is not always simple. In Scandinavia there are two gull species, *Larus fuscus* and *L. argentatus*. If we follow them around the northern hemisphere they are connected by populations showing a continuous transition from one species to the other. A biological species concept was coined by Ernst Mayr during the forties and is formulated as follows: *Species are groups of actually or potentially interbreeding populations, which are reproductively isolated from each other*. As might be understood from this concept it is important that crosses among species are prevented in one way or another.

Box 6-1 Definitions of adaptation, adaptedness, adaptability, fitness

Adaptation = the process that leads to a better adaptedness in a specific environment. The study of adaptation to varying environmental conditions is called genecology.

Adaptedness = the degree to which an organism is able to live and reproduce in a given set of environments

Closely related to adaptedness according to the above definition is the **fitness** concept. Fitness is an expression for an individual's contribution to the next generation in relation to other individuals in the same population. This type of fitness is sometimes referred to as **Darwinian fitness**. This latter term is used to distinguish processes in nature from cultivation (see section *Darwinian* and *Domestic fintess* in Chapter 7).

Adaptability = the ability of a population to respond genetically or phenotypically to changed environmental conditions. The amplitude of a trait of a genotype studied in at least two different environments is called phenotypic plasticity. The term **reaction norm** is used to describe the trait value change of a genotype studied along an environmental gradient.

Box 6-2 Definition of evolutionary factors

Mutation	= inception of a heritable change in a gene or chromosome
Natural selection	= differential transfer of alleles to next generation resulting in increased fitness
Random genetic drift	= random loss of alleles in small populations
Gene flow	= migration to a recipient population from another population with a different allele frequency
Phenotypic plasticity	= the amplitude of a trait of a genotype studied in at least two different environments

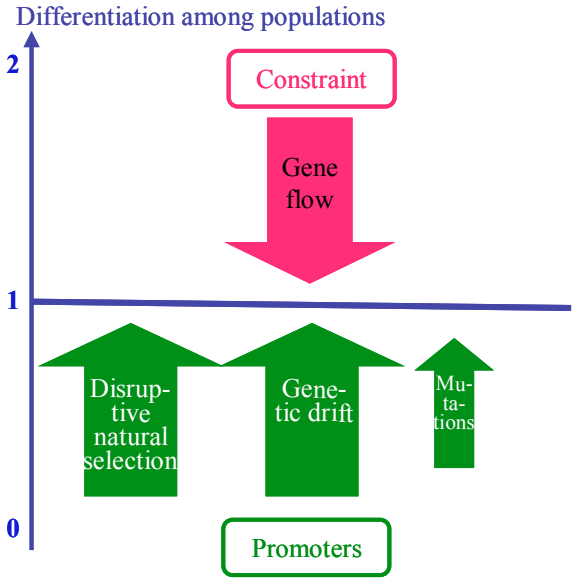


Figure 6-1. Schematic illustration of evolutionary factors promoting population differentiation. The arrows pointing upwards increase the differentiation while gene flow reduces the differentiation between populations. Mutations have a limited impact on differentiation.

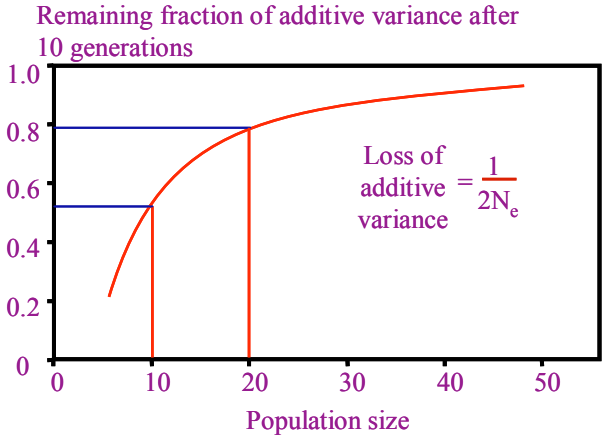


Figure 6-2. Remaining fraction of additive variance after 10 generations as a consequence of genetic drift. It is assumed that the effective population size was constant during these 10 generations.

Terminology

The terms related to adaptation are sometimes used with some differences in meaning depending on the author. It is important to define terms that are frequently used in the literature. The definitions used in our text are those defined in Box 6-1.

Factors influencing evolution

Differentiation among populations is a major issue in evolution. We have tried to visualize the factors promoting and constraining differentiation among populations in Fig. 6-1. Natural selection, genetic drift, and mutations promote differentiation among populations. They raise the horizontal line to a higher level if they are in operation, which is equal to a larger differentiation. If gene flow is in operation the horizontal line is pushed downwards. Via natural selection certain individuals contribute more to the next generation than others and in this way cause a change in gene frequencies. Natural selection is regarded by many as weak. However, a careful scrutiny of many scientific papers on natural selection by an American scientist, John Endler, during the mid eighties indicated that natural selection may take any place on a scale from weak to as strong as in plant or animal breeding.

Random genetic drift is a random process that inevitably causes loss of alleles in small populations. This takes place whether or not the adaptedness of the small population is increased. Genetic drift is important in populations of a size less than 20 individuals (Fig. 6-2).

The mutation rate at individual loci is generally low, mainly within the range of one per ten thousand to one per million. For that reason the probability that the same mutation will arise in two populations is low, which explains why mutations are supposed to contribute very slightly to population differentiation. The mutation rate seems to be higher in conifers than in angiosperms.

Matings among individuals from different populations are part of the gene flow which is a strong obstacle to population differentiation. Transport of seeds, fruits, nuts, and acorns is another component of the gene flow which

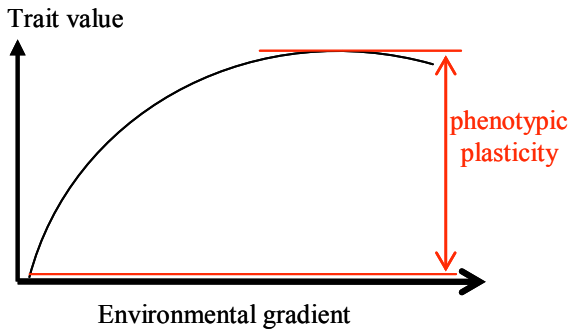


Figure 6-3. The curve describes the change in phenotypic value of a genotype along an environmental gradient. The curve is the norm of reaction within this range of environment of the genotype. The difference between the highest and lowest phenotypic value is the phenotypic plasticity of the genotype.

in many cases is believed to play a minor role compared to the gene flow via pollen. All factors treated above cause changes in gene frequencies.

Plants have a great capability to change their exterior shape depending on the growth conditions. A genotype that is tested in two or more environments may have different heights, crown form, density etc in the different environments. The amplitute of such a variation in a trait is a measure of the genotype's phenotypic plasticity (see Figure 6-3). Many textbooks in genetics do not at all treat phenotypic plasticity. Its role in evolution is somewhat ambiguous: On the one hand the phenotypic plasticity can be regarded as a disguise of the genotype which means that natural selection will not be as efficient as it would be without this disguise. On the other hand phenotypic plasticity may contribute to the fitness of a genotype, especially if it is a long-lived species with a wide distribution encompassing many different site conditions. If this is the case, natural selection will increase the frequency of genotypes with a large phenotypic plasticity.

So far we have discussed differentiation among populations but the same factors operate within a population as well (see Figure 6-4). From this figure it is seen that mutations and gene flow increase the genetic variation within populations while natural selection, genetic drift and inbreeding reduce the within-population genetic variation. The matings that were realized are designated as the mating pattern. Gene flow, genetic drift, and inbreeding can thus be regarded as components of the mating pattern. It should be realised that recombination does not cause any change of gene frequencies but it creates new genetic combinations in the gametes. A comparison of Fig. 6-1 and 6-4 reveals that the same evolutionary factor influences differentiation among populations and genetic variation within populations in different ways. The difference between natural selection at the species level and within an individual population will be discussed when the three types of natural selection are presented below.

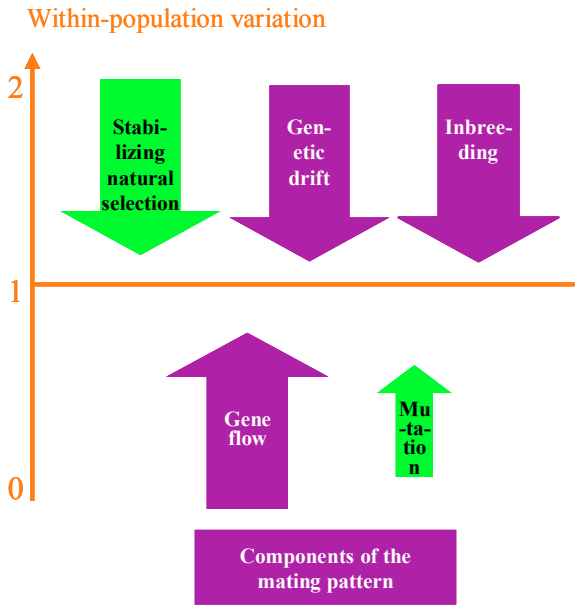


Figure 6-4. Schematic illustration of evolutionary factors promoting variation within populations. The arrows pointing upwards increase the variation while the factors pointing downwards reduce the variation within populations. Mutations have a limited impact on within-population variation.

In conclusion it should be emphasized that natural selection is not the only evolutionary factor. As outlined in the previous paragraph the genetic raw material that can be changed by natural selection depends on the matings that took place. In nature, the five factors discussed above interact in a complex way, and we cannot expect that the adaptedness will be perfect. In most cases evolution is a gradual change of the genetic composition of a population.

Natural selection

Ever since Charles Darwin presented his theory about evolution, natural selection has attracted great biological interest. It has sometimes been misinterpreted. Some have seen it as a dark force while others have seen it as a creative force driving evolution to a greater perfection. Sometimes it has been regarded as an ethical principle that man should not intervene with. None of these opinions is correct. Natural selection is a process that can be expressed by a statistical measure of the differences among individuals in their capacity to transfer genes to the filial generation. Natural selection is not *caused by* differences in transferring genes to the next generation, it *is* differences in transfers of genes to the coming generation. Changes in gene frequencies depend on the existing conditions and have nothing to do with future conditions. Therefore, there is no goal or any predetermined direction of natural selection. It cannot give populations such characteristics that the probability for survival is increased in the future.

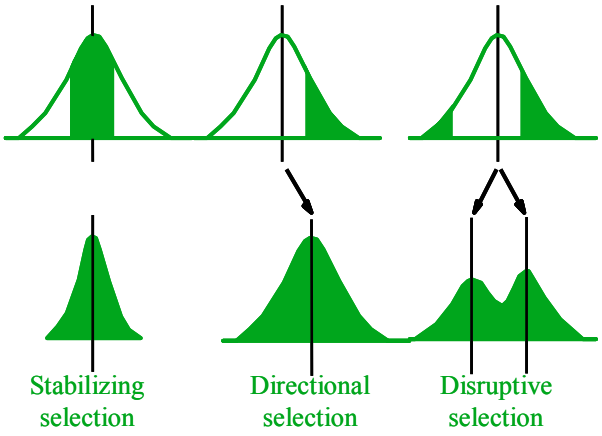


Figure 6-5. The three main types of natural selection and the result of these types of selection.

The great evolutionist Ernst Mayr expressed the essence of natural selection in his book on evolution from 1988 in an excellent way: *Selection is not a forward-looking process but simply a name for the survival of those few individuals that have successfully outlasted the "struggle for existence".*

The target for selection has attracted interest among evolutionists. In most cases it is the fitness of the entire phenotype that is selected or rejected in natural selection. This means that there may be progress in one fitness-contributing trait and regression in another if these two traits are negatively correlated. The concept survival of the fittest has misguided many laymen to believe that natural selection results in improvement in all traits of an individual. This has been strongly criticised by the American Richard Lewontin, one of the leading geneticist during the second part of the 20th century.

Since fitness is the trait that is selected for, there will be no natural selection without genetic differences in fitness. A frequently used example of natural selection is the observed variation in critical night length for budset in Norway spruce seedlings (see Fig. 7-18). It must be assumed that natural selection has favoured genotypes with a date of budset that matches the date for autumn frost appearance at various localities. To give an example in which natural selection is not working we shall present a hypothetical case. Let us assume that we have a large forest population of one single clone growing under very heterogeneous site conditions. As a consequence of this heterogeneity, only some of the clonal members will produce offspring. This is a form of selection but it is not natural selection since there will be no change in allele frequency from the parent to the progeny generation, which is the case for natural selection. In summary, natural selection requires that the phenotypic variation is genetically regulated and that the variation leads to differences in fitness.

We may ask why certain individuals in nature have better prospects than others of transferring their genes to the coming generation. In plants it might be a question of producing a larger number of flowers, to attract more pollinators, having greater resistance against diseases, or standing lower temperatures than others. Among animals it might be a question of attracting individuals of the opposite sex, or of litter size. Certainly, these characteristics are not the only ones of value for the fitness of an individual.

One of the most prominent geneticists, Ronald Fisher, coined in 1930 a concept of great significance for natural selection, namely the The fundamental theorem of natural selection. It is beyond the scope of this book to give the derivation of this concept but in essence it states that the rate of increase of mean fitness at any time is equal to the additive genetic variance of fitness at that time.

The three main types of natural selection

Which individuals in a normal distribution have the highest fitness depends on the conditions. For this reason we can distinguish three main types of natural selection (Fig. 6-5).

Stabilizing selection is the type of selection that is most frequent in stationary populations. This means that the individuals in the two tails of the normal distribution have the lowest fitness values. A prerequisite for stabilizing selection is, as the term suggests, that the mean value of the population remains unchanged. In situations without any heterozygotic advantage, all selections within a population lead to an increase of homozygosity. Since the mean value remains unchanged at stabilizing selection, the selection cannot lead to homozygosity for all positively acting alleles. In order to understand what might take place at stabilizing selection we can make the following assumption: A trait is regulated by alleles with index 1 and index 2 in 26 loci designated **a**, **b**, **c**,....**z**. Let us also assume that the homozygote $\mathbf{a_1a_1}$, $\mathbf{b_1b_1}$, $\mathbf{c_1c_1}$,....$\mathbf{z_1z_1}$ has a tree height of 20 meters at an age of 100 years. Let us further assume that each allele with index 2 increases the height by 0.1 meter. The homozygote $\mathbf{a_2a_2}$, $\mathbf{b_1b_1}$, $\mathbf{c_1c_1}$,.... $\mathbf{z_1z_1}$ would thus be 20.2 meters while trees homozygous for index 2 alleles in all loci, $\mathbf{a_2a_2}$, $\mathbf{b_2b_2}$, $\mathbf{c_2c_2}$,....$\mathbf{z_2z_2}$, would be 25.2 meters. According to the definition of stabilizing selection, the mean value cannot be changed. Stabilizing selection, for this reason, cannot lead to an enrichment of index 2-alleles. If the mean value of the population is 22.6 meters we must assume that the increase of homozygosity that takes place is of the character that 13 loci are homozygous for index-1 alleles while the other 13 loci are homozygous for index 2-alleles. Which loci are homozygous for index 1-alleles and index 2-alleles will vary from individual to individual. Such a situation might occur after a large number of generations and if no other evolutionary factors are in operation. In nature we will have deviations from the 13:13 ratio of index 1- and index 2-alleles such as 14:12, 12 :14, 15:11, 11:15 etc.

Any scientific proof that the selection gives rise to such a situation does not exist. The above described form of increased homozygosity offers an explanation as to why additive variance remains even under stabilizing selection.

Under the harsh conditions that prevailed after the withdrawal of the ice after the last glaciation it is easy to perceive that plants in the "harsh tail" of the normal distribution had the highest fitness. This will favour a directional selection. Directional selection is probably of great significance in populations migrating along an ecological gradient. If it was the tail with the harshest individuals that was favoured after the glaciation perhaps the other tail has the highest fitness today when we are probably in a period of temperature increase owing to increase of greenhouse gases in the atmosphere.

We can look upon directional selection in an analogous way to what we did for stabilizing selection with alleles designated with index 1 and 2. We may assume that individuals in one of the tails mainly have index 1-alleles while mainly index 2-alleles dominate in the other tail of the normal distribution. Depending on the direction of selection the progeny will face, there will be an increase in the frequency of one of the alleles. If alleles with index 1 contribute to fitness under harsh conditions, an increased frequency of index 2-alleles is expected under global warming.

If we assume that the individuals in the two tails have the highest fitness we shall observe disruptive selection. In northern Scandinavia with much snow during the long winters there may be an advantage for a tree either to have a narrow crown so that the snow glides down from the tree, or to have extremely strong branches. The narrow crown might be the result of natural selection. When branches are broken by heavy snow the tree crown will be reduced, which probably leads to reduced photosynthesis and reduced growth. This in turn means fewer flowers and a lower possibility that the genes of such a tree are transferred to the next generation. On the other hand if the branches are very strong they may carry the large amount of snow coming during the winter. This means that trees in the opposite end of the distribution are also equipped with high fitness and we have a situation that might provoke disruptive selection.

It is worth emphasising that stabilizing and directional natural selection cause a reduction of within population genetic variation while the variation among populations becomes larger. It is thus important to distinguish between the selection within an individual population from the selection that takes place at the species level. This is evident from Fig. 6-6 in which we have stabilizing selection in the four populations that are growing along an ecological gradient. When we introduce the phenotypic

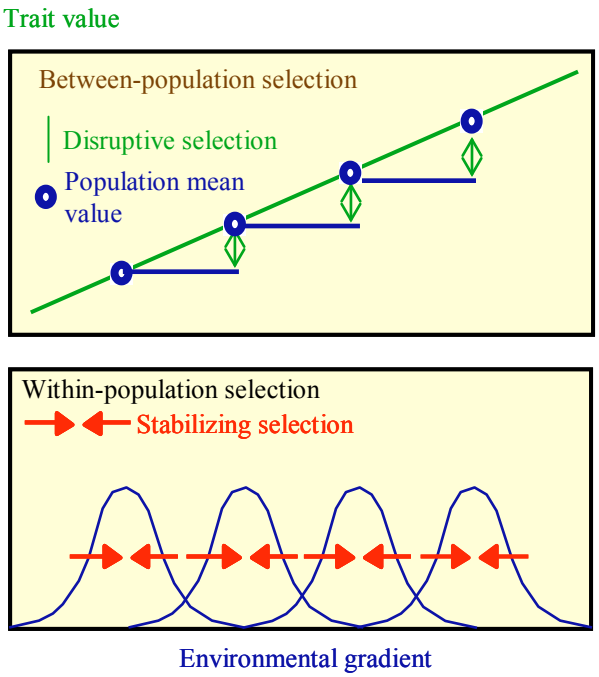


Figure 6-6. In many populations in nature there is stabilising selection. When a series of populations are growing along an environmental gradient, their phenotypic values will differ. This will be perceived as disruptive selection between populations.

values we observe that the stabilizing selection within populations becomes disruptive among populations.

To understand the speed of change caused by directional selection we have to introduce an expression for how strongly the selection influences a certain genotype. For that purpose we assume that the three genotypes **AA, Aa**, and **aa** have the fitness values 1, 1, and 1-**s**; **s** is called selection coefficient and should not be mixed with selection differential. It is frequently expressed in per cent. The allele frequency of **A** is as usual designated by p and the allele frequency of **a** is designated by q. The changes in allele frequency of **A** before and after natural selection may be obtained by some algebra, which is not carried out here. Such a derivation leads to the following expression for the change in **A**, *i.e.* Δp:

$$\Delta p = -spq^2/(1 - sq^2)$$

This formula, which is valid under complete dominance, gives an interesting piece of information on the prerequisites for changes of allele frequencies via natural selection. The maximum speed of change occurs at an allele frequency of one third of the favoured **A** allele. The equation also shows that the speed of change is largest when both alleles are common while the change is minor when one allele is common and the other rare. The equation further tells us that the larger the value of **s**, the larger the speed of change.

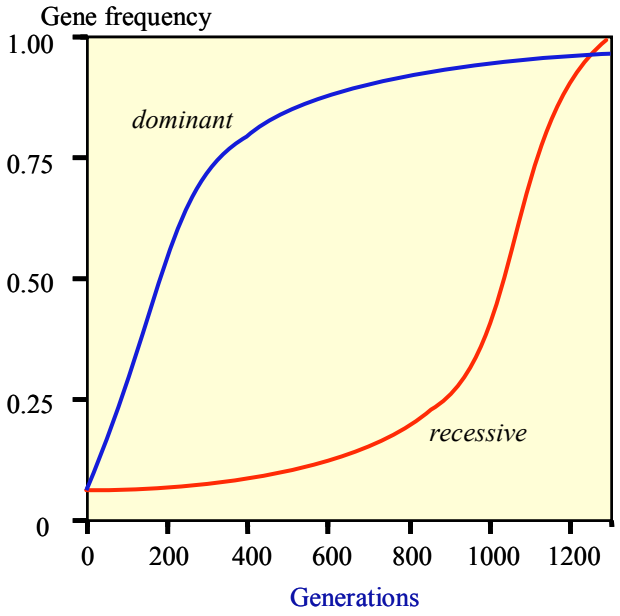


Figure 6-7. The change of allele frequency at a locus in a large population under the assumption that the difference in fitness between the two homozygotes amounts to 3% and that the initial allele frequency is 0.05

In Figure 6-7 the effects of selection on a dominant and a recessive allele are graphically shown. The curves are valid for an infinitely large population with random mating. Moreover, we have assumed that the difference between the homozygotes, **AA** and **aa**, is 3 %, *i.e.* s is 0.03. As expected, the largest change is observed for the dominant allele during the first generations while the increase of the recessive allele is very slow in the first hundreds of generations. This is understandable since to begin with the **a** allele is found only in heterozygotes that are constituents of the favoured part of the population. From the chapter on quantitative genetics (Chapter 5) we also know that low frequency alleles hardly at all contribute to fitness, which is required for natural selection to come into operation. The increase of the dominant allele is very slow when its frequency in the population is high. Both of these courses of events have their origin in the impossibility of distinguishing **AA** from **Aa** individuals.

It might seem as if there is no possibility for a new mutant to become established in a population. If the number of individuals in the population is low there is a higher chance for the new allele to become fixed (= homozygous) than is the case in Fig.6-7. Thus, fragmentation *per se* is no obstacle for evolution, rather it might speed up evolution. Some evolutionary geneticists have the opinion that limited population size is the explanation for much of the speciation that occurs. The positive effects of the integration of fitness-promoting alleles in a population must be weighed against the disadvantages of small populations. As will be shown below, genetic drift may give rise to loss of fitness-promoting alleles as well as to increased inbreeding with accompanying inbreeding depression.

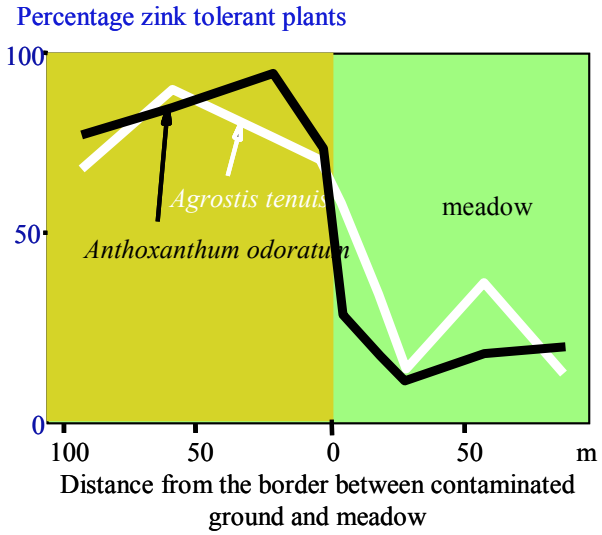


Figure 6-8. Relative zink tolerance in two grass species in populations growing at various distances from the border between contaminated and uncontaminated soil.

Natural selection under severe stress conditions

Studies of plants under severe stress have shown that natural selection can change allele frequencies dramatically. Investigations of heavy metal tolerance in grass species growing on mining wastes show that natural selection must play a major role for the frequency of tolerant plants in adjacent populations growing on non-contaminated soil (Fig. 6-8). Since these species are wind pollinated there is probably a strong gene flow between the two populations. In spite of this the great difference between the two populations remains.

Another example of rapid change concerns pesticide resistance in insects, which was built up after introduction of pesticides on a large scale. In certain cases it did not take more than 5 generations to achieve resistance which might seem extremely few based on the curves presented in Fig. 6-7. This rapid building up of resistance must be attributed to an extremely strong selection for the allele contributing resistance plus that such alleles are dominant. The more normal situations that are depicted in Fig. 6-7 do not apply in this case.

It is probable that there is a high cost for keeping various types of strong stress tolerance. This means that individuals equipped with strong stress tolerance are not very competitive under more normal conditions (cf Figure 6-8); they have lower fitness under ordinary conditions. Therefore, it is unlikely to find genotypes that have a high fitness over the broad span of environmental conditions that a species occupies. For gene conservation it is important that we capture genes of importance for growth under stress conditions in the gene resource population. As presented in chapter 11 this is most easily done by splitting the gene resource population into several subpopulations.

Global warming is a specific stress problem that has raised much concern as to whether species can cope with it. Since evolutionary factors other than natural selection are of importance in the case of global warming it will be treated in a separate section.

Random genetic drift

In populations with a small effective population size ($\mathbf{N_e}$), genetic drift is of more importance than the other evolutionary factors. By small $\mathbf{N_e}$ is meant that there are few trees contributing to the progeny in the next generation (cf Chapter 4). A population may contain many trees but for various reasons only a few of them flower, which makes the population small as regards progeny production.

Numerous simulations have shown that genetic drift leads to allele fixation if a population remains small over a large number of generations. This means that the population either becomes homozygous $\mathbf{a_1a_1}$ or $\mathbf{a_2a_2}$. Such an allele fixation takes place independently of any evolutionary advantage of the homozygote. It is worth mentioning that allele fixation also takes place for the allele of the lower frequency of the two. If the allele frequencies are 0.9 for $\mathbf{a_1}$ and 0.1 for $\mathbf{a_2}$ at a locus and the population consists of a large number of small populations, after a large number of generations there will be 90 % of the populations homozygous $\mathbf{a_1a_1}$ and 10 % of the populations homozygous $\mathbf{a_2a_2}$. It is important to note that genetic drift takes place without an increase of fitness. Genetic drift does not rule out natural selection but by decreasing population size the impact of natural selection drops. In gene conservation and breeding we are interested in limiting the impact of genetic drift. It is therefore important to keep the population size large enough to prevent any major role of genetic drift.

As is illustrated in Fig. 6-2 the remaining additive variance is dramatically reduced at effective population sizes lower than 20. The loss of additive variance per generation is $1/2\mathbf{N_e}$. This means that the loss of additive variance with an effective population size of 50 amounts to 1 % while an effective population size of 10 causes a loss of 5 % per generation.

For wind-pollinated trees with wide and continuous distributions it is expected that genetic drift plays a minor role. The only exception may be populations at the margin of their distribution area. Strong gene flow might compensate to some extent for a low number of flowering trees. Perhaps genetic drift has played a role for speciation in the tropics where many tree species are represented by one or two adult trees per hectare.

Mutations

It has long been assumed that mutations are randomly distributed over the genome. This assumption has been challenged. In some grass species growing adjacent to mining wastes, heavy metal tolerant mutants have been found in some species but not in others. Thus, it seems as if there are restrictions in the genome such that heavy metal tolerance mutants can only be induced in some species.

Estimates of the mutation rate per locus and per generation in higher organisms are mainly in the range of one per ten thousand to one per million. Even if mutations are prerequisites for evolution it is important that the hereditary material is resistant to change. A highly conservative characteristic of DNA is important to avoid chaotic conditions. As regards mutations at loci regulating quantitative traits, the knowledge is for obvious reasons scanty. There are estimates of the pooled mutation rate at all loci involved in the regulation of a quantitative trait. Such estimates are in the range of one per hundred to one per thousand per generation. Since there are many or even numerous loci involved in the regulation of such a trait it is reasonable that the pooled mutation rate is higher than in individual loci but that it differs as much as 10 - 100 times is somewhat surprising.

As regards the influence on population differentiation, mutations play a minor role owing to the low mutation rate at individual loci. Thus the effectiveness of mutations in promoting population differentiation is in most cases several times weaker than natural selection in large populations. Similarly it is several times weaker than genetic drift in small populations.

Gene flow

The meaning of this term is that individuals from one population participate in the procreation of a new generation in the recipient population and that the donor and recipient populations have different allele frequencies. For plants, which are mostly stationary, there are gene flows via pollen, seed or fruit dispersal. Different species vary considerably with respect to distance of dispersal. Studies have shown that pollen grains of the wind-pollinated tree species such as spruces and pines may spread their pollen hundreds of kilometers. Such figures do not tell us how important long-distance transport of pollen is for fertilization in a population. They only tell us that there is a potential for long-distance transfers of alleles which might be lacking in other species with more stationary pollen vectors.

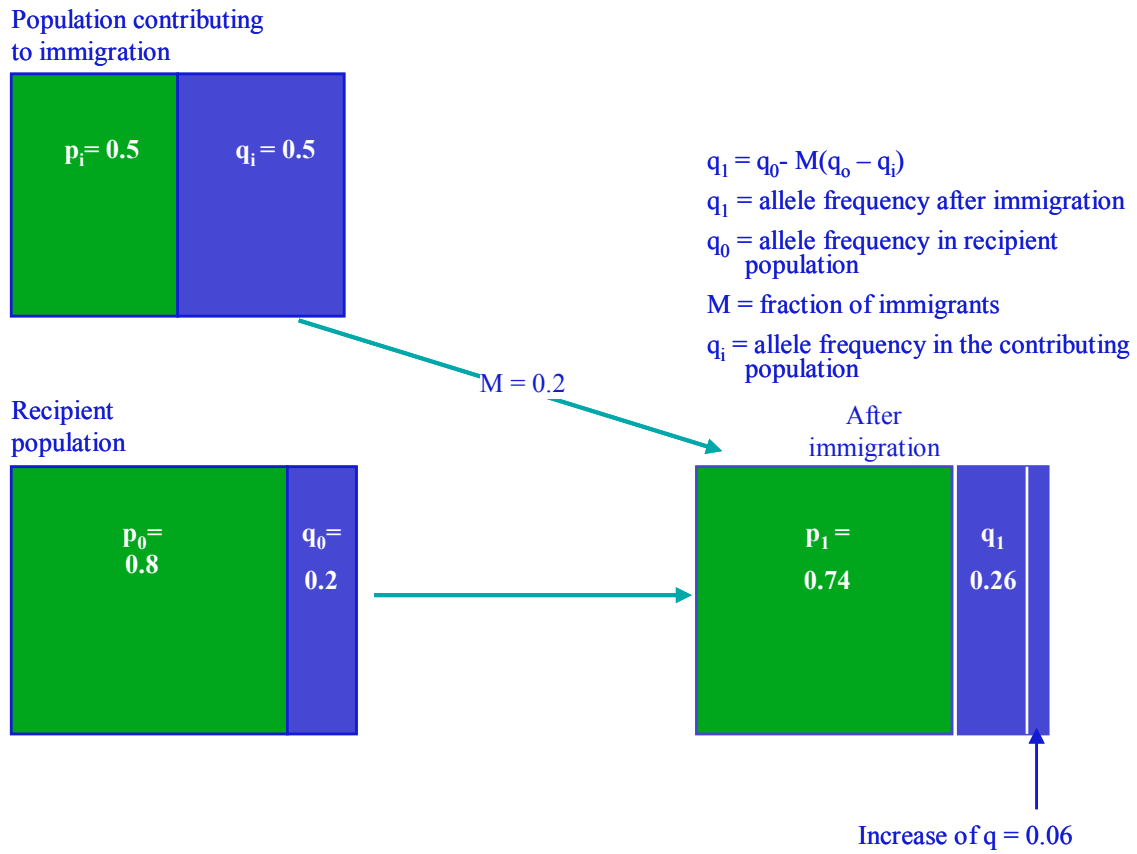


Figure 6-9. Illustration of how gene flow (=immigration) from one population influences the allele frequency in the progeny of a recipient population. The increase of the q allele frequency is indicated.

The effect of gene flow in a recipient population is visualised in Fig. 6-9. As is evident from this illustration, large differences between donor and recipient populations cause a large change in the recipient population. Slighter differences, as well as a low fraction of immigrants, also lead to deviations from what is expected according to the Hardy-Weinberg law. It is worth mentioning that gene flow in most cases has a greater impact on the population structure than mutations have. Exchange of one single gamete between two populations prevents a fixation of neutral alleles in the recipient population. From an evolutionary point of view, it is of great interest to estimate the magnitude of the gene flow among populations.

In a study of 66 populations belonging to 3 subspecies of lodgepole pine, it was shown that the number of migrants between populations was larger than 1. This means that the possibilities for differentiation among these populations are small. Another way of describing this phenomenon is to estimate the effective population size. For *Picea abies* and *Pinus sylvestris* the estimates vary between 8,000 and 16,000. These figures show that these species are efficient dispersers of their genes over large areas. Detailed studies of the pollination pattern were carried out for *Quercus petraea, Q. robur, Tilia cordata,* and *Castanea sativa*. In the two oak species the gene flow into the studied French populations was considerable, above 60 %, while the selfing did not exceed 2% (Fig. 6-10).

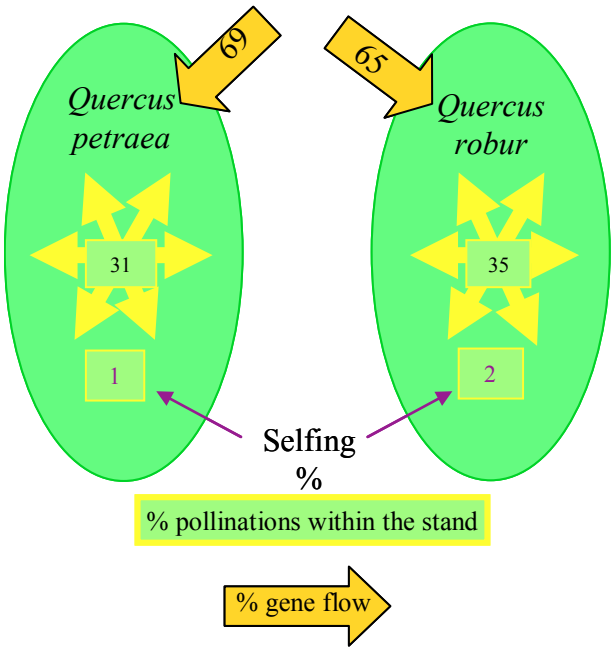


Figure 6-10. The percentage of matings within French populations of Quercus petraea *and* Q. robur. *The percentage of pollination by pollen from other populations is also indicated.*

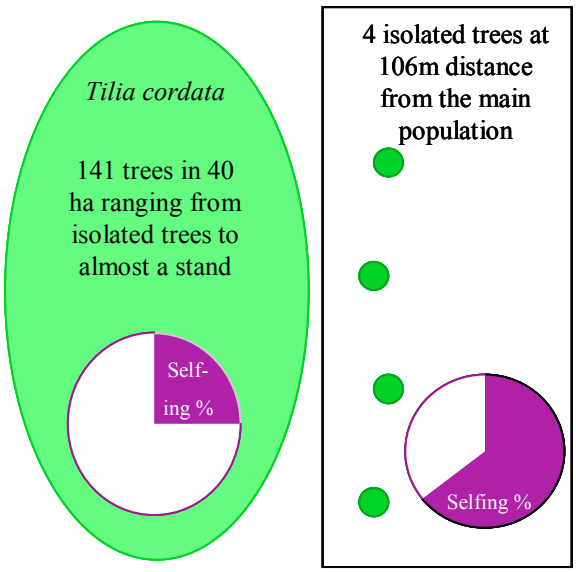


Figure 6-11. The percentage of selfings in a German population of Tilia cordata *and in four isolated trees outside the main population.*

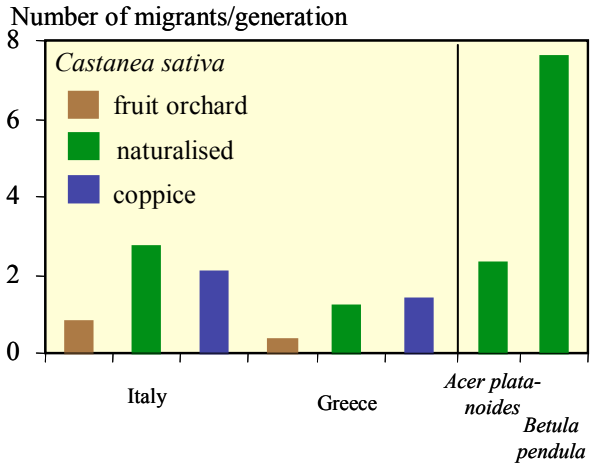


Figure 6-12. Number of migrants per generation in three types of Greek and Italian Castanea sativa *populations, naturalised, orchard, and coppice, and in natural populations of* Acer platanoides *and* Betula pendula.

Such a large gene flow is a serious constraint to adaptation to the prevailing site condition at the growth localities. Contrary to the situation for oak, the selfing was high in *Tilia cordata*, amounting to 25% in the stand studied and to 65% in four isolated trees outside this stand (Fig. 6-11). The average pollination distance was estimated at 150 meters while the corresponding estimate for maximum pollination distance was 1,666 meters. These figures for *T. cordata*, which is an insect-pollinated species, were slightly lower than the results from *Castanea sativa*, which is a wind-pollinated species. Another observation in the *Tilia* study was that the pollinating insects flew preferentially to large trees and stayed longer in them. In *Castanea sativa* the number of migrants exceeded 1 in naturalized and coppice populations from Greece and Italy while the fruit orchard populations had low estimates (Fig. 6-12). (A naturalized population is either a naturally regenerated forest or a coppice or fruit orchard population converted into a ordinary forest). Fig. 6-12 also reveals that the insect pollinated species, *Acer platanoides*, had such a high estimate as 2.3. The high value, 7.6, for the wind-pollinated species *Betula pendula* followed the expectation of a high number of migrants among populations. Studies of *Sorbus domestica and Sorbus torminalis* have indicated that gene flow is not as restricted as may be expected from the scattered distribution of these species. This observation plus the results for *Acer platanoides* suggest that we have to reject the *à priori* assumption that gene flow in scattered and rarely occurring species is rather restricted.

There are other examples of long-range pollen transfer in rarely occurring species (Fig. 6-13). The siring success in two small cohorts of trees and one isolated tree of *Guaiacum sanctum* growing in the north-western corner of Costa Rica was estimated by aid of 12 isozyme loci. This species is insect pollinated and classified as endangered. In Fig. 6-13 the mean siring success of the 8 and 11 trees growing in the two cohorts was estimated at around 4%. In contrast to this, the siring success of the isolated tree was estimated at 14%. Exterior pollen also contributed to 14% of the sirings in these 20 trees. The striking result from this investigation is that long range of pollen transfer occurs in such an insect pollinated species. The mean pollination distance was estimated at 1,864 metres.

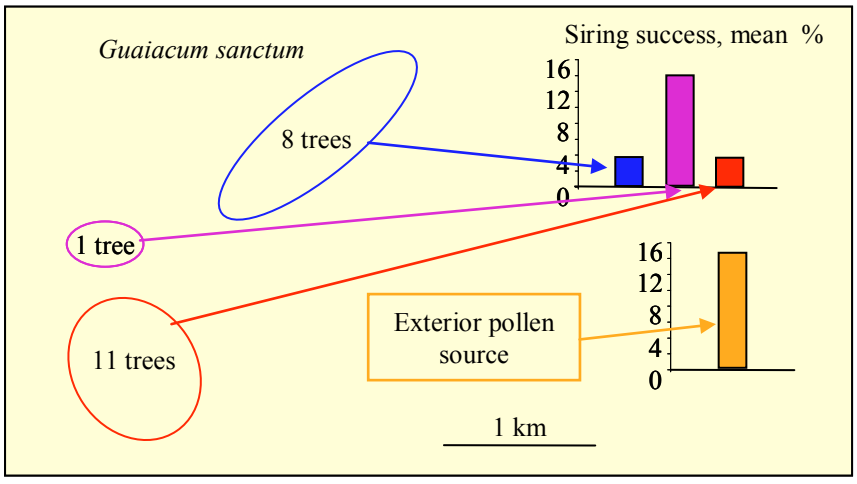


Figure 6-13. The mean siring success was estimated in two populations and one isolated tree of a rare insect pollinated tropical tree species, Guiacum sanctum. *Twelve isozyme loci were used for this study. The selfing in the isolated tree was marginal. The study was carried out on trees from the North Western corner of Costa Rica.*

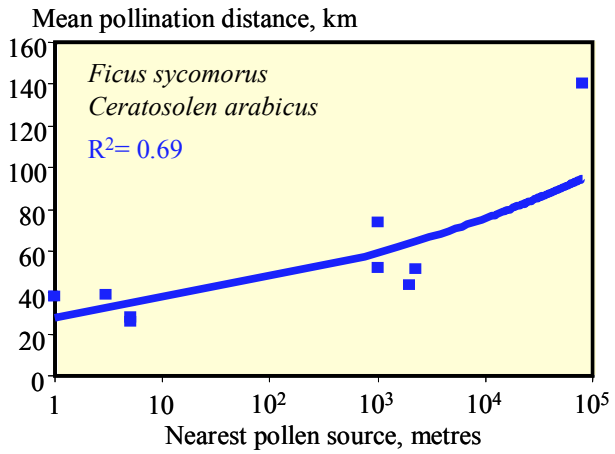


Figure 6-14. Mean pollination distances in rarely occurring and insect pollinated species growing along a river in Namibia. Note the logarithmic scale of the X-axis.

A still more extreme case was reported for a riparian, insect pollinated and rare species, *Ficus sycamorus*. growing along a river in Namibia. Owing to the wind conditions the pollinating insect *Ceratosolen arabicus* flies only in western direction. The longest successful siring was found to be approximately 167 km (Fig. 6-14).

These examples show that successful long-distance pollinations may occur in rarely occurring species.

Data on outcrossing, defined as matings between unrelated individuals, do not give us information about gene flow but such data inform us about the occurrence of matings among related individuals or selfing in populations. Data on outcrossing can be related to life-history traits of various species and therefore give us an understanding of mating pattern in species with different characteristics. In Fig. 6-15 data for several temperate forest tree species are presented based on a compilation by David Boshier. As seen from this figure outcrossings occur in more than 70% of all species in this figure. In conclusion, outcrossing data suggest that most males are unrelated and grow in the vicinity of the seed tree studied, but long-distance pollinations occur. The number of pollen donors varies considerably. Obviously successful mating requires that there is overlap between female receptivity and pollen dispersal.

Especially in southern Sweden a large number of new stands of Norway spruce have been established with eastern European *Picea abies*. Some people have regarded the pollination of the domestic *Picea abies* from the eastern sources as genetic pollution. For traits of high adaptive value the progeny will with high probability be intermediate between the exotic and domestic *Picea abies*. This in turn must be attributed to changes in allele frequencies. The issue will be elaborated somewhat more under Genetic pollution in Chapter 11.

The evolutionary importance of gene flow and phenotypic plasticity is further discussed in the section Ecotypes and ecoclines later on in this chapter.

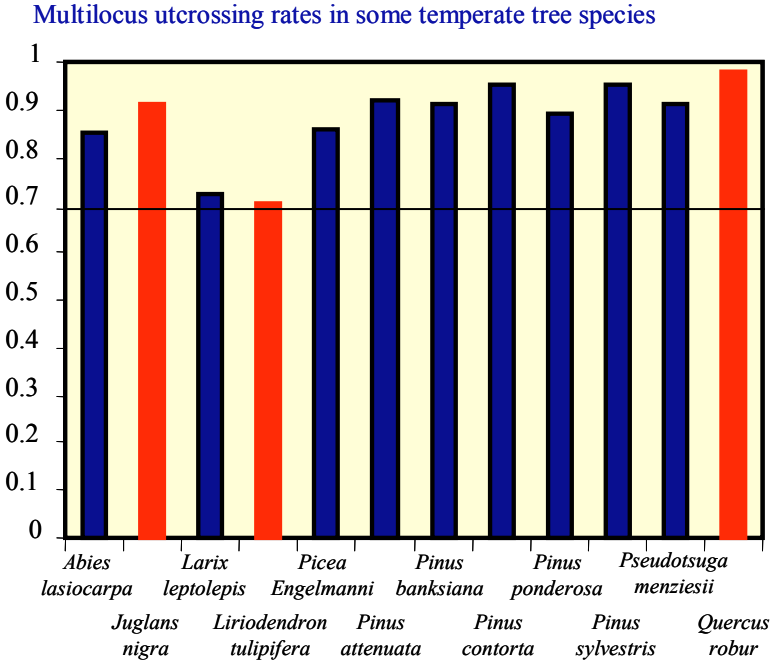


Figure 6-15. Percentage outcrossing rates estimated by several isozymes for Abies lasiocarpa, Juglans nigra, Liriodendron tulipifera, Picea Engelmanni, Pinus attenuata, Pinus banksiana, Pinus contorta, Pinus ponderosa, Pinus sylvestris, Pseudotsuga menziesii, *and* Quercus robur.

Phenotypic plasticity

Especially plants have a great ability to develop in different ways dependent on the environmental conditions. It has become more and more clear that this plasticity in traits such as plant size, flower number etc. can be genetically regulated. The morphology of flowers seems to have less plasticity. If such traits were very plastic it would threaten the reproduction of the species. Different species probably have different phenotypic plasticity according to their ecological characteristics.

Our knowledge about variation in phenotypic plasticity within populations is still limited. From theoretical points of view we expect that it is large in species such as Norway spruce, Scots pine, and lodgepole pine. It may be less in insect pollinated species with a scattered distribution and limited gene flow among populations (See also the section Ecotype and ecocline below).

Will the adaptedness ever be perfect?

Many laymen seem to believe that the adaptedness is perfect at a particular site if the population has grown there for many generations so that natural selection chisels out something perfect for just this site condition. Even among geneticists adaptation lag is discussed, meaning that if natural selection was allowed to proceed over enough number of generations we should eventually observe a perfect adaptedness. Besides, one tries to find adaptive advantage in all traits an individual carries. If a perfect adaptedness should exist it is required that the following prequisites are fulfilled:

* the environment is constant
* all traits in the population are totally independent of each other

That the environment is not constant over time is so evident that there is no need to elaborate much on this issue. Let us just remember that we who live in the far north have different seasons during a year and that conditions during two summers are never the same. Someone might believe that the year to year variations are so slight that they would not make any difference to natural selection. However, we do not know whether subtle differences would be evolutionarily very important. In southern Sweden the winters during the last 25 years are a good illustration of the annual weather variability. Only two harsh winters occurred during this period. After that, most winters have been milder than normal. These conditions have probably had consequences for survival and growth of Norway spruce plants. The mild winters have resulted in melting of the snow and plants were sometimes exposed to high day temperatures while the ground was still frozen. This leads to frost desiccation with death or retarded growth as a consequence. As regards variable weather we must assume that natural selection can change gene frequencies in different directions under different ambient conditions.

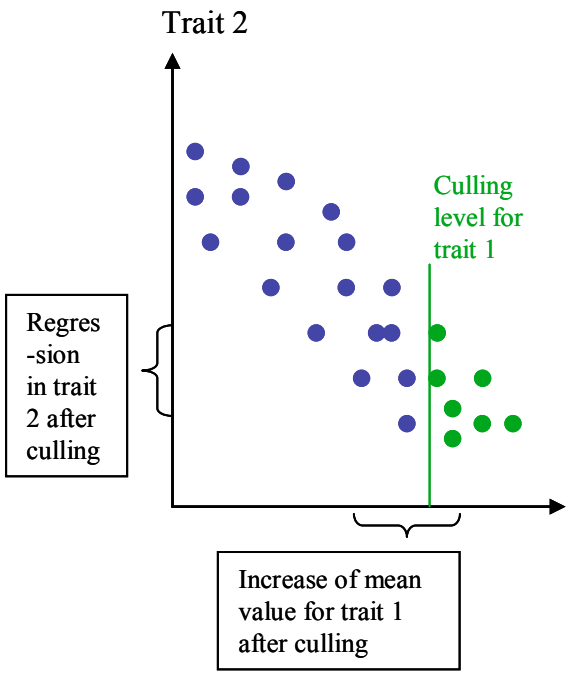


Figure 6-16. The consequences for selection on one trait for another trait, which is negatively correlated with it.

The relationship between two traits may be positive, negative, or absent. If it is negative it means that progress in one trait leads to regression in the second trait as is illustrated in Fig. 6-16. Since natural selection operates on the individual as an entity it means that there may be regression in a trait of adaptive value if it is negatively correlated with another trait of still higher adaptive value. It is the phenotype of an individual as an entity that is the selection target in most cases. Therefore, traits cannot be disentangled from each other and as corollary of this; natural selection may lead to good adaptedness in certain traits but with low adaptedness in others.

Besides the two prerequisites raised above there are other conditions that make it unlikely to find perfect adaptedness. In small populations there is a chance-conditioned loss of alleles owing to genetic drift. Once more it is important to stress that it is the number of the trees that contribute in the formation of the next generation that counts. This number can be considerably less than the census number of trees of a species growing in a forest.

Wind pollination, which is important for avoidance of genetic drift, may slow down the adaptation. This is the case if the pollen emanates from other populations with a certain degree of adaptedness to other site conditions than in the recipient population. The impact of gene flow on among- and within-population variation is further discussed in connection with Figs. 6-17 and 6-18. Similarly the role of gene flow for conferring fitness to phenotypic plasticity is discussed with starting point in these figures.

As mentioned in the chapter on quantitative traits it is expected that several different genotypes can give rise to one phenotype. A simple example might be used to illustrate this. Let us assume that alleles with index 1 contribute equally to the adaptedness and differently from alleles with index 2, which in turn contribute equally much. Under these conditions the gentypes $\mathbf{a_1a_1b_1b_2c_1c_2}$ and $\mathbf{a_2a_2b_1b_1c_1c_1}$ would have the same adaptedness, which is also valid for all other genotypes with four index-1 and two index-2 alleles. The phenotype with highest fitness may differ genetically, and therefore natural selection will not favour just one genotype. The population which is growing in nature as a consequence of natural selection, with all its limitations, must therefore be regarded as one solution of a great number of possible solutions.

It might be a little trivial to remark that most of the newly arisen mutations reduce the vitality and thereby the adaptedness of its carrier.

The conclusion from the discussion above is that we can never regard the present genetic constitution of a population as perfect or sacred, rather it must be regarded as transient and one of several possible. Therefore, the present genetic constitution should not be targeted in dynamic gene conservation (cf. Chapter 11).

Ecotype and ecocline

The Swedish geneticist Göte Turesson was probably the first to discuss genetic adaptation to different site conditions in the early 1920s. He introduced the term ecotype for this type of adaptation. He mainly studied perennials growing under quite distinct site conditions, such as rocky localities as contrasted to beach meadows. After cultivation at other site conditions the ”rocky” and the ”meadow” ecotypes kept their morphology, proving that their characteristics were genetically conditioned.

As will be shown in Chapter 7, growth rhythm, budburst during spring and inwintering at the end of the growth period show a continuous variation in Norway spruce and Scots pine. Such a variation is designated as clinal, and instead of ecotypes we have ecoclines in Norway spruce and Scots pine.

For most tree species from the northern hemisphere that have been studied, the night length is the primary trigger for onset of growth cessation. If the ambient conditions are harsh *e.g.* owing to drought this might also induce growth cessation for obvious reasons. The regulation by the night length of growth cessation means that a population transferred northwards gets a longer growth period than at its original site. Similarly, transfers southwards reduce the duration of the growth period since the critical night length for growth cessation occurs earlier than at the original site. The growth cessation triggered by long nights is coined photoperiodic response

Are there any proven cases of specific adaptation to edaphic conditions within tree species? There are a few reports suggesting this. However, later reports describing the same materials have disclosed that there were no longer any indications of specific adaptation to edaphic conditions. This does not exclude that individual genotypes differ in their ability to take up or utilize nutrients.

Detailed studies of Scots pine seedlings cultivated at different availability of nitrogen, which is a limiting nutrient element for pine growth in Sweden, resulted in some genotype x nitrogen treatment interaction but the interaction was not larger than the variation among families in the experiment. Is it possible to understand such a result evolutionarily? In southern Sweden Scots pine grows at various site conditions, which ought to give rise to specific adaptedness. However, the sites occur in mosaics and there is a large gene flow between trees growing at the different site conditions. As may be remembered from the section on gene flow, it is a strong factor tending to eliminate population differentiation. Thus, to allow a specific adaptation to take place there must not be any gene flow among the different types of site. It might even be an evolutionary advantage to develop genotypes that give rise to progeny that grow well over a broad span of site conditions. This means that phenotypic plasticity will contribute to fitness.

The above example illustrates well that the mating pattern is of utmost importance for the genetic structure. The American philosopher Robert Brandon, who has devoted much of his research to adaptation, has introduced the concept of selective environmental neighborhoods (SEN). Within such an area there is no genotype x environment interaction as regards fitness which means that there is a large environmental homogeneity within a SEN. In Figs. 6-17 and 6-18 two contrasting situations as regards gene flow among different SENs are illustrated. In the first there is a gene flow among all SENs, in the second there is no gene flow between the two SENs. When there is no gene flow between the two SENs there are good opportunities for specific adaptation to the site conditions in each SEN. The examples in figures 6-17 and 6-18 were selected consciously to illustrate a situation that is typical for northern conifers and broadleaved trees, respectively. If the broadleaved tree species consists of isolated populations and is pollinated by insects which are flying over short distances only, the schematic picture becomes close to reality. This type of tree has higher probability for specific adaptation than tree species which do not share these characteristics.

We have tried to illustrate schematically a situation that is typical for a species with wide and continuous distribution in Fig. 6-19. In such a case the environment changes gradually, *e.g.* there is frequently a gradual change from south to north with respect to climate. There will be no sharp boundary between SENs and pollination and seed

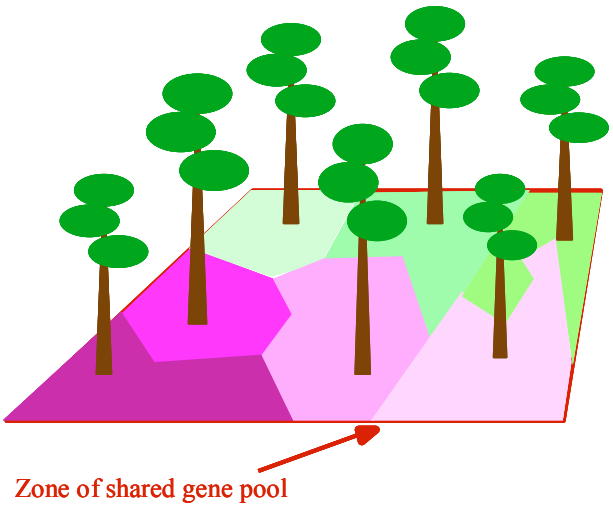


Figure 6-17. Schematic illustration of the importance of gene flow for the possibilities of population differentiation. In the figure there is free gene flow among the seven selective environmental neighbourhoods illustrated with different colours. Such a situation is a great constraint for population differentiation.

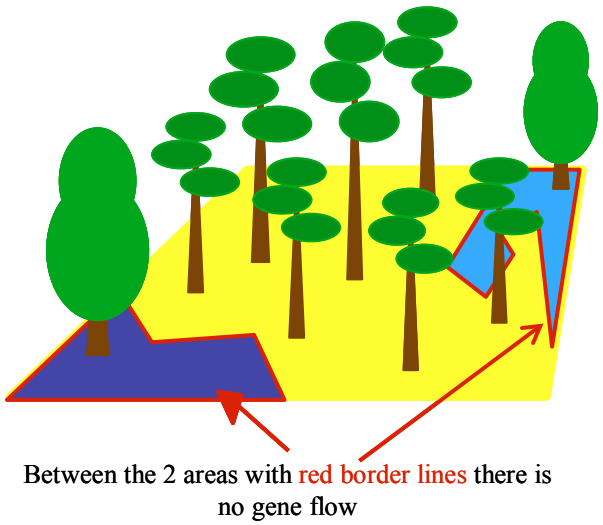


Figure 6-18. Schematic illustration of the importance of gene flow for the possibilities of population differentiation. In the figure there is no gene flow between the two areas with a broad-leaved tree species, which facilitates a specific adaptation within each of the two areas.

transfer may take place between adjacent SENs. If the environmental conditions are fairly stable, natural selection will improve the adaptedness along this gradient but gene flow will slow down this adaptation. However, such a gene flow may be useful under rapid global change as will be discussed in the next section of this chapter.

Many tropical tree species are represented by one or a few trees per hectare. Huge areas may constitute one selective environmental neighborhood in wet tropical forests. In such a case the zone for shared pollination may be much smaller than a SEN (Fig. 6-20). The situation for many tropical tree species constitutes a great contrast to the situation for a species with continuous distribution as depicted in Figure 6-19.

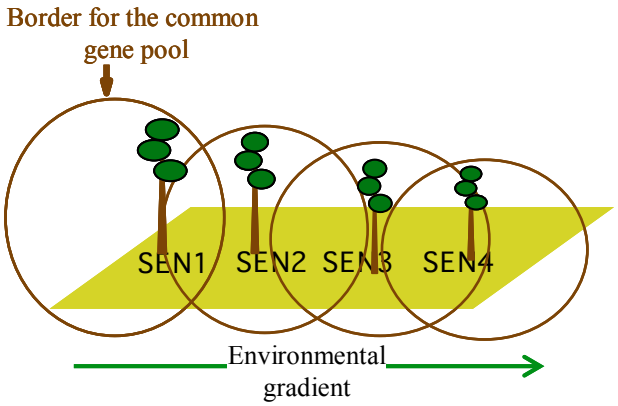


Figure 6-19. Schematic illustration of a common situation for many widely and continuously distributed species, which occupy a gradually changing environment

In ecological texts the niche concept is frequently used to describe site conditions. The advantage with the selective environmental neighborhood concept is that it is not bound to one specific geographic area but it may vary dependent on which trait is under consideration. Thus, for a strictly neutral trait in a species there is just one SEN whereas there may be many SENs for adaptive traits.

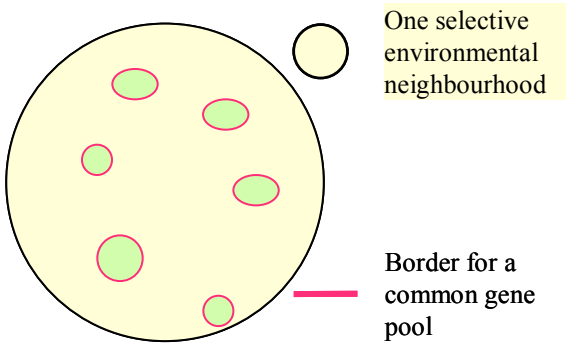


Figure 6-20. Schematic illustration of a common situation for many tree species from the wet tropical forests with one or a few tree species per hectare. It is assumed that the environmental conditions are fairly uniform over a huge area, which thus constitutes one selective environmental neigborhood.

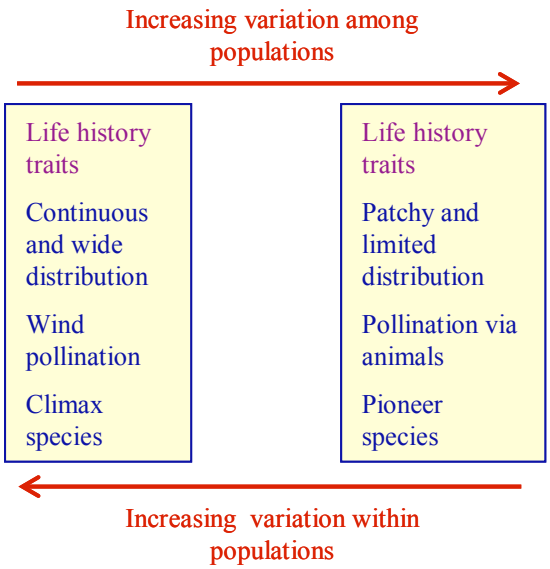


Figure 6-21. Schematic illustration of combinations of life-history traits promoting population differentiation and within-population variation, respectively. See the text.

It has been hypothesized that life history-traits such as type of distribution, pollen and seed dispersal, and stage in ecosystem may influence the variation within and between populations. In Fig. 6-21 we have summarized the life-history traits that are believed to promote or decrease the ratio *among-population differentiation/*(*within-population variation*). In a wind-pollinated species with a wide and continuous distribution, gene flow may be considerable, which means a leveling of allele frequencies between populations. This may be strengthened if the species is one of the climax species in the ecosystem under consideration. Contrary to this, a species with scattered distribution and with limited dispersal of pollen and seed there is room for a larger population differentiation than in tree species with the life-history trait combinations shown to the left in Fig. 6-21. Some studies give support to this but there are data,indicating that many species sharing the life-history traits to the right in Fig. 6-21 have ecoclinal rather than ecotypic variation. It must be assumed that these species have passed the threshold, that causes ecoclinal variation, even if their gene flow is lower than in wind-pollinated species.

Alnus maritima is a North American species with a patchy distribution. In one study comprising three different regions in USA - Delaware/Maryland, Georgia, and Oklahoma - microsatellites were used to study variation within and between the three regions. Only one population from Georgia was included in the study. A relatively high differentiation among the populations was noted, F_{ST}=0.26. The so called Genetic Identity index according to Slatkin was estimated. In Fig. 6-22 this index within regions and between regions reveals that there is a few times higher similarity within regions than between regions. Moreover, the geographically most distant of the four Delaware/Marilyn populations had a lower similarity index with the three other Delaware/Maryland populations, mean value, 0.89, compared to the mean for the three other populations, 3.24. The data from this species support the hypothesis that a patchy distribution leads to large genetic differentiation.

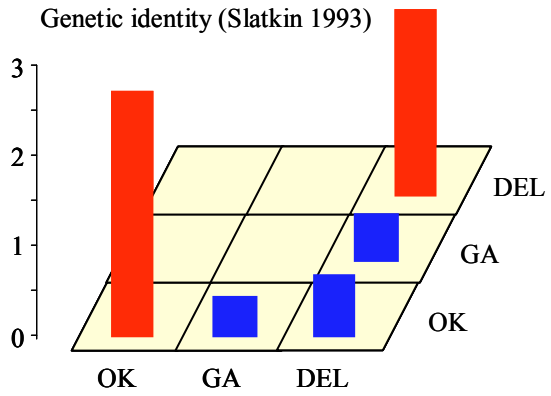


Figure 6-22. Slatkins genetic identity estimates in four populations in each of Oklahoma (OK) and Delaware/Maryland (DEL), and one Georgia population (GA) of Alnus maritima*. Within-region is shown as red columns and between regions as blue columns.*

Pioneer species such as weed species, which invade bare ground, may benefit from great uniformity to effectively utilize the open land. Dependent on the variation of the conditions at each locality, different clones will invade different localities. Asexual propagation, such as in *Taraxacum vulgare*, results in well-adapted clones that are adavantageous in the short-term. Pioneer tree species are rarely or never exposed to such uniform reforestation conditions that total uniformity would be advantageous. Generally there is an inverse relationship between adaptedness and adaptability. Adaptedness may reach a high level by eliminating what is referred to as genetic load, resulting in high genetic uniformity. Such a uniformity means that the additive variance and thereby the adaptability goes down. Thus, high adaptedness may be very useful under constant environmental conditions but may be disastrous under rapid change of the environment.

Some proponents of the ecotype concept have claimed that what we observe as continuous variation is actually a stepwise variation which should be designated as ecotypic. The prerequisite for us to detect stepwise variation is that there is no gene flow among populations that are growing under different site conditions. The pattern of pollination and seed dispersal are decisive as to whether there will be an ecotypic or an ecoclinal variation along an environmental gradient. It is highly unlikely to find ecotypes in wind-pollinated species which have a broad and continuous distribution. If plants are exposed to extreme stress, like the grass species growing on mining wastes, there can be ecotypic variation as was earlier shown in this chapter. In summary, the lengthy and animated controversy among scientists about whether forest trees re-

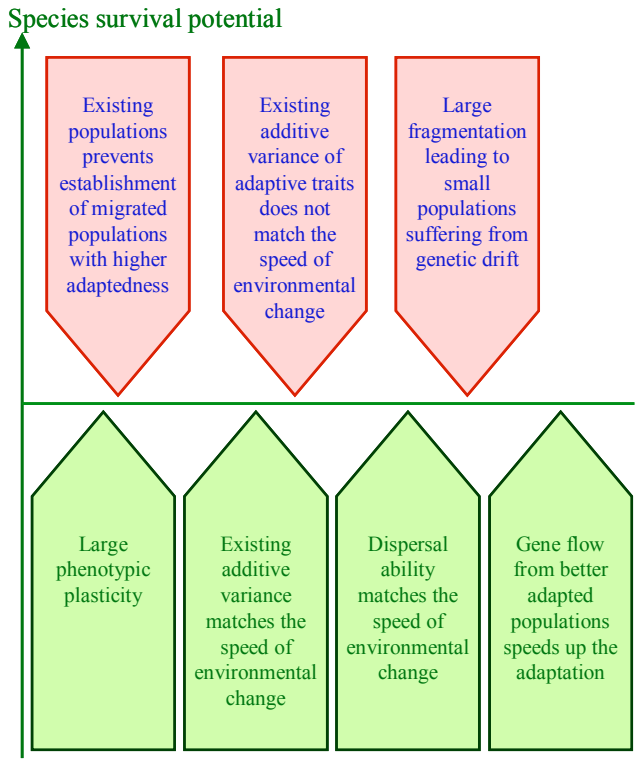


Figure 6-23 The arrows pointing upwards promote survival of a species in case of global warming and the arrows pointing downwards are constraints to adaptation.

ally show continuous variation or not is best resolved by trying to identify the evolutionary factors of significance for each species separately.

Evolution and global warming

As components of ecosystems, trees and plants are continuously exposed to environmental changes. Under global warming the speed of change might be faster than before. The changes connected with a greenhouse effect are more a question of degree than of new types of genetic processes differing from those occurring under ”normal” changes in the environment.

It is evident that long-lived tree species under global change will be exposed to a gradual change of weather conditions during their life times. To endure such a change trees have to be equipped with large phenotypic plasticity, which thus is of great importance for trees in the forests today. However, the phenotypic plasticity is simultaneously a constraint if it allows continuous existence of an already existing population, which prevents establishment of a new population with better adaptedness to the changed conditions (cf Fig. 6-23). For long-term success trees must rely on two other options. The first is dispersal ability and the second is the ability to respond genetically, *i.e.* that there is ample additive variance for traits of adaptive significance. When either of them is large enough to cope with the changes in the environment the species will survive. A continuously distributed species with long-distance pollen transfer may benefit from this gene flow in contrast to a species with scattered distribution and no or limited gene flow (cf Fig 6-24). However, for the population from the warmest location there is no pollen donor to benefit from.

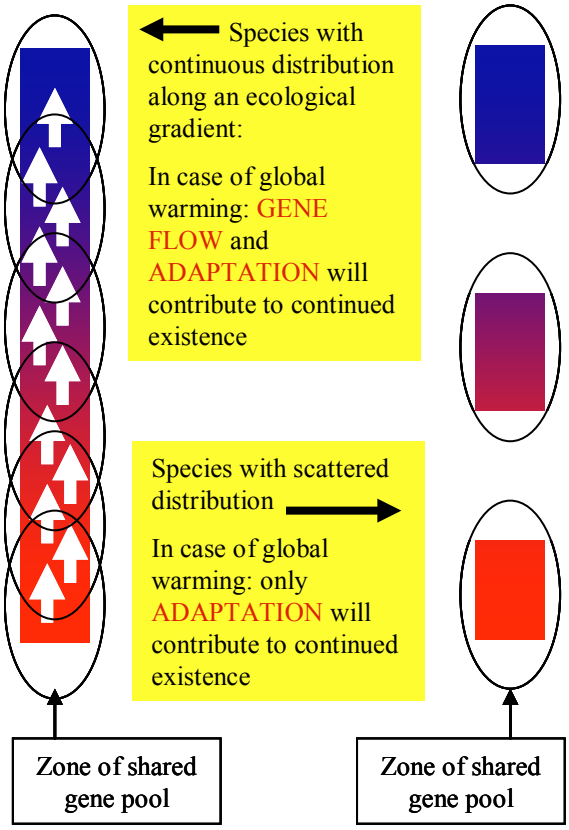


Figure 6-24. Illustration of the possibilities for adaptation in case of global warming in a continuously distributed species and a species with scattered distribution. The white arrows indicate main direction of pollen flow.

Phenology of growth and flowering are critical for survival and good growth of many forest tree species from the temperate part of the world. We shall discuss growth phenology for *Picea abies*, which is a most important forest tree in Scandinavia but first we shall give some comments on flowering phenology.

Flowering intensity and flowering time are of great significance in any sexually reproducing species. It has been proven that flowering initiation is dependent on high temperatures in several tree species, at least at high latitudes. It is likely that flowering will take place earlier during the season in case of global warming since flowering like many other phenology traits is triggered by the heat sum. The expectation following a temperature increase can be phrased in the following way: With a prediction of more temperature extremes, this early flowering may lead to exposure to low and damaging temperatures. In consequence severe frost damage may occur owing to early flowering followed by a frost spell.

Table 6-1. A summary of possible effects of global warming on phenology in Picea abies.

	occurs earlier	occurs later
Triggering of growth cessation	No difference since night length, which is the triggering factor, is not influenced by climate change Sustained drought may provoke earlier growth cessation	
Reaching of dormancy	If the low temperatures are present and the high temperatures speeds up the development	If the low temperatures occur later than under present conditions
Breaking of dormancy and start of growth activities	If temperatures low enough occur and high temperatures occur during winter	If dormancy is built up later and low temperatures are less frequent than under present conditions

Most forest tree geneticists agree that growth cessation in boreal and temperate tree species is triggered by night length and that budburst is dependent on the heat sum. The northern populations require shorter night lengths for triggering of growth cessation than southern populations. For *Picea abies* there is a clinal variation for growth cessation.

In Table 6-1 possible effects of global warming on *Picea abies* phenology are summarized. Since growth cessation is triggered by long nights global change will not cause any change of this phenomenon. Building up of dormancy is dependent on both low temperatures and high temperatures. Therefore, it is hard to predict whether dormancy is reached earlier or later in case of global warming. Breaking of dormancy may occur later if low temperatures are infrequent or earlier if low temperatures are frequent enough and dormancy is obtained early. One problem with early breakage of dormancy is that fluctuations between mild weather and frost exposure are expected to be more frequent during global warming. This in turn may lead to severe frost damage of the highly frost sensitive shoots.

Phenological gardens, *i.e.* plantations located in different climatic zones with the same genetic material, give useful estimations of effects of global warming on phenology traits. Based on data from phenological gardens distributed over Europe it was estimated that budburst in *Prunus avium* would take place 5 days earlier per degree of temperature increase. The corresponding estimates for *Tilia cordata* and *Sorbus aucuparia* were 2-3 days. The prediction that leaf fall will not be changed by increased temperature was confirmed for *Tilia cordata*. Strong temperature dependence for budburst in some *Fagus sylvatica* populations was also reported. This means that budburst will take place earlier in the case of global warming. Many models have been put forward to predict effects of global warming on phenology traits. In one paper, possible outcomes of models that try to predict effects of global warming were formulated in the following way: *Both models and experiments show that the response of phenology to climate change, and in particular to global warming, will depend on the species, the latitude at which the populations are observed and the intensity of changes*. It seems as if the effects on phenology will be more pronounced at higher latitudes after a temperature increase than in the Mediterranean region. In analogy with this, the effect in the latter region will be largest at high elevation.

One possible consequence of global warming is fragmentation of a continuously distributed species. This could lead to lower effective population sizes with increased importance of genetic drift in the scattered and sometimes small populations. This means that the mating pattern may be changed. Mating pattern is defined as the matings that are realized, *i.e.* the zygotes formed in a population. Fragmentation of a previously continuously distributed species may have consequences for its mating pattern. One leading scientist has stated that fragmentation might be of importance only if the fragmentation results in populations with effective population sizes less than 100. However, general predictions are hard to put forward owing to limited empirical data. Generally, the effect is dependent on the gene flow before fragmentation, the pattern of migration between separated populations after fragmentation, as well as local recolonisation and extinction. This will be discussed by the aid of Fig. 6-25.

In the centre of this figure the gene flow between populations is illustrated by arrows of different thickness, the thicker the arrow the larger the gene flow. There is no direct gene flow between the two most distant populations. However, there is a possibility for a stepwise gene flow between these populations via the central populations. If the two central populations become extinct there may be no gene flow between the most distant populations. Intuitively, the change in mating pattern depicted to the right in Fig. 6-25 is expected to result in an increased dif-

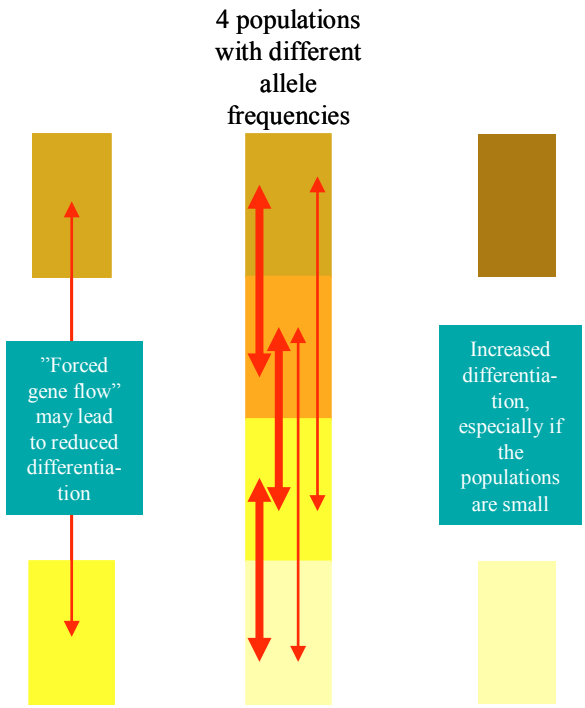


Figure 6-25. Two contrasting consequences of loss of two central populations on the variation between populations after fragmentation; differentiation between populations is reduced if the fragmentation forces the pollen vector to longer transfers to catch nectar while there may be an increased differentiation if gene flow is terminated.

ferentiation between populations thanks to adaptation in absence of gene flow by pollen from other populations. If this break of gene flow is associated with reduced population size, genetic drift may also contribute to increased population differentiation. Some of the empirical results and results from simulations related to the effect of fragmentation are presented below (Chapter 12).

However, there are results pointing to a reduced differentiation between distant populations (Fig. 6-25, left part). If fragmentation occurs in an insect pollinated species, loss of central populations may force insects to fly further than before to find food, which will result in gene flow between earlier isolated populations with reduced population differentiation as a result. Therefore, it is unlikely that a general prediction for the outcome of fragmentation can be put forward.

Climate change may also have consequences at the species level. Allopatric (see below), related species may after climate change migrate in such a way that they will occupy the same habitat. If they have no means of isolation except for the previous geographic isolation, interspecific hybridisation may occur. There are supposed examples for this in the *Abies* and *Pinus* genera.

From the study of fossil records many ecologists have come to the conclusion that most species will not be able to migrate fast enough to cope with the speed of change caused by global warming. If this conclusion is true, species have to rely on the genetic ability to respond to the changes caused by global warming.

In conclusion, for long-term survival of a species under global climatic change one of the two following conditions must be fulfilled:

* the dispersal ability is greater than the speed of environmental change
* the genetic response is greater than the speed of environmental change

It should be noted that these conditions apply irrespective of the duration of the environmental change. However, it must be remembered that a tree species with a generation time of 25 years needs a much larger amount of additive variance than an annual species that can respond 25 times during this period. Species with exclusively asexual reproduction have to rely on dispersal ability to cope with global change.

Coevolution

Coevolution is usually defined as: *Mutual evolutionary changes in two interacting species as a response to changes in these species*. The typical example is that a host plant builds up a defence mechanism against one of its herbivorous species. This is then followed by development of a mechanism in the herbivore to overcome the defense system of the host plant. In turn the host plant develops a new defense mechanism which again is overcome by the herbivore. Coevolution has been regarded as an arms race. In the breeding of agricultural crops such a kind of arms race is quite common. The resistance against a harmful organism is followed by a change in the pest or disease organism to overcome the defense. This has led to a constant search for new resistance alleles since one crop variety after the other has lost its resistance. There has been a constant struggle to be ahead of the pest or disease organism. Does such an arms race occur in nature? Many investigations of herbivores and their host plant have been carried out. Some of the results are presented below.

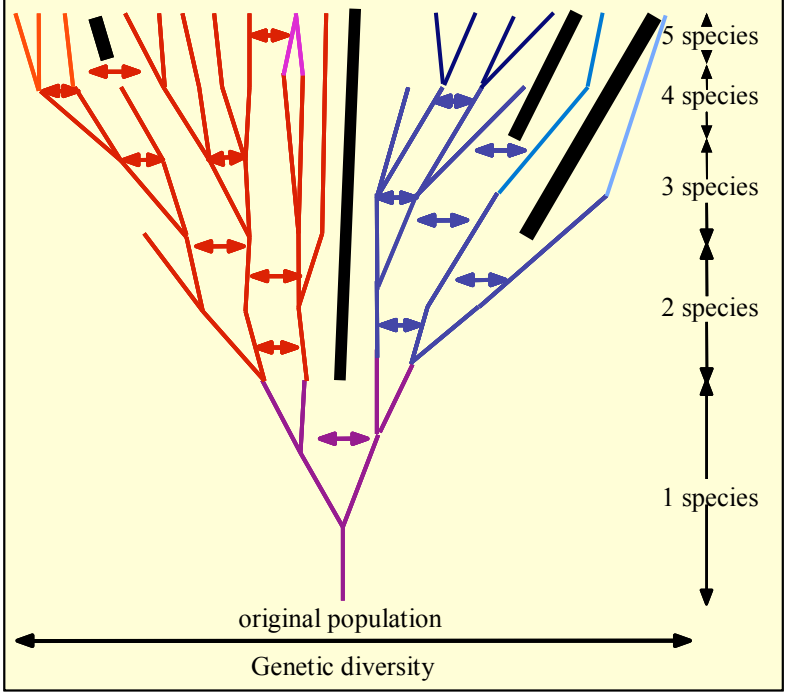
Coevolution seems to be an exception among host plants and herbivorous insects. Many plant species have secondary metabolites which are sometimes toxic and may slow down the digestion in herbivores. Especially among herbivores of families belonging to *Brassicales* (*Cruciferae*) and *Apiales* (*Umbelliferae*) it is common that the toxic substances are signals for recognition such that the insects are enticed to visit these plants.

As a rule it does not seem as if the host plants have developed their defence mechanisms against their own herbivore. Both among host plants and herbivores it seems that

Box 6-3 Speciation

The horisontal double arrows indicate gene flow between populations.

Fat black line shows that there is no gene flow between populations.



the defense mechanisms and the means to overcome the defense are more general than a strict coevolution requires. Thus it is likely that vascular plants early during their evolution produced secondary metabolites that raised the fitness of its carriers. Long-lived tree species, such as the conifers, rarely have specific toxic substances but they have secondary metabolites which slow down the digestion in the herbivores.

Specialization of the herbivorous insect on certain host plants might suggest that coevolution would be beneficial. However, there are other reasons why a specialization might be advantageous. Certain of the toxic metabolites, which the herbivore gets from feeding from plants with toxins, might protect it from its own parasites or from other animals which have the herbivore as a prey animal.

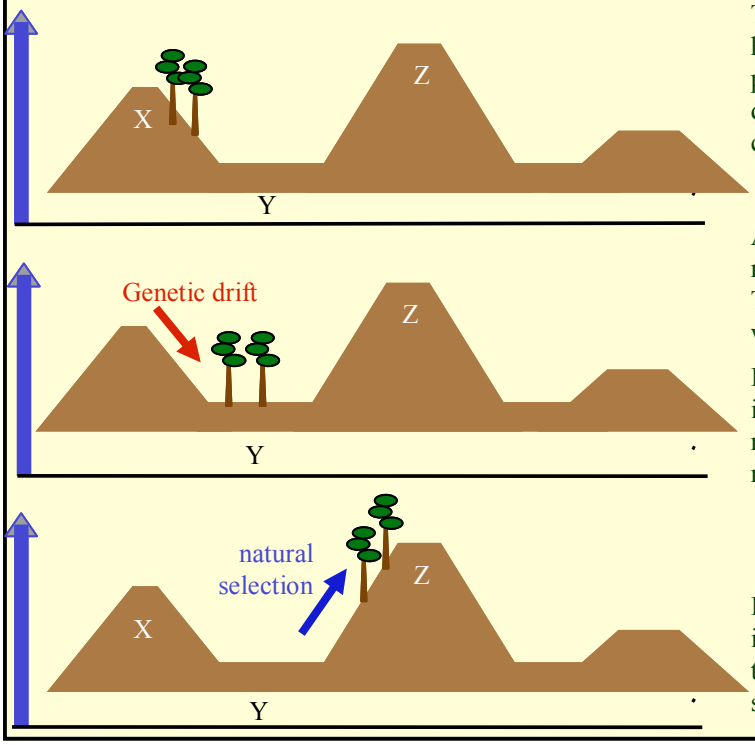
Speciation

Speciation must be regarded as a logical continuation of population differentiation (See Box 6-3). When the differentiation between populations has gone so far that there is no gene flow between the two populations the main condition for speciation is fulfilled. If no gene flow occurs the populations will become reproductively isolated after some time. In Box 6-3 it is illustrated how one homogeneous population (below) after some time converts into two populations that are differentiated genetically although some gene flow occurs (horisontal arrows). When there is no longer any gene flow between the two populations, two new species have arisen. Each of these two species becomes internally differentiated over time. A little later the population to the furthest right becomes isolated from the rest of its species and a new species appears on the arena. After still further time a new speciation takes place in the right part of the box while the speciation in the left part of the box does not occur until the most recent time. The end result of this hypothetical example is that we now have 5 species from the once homogeneous population. In the left part it is also illustrated that differentiation between populations might cease. A prerequisite is that the gene flow becomes strong enough between the two populations so that the matings can be regarded as totally random.

The major difference between speciation and population differentiation is that the former implies reproductive isolation from other species. The isolation mechanisms can be of various kinds. The requirement for reproductive isolation is easy to understand if we remember what was stated about gene flow above, *viz.* that it is a strong constraint to differentiation among populations.

Usually three types of speciation are distinguished, allopatric, sympatric, and parapatric. Allopatric means that the speciation takes place in populations which exist in

Box 6-4 Adaptive landscapes



Sewall Wright introduced the concept of adaptive landscapes, which consists of peaks and valleys. The higher the position on a slope a population has reached the better the adaptedness. A population in which natural selection is the dominating evolutionary factor will figuratively climb higher and higher on the slope

A condition for the population on the X slope to reach peak Z is that it passes the valley bottom Y. This cannot take place via natural selection, which only results in improved adaptedness.

If the effective population size of the population is reduced such that genetic drift outweighs natural selection the population may theoretically reach valley bottom Y.

If the effective population size of the population increases such that natural selection can operate the population may theoretically climb the Z slope and in this way reach a higher peak.

different regions. Sympatric is the opposite, *i.e.* speciation in a common area. Parapatric means that two different alleles in adjacent populations are favoured. This type of speciation will not be further commented on.

Allopatric and sympatric speciation.

Indications of the occurrence of allopatric speciation are numerous and are built on information about geographic variation. Distant populations often have sterility barriers or differ more in ethological behaviour than adjacent populations. A good example is that of the seagulls referred to in the beginning of this chapter. Often biological differences covary with geographic barriers. This is the case for many fresh water fish species inhabiting different mountain lake systems. One of the most prominent evolutionists, Ernst Mayr, has very strongly argued that geographic isolation is a prerequisite for speciation. He has taken examples from populations at the periphery of the distribution of the species. These are designated as marginal populations. They frequently differ from central populations in many respects. Marginal populations are often characterised by a small effective population size. Genetic drift might be important giving them different characteristics from those of central populations. For a considerable difference to develop between marginal and central populations owing to drift it is required that the effective population size remains small over several generations; otherwise the characteristics of the small population may rapidly vanish when natural selection operates again.

When the differences have been accumulated between two populations they have also obtained differences in alleles which may not be of immediate importance for survival. Such changes can lead to inferior vitality of hybrids between the two populations. This is referred to as outbreeding depression. Such a hybrid inferiority means that the parental populations are more or less reproductively isolated from each other. Another means to prevent gene flow among populations is that their flowering times do not overlap. At the species level this seems to be the case for European and Siberian larch growing in Sweden. It should be noted that neither of the two species is native in Sweden.

Adaptive landscapes

Sewall Wright introduced the concept of adaptive landscapes, in which peaks and valleys occur (Box 6-4). According to this concept natural selection can only result in a climb towards a peak. Once a population has started such a climb it is impossible to reach an adjacent higher peak via natural selection since natural selection only favours an upward climb. If the effective population size is so small that genetic drift becomes important the population might figuratively pass a valley bottom and later at larger effective population sizes start climbing a higher peak by the aid of natural selection. If such a migration is carried out by a limited part of the population it may result in two species. This kind of reasoning has led to the perception of genetic drift as an important factor for speciation.

Sympatric speciation is debated. One condition for sympatric speciation is that there is limited or no gene flow between groups of individuals in a population inhabiting a certain area. Such a situation may occur if the two groups of individuals have totally different flowering times. There is one unquestionably important means of sympatric speciation, hybridisation between two species followed by polyploidisation. This kind of speciation is treated in a separate section below.

Speciation *per se* is seldom adaptive, rather it is a biproduct of adaptation to different site conditions. Speciation, though, is a good starting point for future evolution. Some scientists claim that speciation can be due to single alleles. Above all, this might be the case for alleles influencing floral structure, which in consequence must lead to reproductive isolation between individuals with the original floral shape and the mutant form individuals. Speciation via a difference at just one locus is probably rare.

Speciation by polyploidy

Already early in the 20th century it was detected that species belonging to the same genus had multiples of the somatic chromosome number. In one of the first studies the Swede Otto Rosenberg found that *Drosera rotundifolia* had 20 chromosomes while *D. longifolia* had 40 chromosomes. Later on it was found that many cultivated plants such as wheat, oats, cotton, banana, sugarcane, coffee, potato and tobacco are polyploid. Already during the second decade of the 20th century the hypothesis on speciation via doubling of the chromosomal number after species hybridisation was presented. The first example of a replication of spontaneous speciation via polyploidy in nature was the creation of an artificial *Galeopsis tetrahit* after crosses between two other *Galeopsis* species *G. pubescens* and *G. speciosa* by Arne Müntzing during the thirties.

Meiosis is frequently disturbed in species hybrids since there are no homologous chromosomes available for pairing. Sometimes so called restitution nuclei are formed, in which all chromosomes from the two crossing partners are included. These nuclei have twice as many chromosomes as the normal gametes. Even if only the egg cell is diploid, a polyploid (triploid) embryo will be formed. A plant developed from such an embryo usually differs much from the parental species. Progeny derived from spontaneous back crosses with the parental species are rare if they occur at all since the hybrid is highly sterile owing to the problems with bivalent formation during meiosis. Therefore, a tetraploid plant that has arisen after a doubling of the chromosome number in a species hybrid will be reproductively isolated from the parental species. This as pointed out above, is a prerequisite for speciation. Species created in this way are usually referred to as allotetraploids. To simplify the description of polyploids we use one letter to designate an entire genome of a polyploid species. An allotetraploid is thus designated as **AABB**.

Spontaneous doubling of the chromosome number may occur without a preceding species hybridisation. This is designated as autotetraploidy and is written as **AAAA**. The meiotic division in such a polyploid is disturbed since four chromosomes try to find their homologue for pairing. This leads to variable chromosome numbers both in eggs and pollen grains.

The speed of speciation

The speed of speciation seems to vary much among different groups. The European and American species of *Platanus* have been separated for numerous generations. In spite of this they do not differ much morphologically from each other. Moreover, it is easy to obtain hybrids between the two species. The speciation among ciclide fish species in lake Victoria and the nearby lake Nabugabu in Africa is an example of recent time rapid speciation. A limited gene flow and small populations seem to be the best conditions for fast speciation, which also promotes population separation. In animals the speciation can be speeded up if the animal has specific mating rituals, which causes a strong sexual selection. Isolation in the form of islands or lakes is a good starting ground for rapid speciation. The far-reaching differentiation as regards choice of foodstuff among bird species in Hawai and Galapogos islands shows that isolated populations can rapidly become species by occupying different selective environmental neighbourhoods. In this case the type of food is the selective environmental neighbourhood.

Summary

If we look upon adaptation from an analytical perspective we can distinguish two steps. During the first step genetic variation is created and recombination of alleles takes place. This is mainly a random process. Natural selection constitutes the second step, during which the allele frequencies of populations are changed.

It is stressed that natural selection is one of several factors that influence genetic variation within and among populations. Natural selection is a change of gene frequencies and it reduces the genetic variation within populations. There are three types of natural selection. Stabilizing selection means that phenotypes close to the population mean are favoured. In directional selection individuals in one tail of the distribution are favoured. Finally, disruptive selection favours individuals in both tails of the distribution. Stabilizing selection is common within stationary populations. A stabilizing selection within a series of populations growing along an environmental gradient will be experienced as disruptive selection among popu-

lations. Natural selection improves the adaptedness but other evolutionary factors participate in the evolution. Therefore, perfect adaptedness will never be observed in nature.

Genetic drift is a random process that leads to allele fixation independent of the fitness contribution of the fixed allele; this reduces the within-population genetic variation. By chance different alleles will be fixed in different populations, contributing to among-population variation. The effect of genetic drift increases exponentially with decreasing effective population size.

Mutations occur at a low frequency and increase the genetic variation within populations. Since the mutation rate per locus and generation is so low, the probability for the same mutation to arise in two populations is infinitesimal. Therefore, mutations will give rise to a small difference among populations.

Gene flow is a strong constraint to among-population differentiation. At the population level it is a strong contributor to increased within-population variation. Data on gene flow and outcrossing suggest that a large gene flow is not restricted to wind-pollinated species with a wide and continuous distribution but also occur in scattered and insect pollinated tree species. Ecotypic differentiation occurs if gene flow among populations is much restricted. Ecoclinal variation occurs in widespread species with a large gene flow among populations.

The role of phenotypic plasticity is ambiguous. On the one hand it can confer fitness to its carrier and thus is favoured by natural selection. On the other hand it may be regarded as a disguise of the genotype. In this way natural selection becomes less efficient in the presence of pronounced phenotypic plasticity.

In Fig. 6-26 the requirements for natural selection and genetic drift to accomplish evolution are illustrated. Difference in fitness is what characterises natural selection and separates natural selection from genetic drift. Differences in fitness facilitate evolution but are not a prerequisite. Evolution means that genetic change has taken place.

Dependending on the ecological characteristics of a species they show ecoclinal or ecotypic differentiation. The latter have distinct differences among populations growing under different site conditions. Ecoclinal variation means a continuous variation along environmental gradients.

From an evolutionary point of view differentiation of populations and speciation are related. The difference is that reproductive isolation exist at the species level whereas some gene flow might occur among populations within a species. Small populations with no or restricted gene flow are a good basis for rapid speciation.

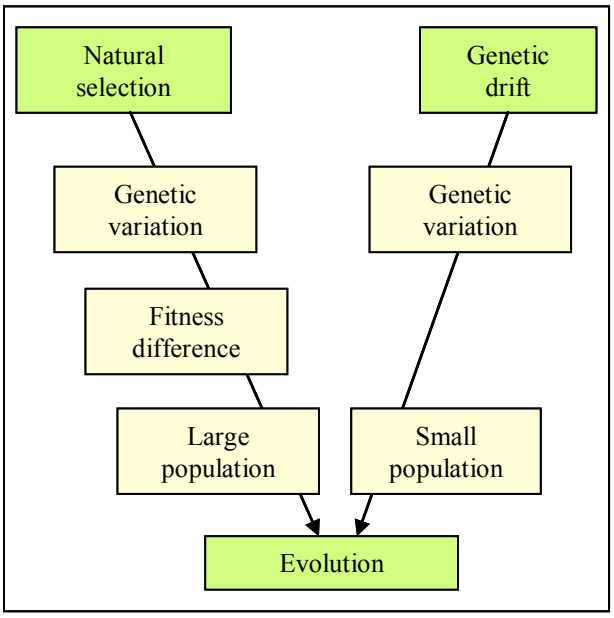


Figure 6-26. The requirements(yellow boxes) for natural selection and genetic drift to accomplish evolution.

Speciation is facilitated by geographical isolation. Doubling of the chromosomal number in species hybrids has been shown to have occurred frequently in plant speciation and is an outstanding example of speciation without geographical isolation.

Evolution in the past has created various patterns of population structure in different tree species. Some of the types of population structure are illustrated schematically in next chapter, Box 7-1. Examples of species having the various patterns are also given. These patterns are discussed in the next chapter while variation within populations is discussed in Chapter 8.

Further reading

Brandon, R. 1990. Adaptation and environment. Princeton Univ. Press, Princeton, New Jersey.

Futyuma, D.J. 1997. Evolutionary biology. 3rd ed. Sinnauer Ass, Ltd, Sunderland MA 01375 USA.

Hartle, D.L. and Clark, G. 1989. Principles of population genetics. 2nd ed. Sinnauer Ass, Inc, Sunderland MA 01375 USA.

Mayr, E. 1988. Toward a new philosophy of biology. Harward University Press, Cambridge, Massachusetts, and London, England.

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264-279.

Genetic variation and provenance research

In Chapter 2 we have presented the origin of genetic variation. In this chapter the main emphasis is on the genetic variation within a species and particularly provenance differences. The important distinction between Darwinian and domestic fitness is also outlined.

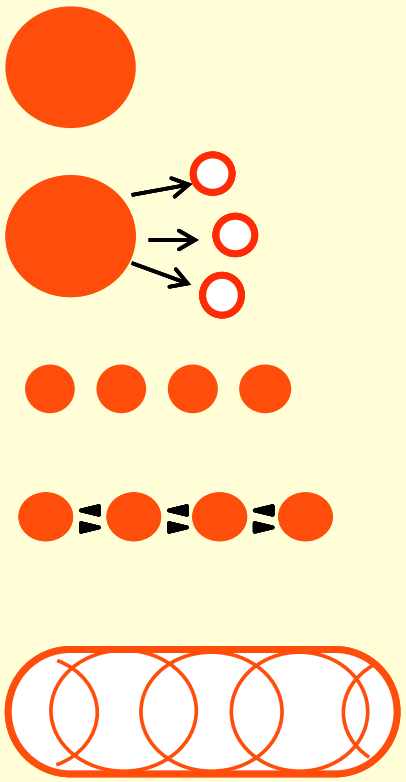
Genetic structure and how it is estimated

By genetic structure we mean how alleles and genotypes are distributed among and within populations. The previously described evolutionary processes have contributed in different ways to the present genetic constitution in nature. The history of a population or a species is therefore important for the genetic variation we can observe today.

Some geneticists have the opinion that we should only talk about genetic structure when we have identified the alleles that affect different traits. In this narrow sense the quantitative traits are excluded which are affected by alleles that we cannot identify with certainty. Since most of the traits with high adaptive value are quantitative, it would be unfortunate if they were not included in estimates of the genetic structure. Below, genetic structure is used to describe genetic variation in both qualitative and quantitative traits.

In Box 7-1 we present a number of possible population structures as well as examples from tree species representative of the various structures. In nature the structure is often not as distinct as illustrated; rather we can observe transitions between the different structures of Box 7-1.

Box 7-1 Potential population structures and gene flow



One large contiguous population; Example: *Pinus resinosa* from northeastern North America

Continent-island, gene flow occurs mainly from the large population to the small island populations; Example: *Picea omorica*

Small disjunct populations without any clear gene flow between them; Example: *Pinus radiata* in Californien

Stepping stone structure, where gene flow occurs between adjacent populations; Example: *Abies fraseri* in the Appalachian mountains in eastern USA

A large continuous population, where geographic distance affects the similarity between populations; Examples: *Picea abies* and *Pinus sylvestris* in Europe

In gene conservation it is important to include the entire genetic variation in a species. To enable this we must know the population structure of the species. In reality the population structure is rarely as clear as might be assumed from the population structures visualised.

In order to detect a differentiation among populations it is required that the traits are polymorphic. This means that there are different forms of one trait. Human eye color among Nordic people is polymorphic while in many peoples from warmer parts of the world it is monomorphic. Usually there is a lower limit required for a trait to be classified as polymorphic. At frequencies below 1 % the trait is not regarded as polymorphic.

Different types of traits might be used to estimate the genetic structure:

quantitative or metric traits for which alleles in many loci usually affect the trait
morphological, one or a few loci involved
biochemical markers
DNA-markers

As discussed previously we cannot distinguish any classes in the progeny for a quantitative trait. Instead we have to carry out measurements to reveal the variation of such a trait. Alleles at different loci regulating a quantitative trait cannot usually be identified (see the discussion of QTL in Chapter 5). The majority of morphological traits are probably also affected by alleles at a large number of loci. Certain morphological traits such as chlorophyll mutants with white or yellow seedlings which usually die shortly after germination, "snake spruce phenotype" with strongly reduced branching, and "strawberry spruce phenotype" in which the young shoots are red during a short period, are all examples of traits that probably show monohybrid segregation.

As the name suggests, the alleles regulating biochemical markers can be identified. The groups of biochemical markers that have most frequently been used are the so called isozymes. In scientific literature they are frequently referred to as allozymes. A pair of isozymes may differ in one single amino acid, which often leads to a difference in their electric charge. If so it is possible to separate them after migration on an electrophoresis gel with an applied voltage (electrophoresis). Subsequently the bands of enzymes in the gel are stained by certain chemicals. In forest genetics, isozyme research was mainly introduced during the sixties. A limitation with isozymes is that only a few enzymes can be stained histochemically so that the number of isozyme loci in a tree genome that can be studied seldom exceeds 25. Isozymes analyses therefore only give a rough estimate of the total genome.

DNA markers are mainly obtained by cleaving the DNA into smaller segments that can be distinguished by *e.g.* gel electrophoresis. DNA can originate from the cell nucleus, nuclear DNA, or from mitochondria (mtDNA) or plastids (cpDNA). There are different techniques to cleave and analyse DNA.

RFLP (Restriction Fragment Length Polymorphism) is the first DNA technique that produced markers. The name derives from the use of so called restriction enzymes for cleaving DNA (see Chapter 2). This technique is rather laborious and it does not limit the DNA analysis to coding regions only. For Scots pine and Norway spruce it is estimated that the coding part of DNA is only 0.5 % of the nuclear DNA. It is likely that the ratio between coding and non-coding regions in other conifers is of a similar magnitude. Theoretically a large number of fragments might be identified with this technique. RFLP is a codominant marker, which means that both alleles at a locus can be detected.

Unlike the RFLP technique the RAPD technique (Random Amplified Polymorphic DNA) is faster and it does not require work with radioactive labelling. It is not possible to separate coding from non-coding regions of DNA. The PCR technique is used for amplification of the DNA segments. This technique has come into frequent use in forensic applications. A disadvantage with RAPD is that the segments amplified are dominant. Therefore, it is not possible to discern if there is any difference between two homologous chromosomes as regards a particular segment.

AFLP (Amplified Fragment Length Polymorphism) is a more recent (1995) type of DNA marker where certain DNA segments are amplified by the PCR technique. AFLPs are dominant and coding segments cannot be identified. A larger number of polymorphic fragments can be obtained than with RAPD. This means that genetic linkage maps obtained from AFLP are of higher quality than those obtained from RAPD. This is attributed to close location of the AFLP markers, which gives a so-called high density map.

Microsatellites (SSRs simple sequence repeats) are regions of DNA containing short segments (2-6/8 base pairs) replicated after each other a variable number of times. Such replications are called tandem repeats. They occur all over the genome, mainly in non-coding regions of DNA. A very large number of variants occur which makes them useful for identification of single individuals. Therefore, they are also very useful for studies of gene flow among populations.

The methods for cleaving DNA from mitochondria and chloroplasts do not differ from those for nuclear DNA. Unlike nuclear DNA, mtDNA and cpDNA are not very polymorphic.

EST (Expressed Sequence Tag) is a partial cDNA sequence, *i.e.* a sequence within the coding region of a gene. ESTs are used for recognizing active genes in a tissue and may also be used for constructing comparative genetic maps of conifers. They can, for example, be labelled and used as probes for RFLP.

Recently a large number of single nucleotide polymorphisms (SNPs) distributed throughout the human genome

Table 7-1. Schematic summary of the possibilities to identify population differences and single genotypes using different traits.

Type of trait	*Differentiation of populations*	*Identification of single genotypes*
Metric	significant for traits of adaptive value	non-existent
morphological	insignificant	insignificant
single isozyme locus	insignificant	insignificant
simultaneous analysis of many isozyme loci	limited	the more loci the better
Nuclear DNA:		
RFLP	limited	significant
RAPD	limited	significant
AFLP	limited	significant
EST	limited	insignificant
SNP	limited or significant depending on number	depends on number examined
Microsatellites*	limited	highly significant
Chloroplast DNA	limited – when inherited paternally significant - when inherited maternally	the more loci the better
Mitochondrial DNA	significant	the more loci the better

* If several microsatellites are identified for cpDNA or mtDNA they have the same characteristics as nuclear microsatellites

have been mapped. They will be used *e.g.* in studies of human population genetics. Their role in forest genetics is under investigation.

In Table 7-1 we present our opinions about the usefulness of different traits for estimates of among-population differentiation and for identification of individuals.

Metric traits are superior when there is an interest in revealing differentiation as a result of adaptation to various environmental conditions. This is particularly the case if natural selection played the major role for the present population structure. The overwhelming majority of markers are neutral, which means that they are not affected by natural selection. The possibilities of detecting differences are limited if few markers are available but increase with higher numbers of markers. If there is linkage between a marker and traits of value for adaptedness it is possible to detect differentiation for markers, too. The higher the number of marker loci analysed, the greater the probability that some marker loci are linked to loci affecting adaptive traits. Neutral markers may therefore reflect previous adaptation.

The assumptions given in Table 7-1 as regards such metric traits as growth rhythm, survival, and tree growth or isozymes may be analysed using available data for Norway spruce and Scots pine. Growth rhythm is the point of time for onset of growth during spring and cessation of growth during autumn. These points of time are important for avoidance of exposure to late spring frosts or early autumn frosts. All isozyme studies show a much smaller differentiation than for the adaptive traits mentioned above. It is worth mentioning that the statistical technique used for markers is less precise than for metric traits. This means that the differences might be underestimated. In spite of this it is evident that studies of isozyme variation and variation in metric traits give different types of variation pattern. The variation we observe for neutral markers may be attributed to linkage as mentioned above or to the fact that it takes some generations to reach equal allele frequencies in different populations via gene flow.

Even if metric traits well reflect past adaptation in populations they are more or less useless for genetic identification of individuals. This is because many alleles at many loci affect a quantitative trait, each allele contributes little.

As with identification of a human father using markers, the genetic identification of a tree is facilitated if numerous markers are available. Microsatellites with their hyper-variable DNA seem to be the best choice for such identification.

Since mitochondrial and chloroplast DNA provide only a few markers, they are not particularly well suited for genetic identification of individuals. They have, however another characteristic, that they are transmitted to the progeny via one parent only. In angiosperms they are transmitted by the female while in conifers the male transfers the chloroplasts to the progeny. This makes it possible to distinguish gene flow via pollen from that via seeds. Usually the dispersal is faster via pollen than by seeds, acorns, or nuts.

The development of the so-called neutral theory was one consequence of the results of isozyme research. According to this theory most of the molecular changes in DNA are selectively neutral and their future existence in a population depends on genetic drift. The neutral theory is not accepted by all geneticists. The probability for loss of a molecular change in the genetic code per generation is much higher [$(2N_e/N)\ln(2N)$] than for fixation of the change ($4N_e$). N is the total number of trees while N_e is the effective population size. An example will be used to illustrate this. If N = 100 and N_e = 80, the probability for loss is only (2 x 0.8 x 5.08) ≈ 8 generations while the time to fixation is (4 x 80) = 320 generations.

The conclusion of this discussion is that neutral changes in the genetic code and changes of amino acids (isozymes) are suitable for phylogenetic determination while they are much less suitable for determination of adaptive variation. To investigate adaptation, studies of traits that influence fitness are required. In next section we will present results obtained for quantitative traits and markers studied in the same populations.

Comparison of markers and quantitative traits

In 1984 the American geneticist Richard Lewontin carried out an analysis of the discrimination power of markers, such as isozymes, and quantitative, *i.e.* metric traits. To have the same discrimination power as the metric trait, this trait must not be regulated by more genes than given by the ratio: $1/h^2$. If the heritability of a quantitative trait is 0.2 the markers will have the same discrimination power as the quantitative trait if the latter is regulated by no more than five loci. Since it is expected that more than five loci regulate most quantitative traits, it is anticipated that isozymes and many other markers show lower differentiation among populations than quantitative traits. A few examples of estimates of population differentiation for markers and quantitative traits in the same populations are shown in Figs. 7.1-7.5.

The first example concerns a Canadian study of *Pinus contorta*, in which 2 growth traits and four quality traits were compared with isozymes (Fig. 7-1). Except for branch angle all quantitative traits had much larger $\mathbf{Q_{ST}}$ estimates than the $\mathbf{F_{ST}}$ value for isozymes. The interpretation of this is that isozymes and branch angle seem to be neutral traits, which are not changed by natural selection whereas the rest of the traits are strongly affected by natural selection.

Growth rhythm, such as budburst and budset, is extremely important for northern tree species. In a Finnish investigation with *Pinus sylvestris* populations, originating from entire Finland, 34% of the variation in budset of *Pinus sylvestris* was attributed to population differences while

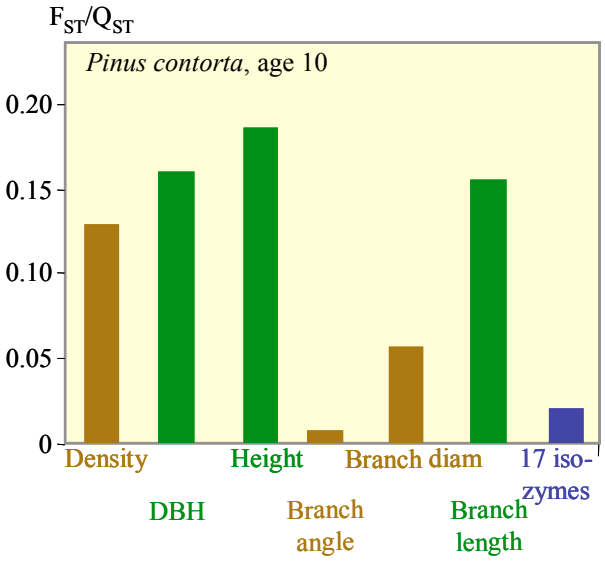


Figure 7-1. Comparison of population differentiation estimated by quantitative traits and isozymes in Pinus contorta.

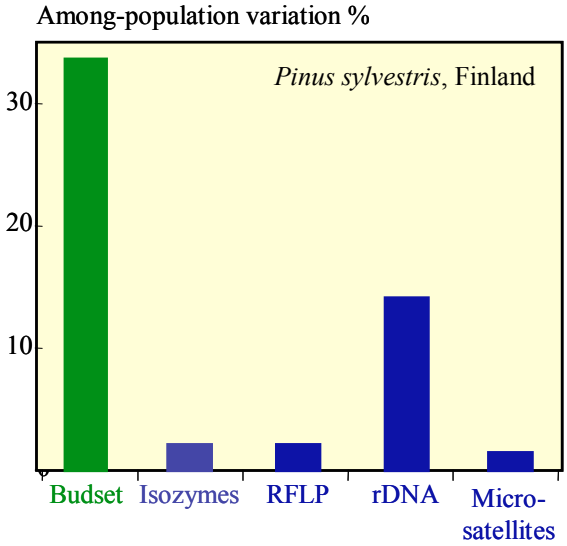


Figure 7-2. Comparison of population differentiation estimated by various markers and one quantitative trait, budset, in Finnish populations of Pinus sylvestris.

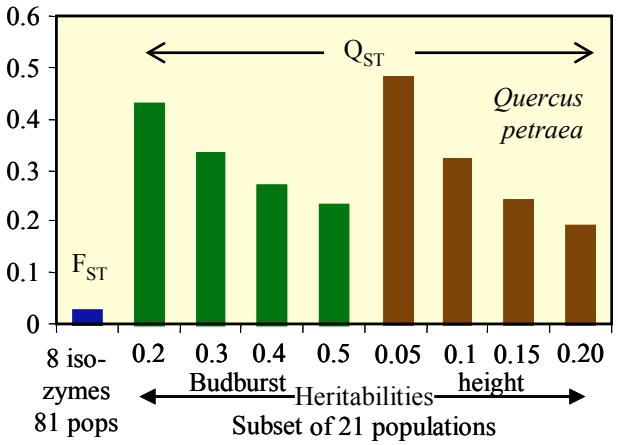


Figure 7-3. Differentiation estimated for isozymes, budburst, and height of Quercus petraea *populations. The quantitative trait estimates are given for the range of heritabilities usually found in this species. The isozyme analysis comprised a larger number of populations than used for the two quantitative traits.*

markers such as isozymes, RFLPs, and microsatellites showed limited population differentiation in agreement with expectation that they are neutral (Fig. 7-2). Ribosomal DNA (rDNA) took an intermediate position. However, no geographic differentiation was noted for rDNA.

In France oak species have played a great role in forest genetics studies. In one case population differentiation by isozymes was compared to the $\mathbf{Q}_{ST}$ for two quantitative traits, budburst and height in *Quercus petraea*. As outlined in chapter 4, $\mathbf{Q}_{ST}$ is dependent on the heritability of the trait under study. Heritability is a term in the denominator of the equation used to calculate $\mathbf{Q}_{ST}$. $\mathbf{Q}_{ST}$ estimates for the range of heritabilities noted for budburst and height are illustrated in Fig. 7-3. It is evident that the population differentiation estimated by isozymes is

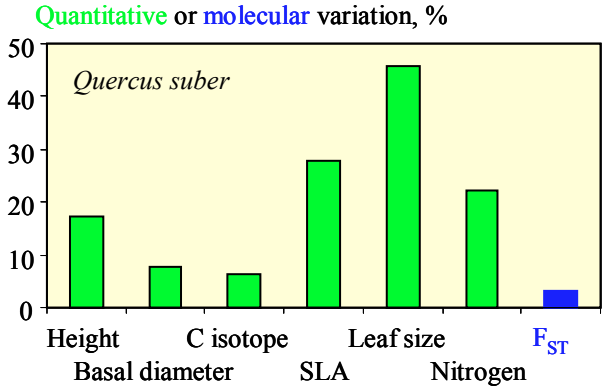


Figure 7-4. Estimates of the differentiation among populations for six quantitative traits and for microsatellites (F_{ST}) among 13 Spanish cork oak populations. The populations were selected to be representative for the distribution of cork oak in Spain. Carbon isotope is considered as an estimate of water use efficiency; SLA = specific leaf area (cm^2/mg); nitrogen stands for nitrogen content in the leaves. Data from assessments at age 9.

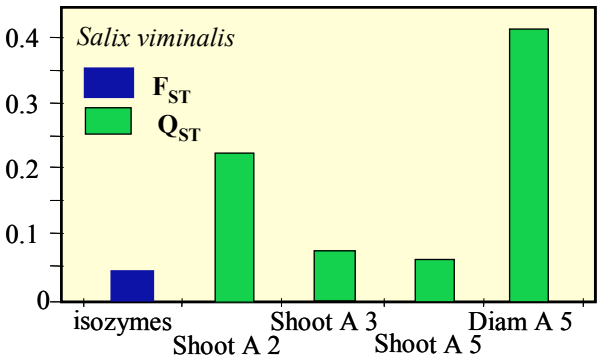


Figure 7-5. Differentiation estimated for isozymes, number of shoots at ages (A) 2, 3, and 5, and stem diameter of Salix viminalis *populations.*

several times lower than for the two quantitative traits even at the highest heritabilities. It should be remarked that populations included in the isozyme study originated from a wider range than the populations included in the study of budburst and height growth. This means that the differences between the two types of traits, markers and quantitative traits, were probably underestimated.

In some cases microsatellites show lower differentiation than is observed for quantitative traits, as is evident from the Spanish cork oak (*Quercus suber*) study (Fig. 7-4).

The shrub species *Salix viminalis* has attracted much interest in Sweden as a source for energy production. Even in this species, which differs considerably from the long-lived tree species, it is evident that $\mathbf{Q}_{ST}$ estimates are higher than the $\mathbf{F}_{ST}$ estimate for isozymes (Fig. 7-5). It should be noted that the drop in $\mathbf{Q}_{ST}$ for number of shoots at ages 3 and 5 may be explained by the increased competition in the field trial and its accompanying increase in heritability.

One example of a study with higher population differentiation of markers than for quantitative traits will be given. *Cedrela odorata* is a Central American tree species growing from Mexico to Panama. Population differentiation in this species was studied via chloroplast DNA, nuclear AFLP, and 17 quantitative traits, both growth and morphology traits. Most populations, 26 of 29, were monomorphic for cpDNA. A consequence of such a high frequency of monomorphic populations is that high $\mathbf{G}_{ST}$ estimates are expected. In agreement with this expectation, $\mathbf{G}_{ST}$ was estimated at 0.96 while the $\mathbf{Q}_{ST}$ estimate was much lower, 0.34. The growth traits are most probably of adaptive significance, whereas this is uncertain for the leaf shape traits. The $\mathbf{Q}_{ST}$ may be somewhat higher if only truly adaptive traits are included in the derivation of this parameter. AFLP was analysed for Costa Rican populations only and the differentiation was estimated at approximately 83%. These data suggest that many neutral substitutions have taken place in DNA without a corresponding change in adaptive and morphological traits.

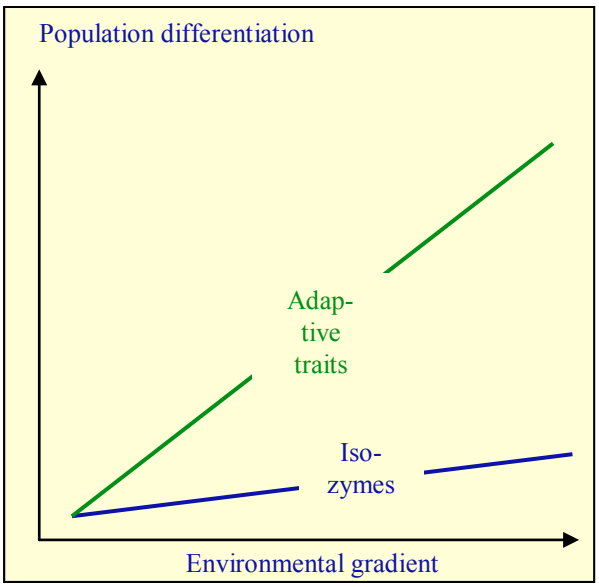


Figure 7-6. Expected population differentiation along an environmental gradient estimated by isozymes or adaptive traits.

In conclusion, population differentiation estimated by isozymes is several times lower than for traits that are of adaptive significance. Along an environmental gradient, clines for adaptive traits are expected to be steeper than clines for isozymes (Fig. 7-6). This agrees with the assumption that isozymes are neutral markers.

Variation among populations in metric traits

Most of the information about among-population variation derives from provenance research, which has played a great role in forest research. One definition of provenance is a population or group of individuals of the same species occurring within or originating from one more or less rigorously defined geographic area. The important thing is that seeds were harvested from a geographically identified area. It should be noted that the term provenance is not always identical with the term origin. Thus the seed of *Pinus contorta* harvested in the province of Lapland in Sweden is provenance Lapland although this species originates from North-America. Therefore, provenance experiments contain genetic entries whose seeds were collected in geographically different localities and should represent a much larger area than an individual stand. Even in those cases where the seed collection is limited to one stand within a provenance, the experiments are usually referred to as provenance experiments. Population would be a more accurate designation when seeds are collected in individual stands.

Provenance trials generally comprise a large number of provenances (populations) from geographically widely separated areas. Mostly such experiments are located at a series of test sites. Thus, most provenance experiments are a part of a series of experiments.

During an international conference in 1965, provenance researchers agreed on the requirements that should be fulfilled by provenance tests. Each provenance should be represented by progenies from at least 20 trees but preferably from 50 trees. The following objectives were also agreed upon:

1. The primary objective of provenance research is applied, concerned with identifying the provenances giving the highest value production within a certain area.
2. There is also a scientific objective of provenance research to trace the adaptation that has taken place as well as the environmental factors that have influenced the adaptation.

As soon as the identification of the best provenance(s) has been carried out, the best stands within the provenance area should be selected for seed harvesting. This is a complicated task since we frequently neither have access to the history of the stand nor to the silviculture applied within a seed tree stand under scrutiny. For approval the stand should be of such an age that an evaluation of tree quality could be carried out. Moreover, the stand should have such a size that selfing is unlikely. Generally, seed harvesting is only carried out in stands fulfilling certain phenotypic standards. Stands in which segregation of phenotypically inferior trees occurs are excluded since this suggests that vitality-reducing alleles occur in such a stand. In many countries a federal organisation approves stands for seed harvests. Sometimes this approval is also given to stands in other countries, from which imports can then take place.

Pinus sylvestris and *Picea abies* provenance research

Already during the early part of the 1900s it was clear to Central European researchers that there was a large variation among populations of Scots pine and Norway spruce. The Austrian forest researcher Cieslar concluded that *the physiological varieties were hereditarily adapted to the length of the vegetation periods in their respective native habitats*. Based on their experiments, both Cieslar and his contemporary colleague from Switzerland, Engler, were aware that there was a continuous variation of Norway spruce and Scots pine from north to south and from valley bottoms to high elevations.

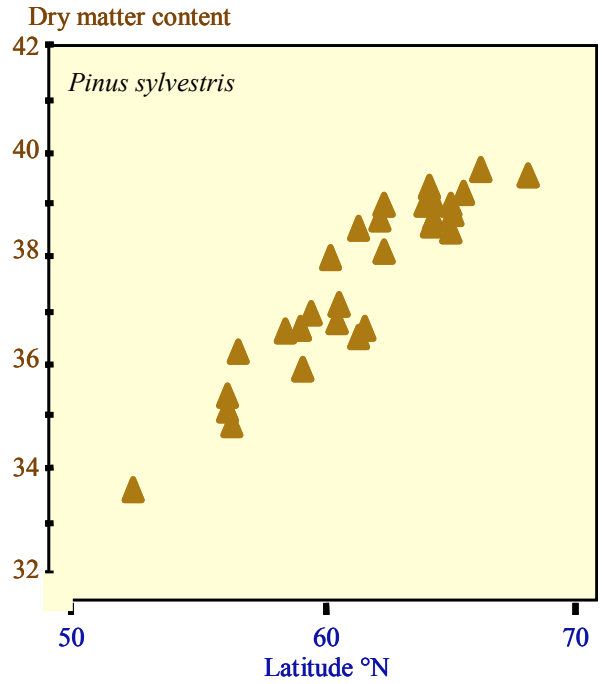


Figure 7-7. The relationship between dry matter content and original latitudes of Pinus sylvestris *populations at a certain date during inwintering.*

A pioneer achievement was that of the Swedish forest geneticist Olof Langlet who presented his thesis *Studies on the physiological variability in Scots pine and its relationship with the climate* (translated from German) in 1936, a publication that has attracted much international attention. Langlet demonstrated that the dry matter at a certain point of time during the autumn varied continuously from south to north in Sweden (Fig. 7-7). The dry matter content reflects the degree of hardiness obtained in a certain material. Thereby, the frost tolerance attained is indirectly revealed. Langlet was probably the first to introduce replications in provenance trials.

The Swedish forest researcher Gunnar Schotte, who worked in the early 20th century, was probably the first who established real provenance trials in northern Europe, starting in 1904. It took a few decades before forest researchers were aware of the need for establishing experimental plantations with replications. In spite of this, his results give us some guidance about survival and yield of different provenances. His pioneering work was followed by others and during the 1930s it was evident to provenance researchers that the local Scots pine in northern interior Sweden did not have satisfactory survival. Some researchers even observed that there was a large variation within a provenance as well. It was not until the 1960s that foresters in Sweden realised that Scots pine seed transfers to the south must take place in the northerly harsh areas of Sweden to get satisfactory regeneration. The credit for this must be given to Vilhelms Eiche who during the late 1940s carried out a country-wide collection of seeds in approximately 100 stands for establishment of a country-wide experimental series of provenances. The series contains a few non-Swedish populations as well. This series of provenance trials differs from conventional ones by including different provenances in different test plantations. Eiche's intention was to evaluate the effect of transfer on the provenance performance. He included different transfers in latitudinal (to north or south) and altitudinal (up or down cf Fig. 7-8) direction. Evidently trials close to the timber line or to sea level could not have all possible transfers. Each provenance in this series is represented by open-pollinated progenies from 20 trees per stand which makes it unique. Thanks to Vilhelms Eiche we have good knowledge not only about effects of transfer but also about variation within each population for a large number of traits.

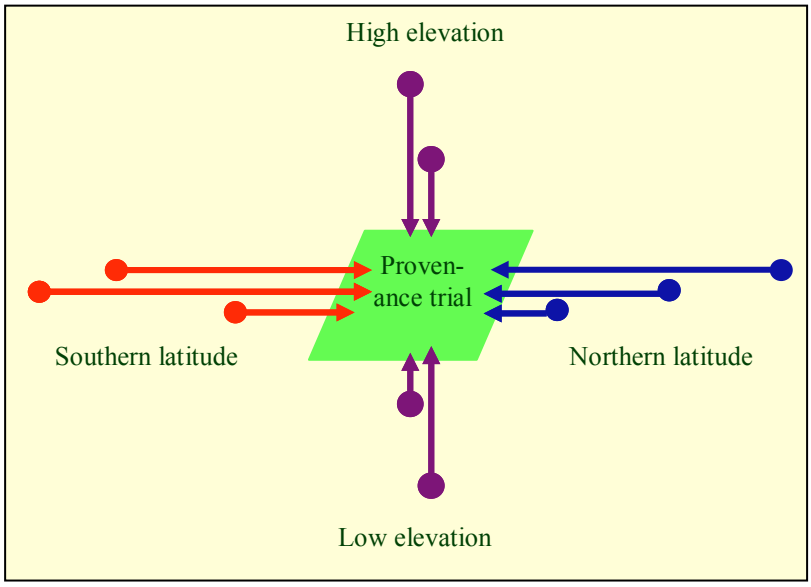


Figure 7-8. The principle for testing of transfer effects – north-south, upwards-downwards, in the Eiche series of Pinus sylvestris *provenance trials.*

Picture 7-1. A picture from one of the trials in the Eiche series. The area indicated with the encircled dead trees was planted with material originating far south of this trial. Photograph Vilhelms Eiche.

Picture 7-1 gives an indication of the large mortality in a population transferred in northern direction. For the northern part of Sweden the results as regards survival agree extremely well within the provenance series established by Eiche.

Transfers to a northern test locality reduce survival while an opposite transfer improves survival (Fig. 7-9 and 7-10). Fig. 7-9 reveals that the mortality in the best populations was above 40% at this harsh site. Data from the series established by Eiche suggest that one degree of latitudinal transfer causes a change in survival of approximately 10 percentage units while a change of 100 meters in elevation gives a change of approximately 3 percentage units.

The building up of frost hardiness in two northern (latitudes 66.42 and 67.50°N and two southern Finnish populations (60.42 and 61.67°N) of *Pinus sylvestris* was studied at an age of 60 years. There was a clear difference between the two origins, northern and southern (Fig. 7-11). No difference in dehardening during spring between these two origins was observed. The difference in hardening between northern and southern populations was interpreted as a difference in night length triggering onset of hardening. Besides, the variation in hardening between years suggests that temperature also influences the triggering of hardening.

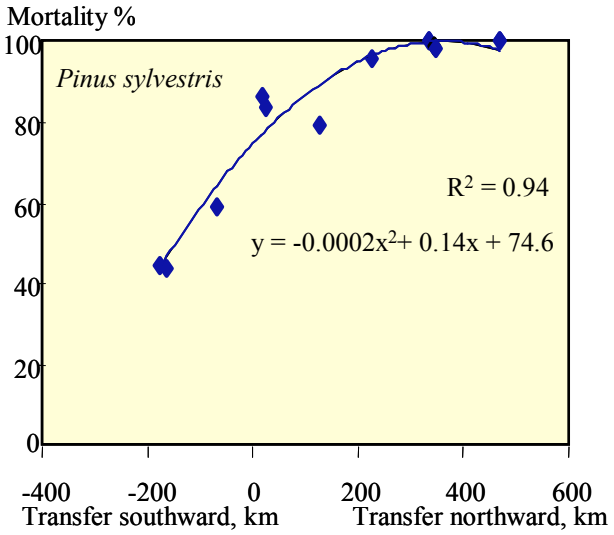


Figure 7-9. The percentage mortality of transferred Pinus sylvestris *provenances at one of the harshest trials located at latitude 66°16', 440 masl.*

Fig. 7-12 is an illustration of the impact of transfer on yield per hectare from the largest experimental plantation in the series established by Eiche at 400 masl close to latitude 64°. As seen from this figure a long transfer southwards seems to give the best yield in this plantation. However, another Swedish study of juvenile material did not show any effect of altitudinal transfer southwards.

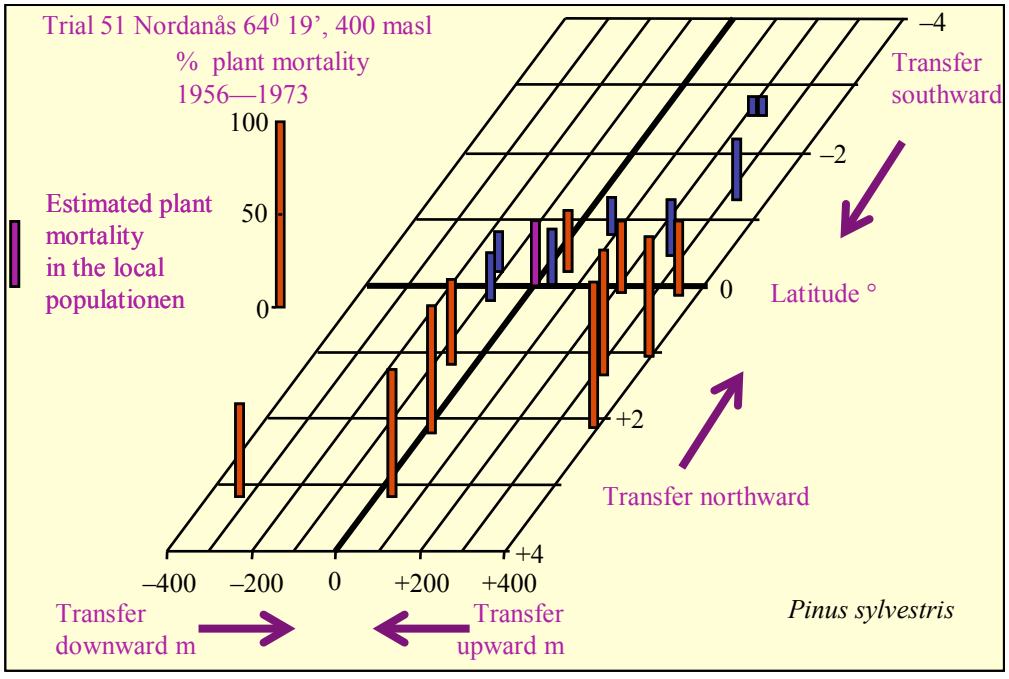


Figure 7-10. The percentage tree mortality in a Pinus sylvestris *provenance trial at latitude 64°19', 400 masl. The position of the bars indicates the transfer in latitudinal and elevational direction. The provenances above the horizontal 0-line were transferred to the south and provenances to the right of the diagonal 0-line were transferred upwards.*

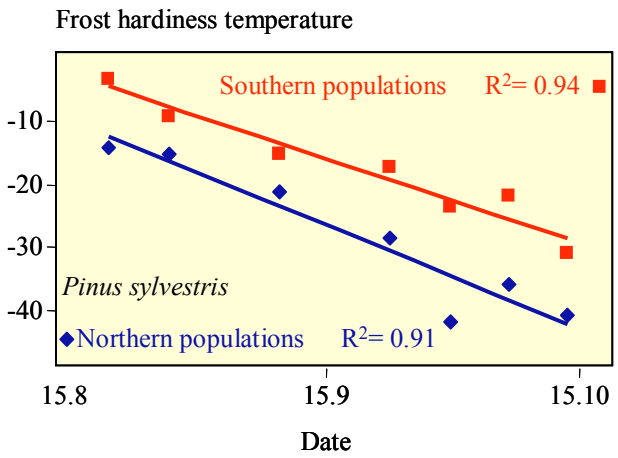


Figure 7-11. The relationship between frost hardiness in pairs of Pinus sylvestris *populations, northern originating from latitudes 66.42 and 67.50°N and southern originating from latitudes 60.42 and 61.67°N. When 50% of the seedlings lacked symptoms it was classified as hardy.*

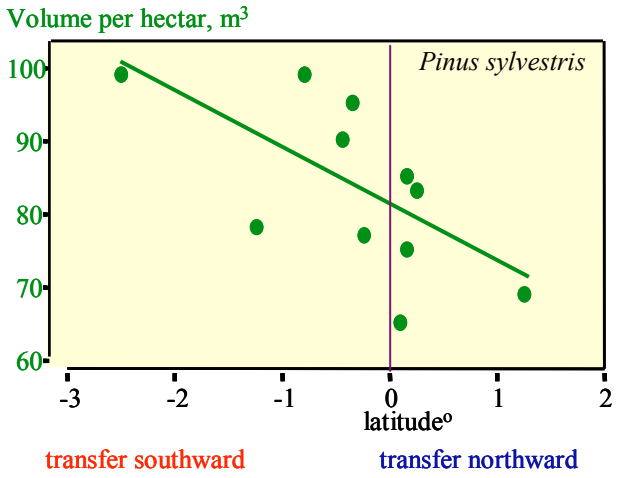


Figure 7-12. The relationship between volume per hectare and latitudinal transfer of Pinus sylvestris *provenances in a trial at latitude 64°19', 400 masl.*

We have used the results from the provenance trials to map biologically the harshness of individual test plantations. In other words we can use the results to map Sweden biologically with respect to Scots pine hardiness to provide a severity index (Fig. 7-13). Severity index is the expected plant mortality in per cent of the local population 20 years after establishment of the test plantation. The reason for using such a high age as 20 years for establishment is that the results have shown that it may take 20 years until full knowledge about hardiness can be reached. The reason for the poor survival of the local population at high latitudes and a few hundred meters

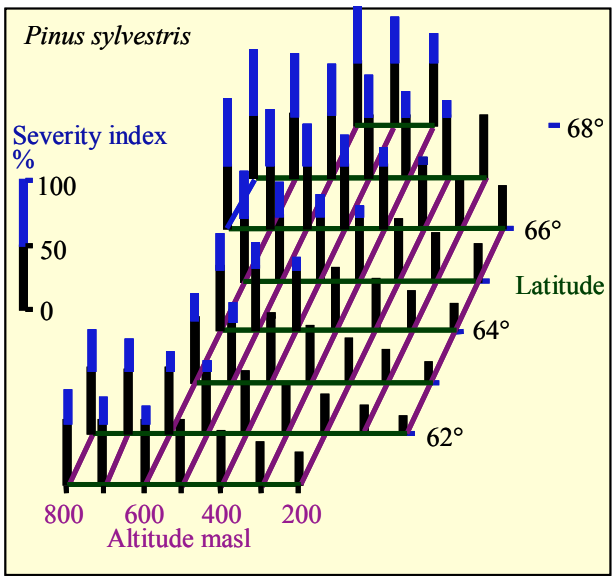


Figure 7-13. Severity index of Pinus sylvestris *provenances in northern Sweden. Severity index is an estimate of the plant mortality in the local population during the first 20 years after planting. The estimate is derived from results of provenance trials within this area.*

above sea level will be discussed in the section *Darwinian fitness and domestic fitness* below.

At an age of 20 or more it has been possible to get information about stem quality traits in the provenance trials. Also for this kind of trait there are large differences among provenances. From the series established by Eiche we know that transfers to southern test localities increase the number of high quality trunks. The effects of transfer are most pronounced at southern hilly plantations and northern low-level plantations.

In 1992 the Polish scientists Giertych and Oleksyn published an historical paper on early international provenance trials with *Pinus sylvestris*. There were many failures over the years to get coordinated assessments and evaluations of the various series. They suggested a useful way to overcome that problem of different assessments and evaluations by standardising data in each trial. Thus, a population performing well in a trial will have a positive standard deviation in this trial. If it has positive standard deviations in most trials it is a population that may be recommended for cultivation all over the range where it was tested. Such an evaluation was carried out for an international *Pinus sylvestris* provenance series from 1982. This series contained populations from lat 40°N, 1400 masl to 60.25°N, 80 m asl. The trials in this series was established from lat 45.55°N, 210 masl to 53.2°N, 160 masl with most test localities in a narrow latitudinal range in Poland and Germany, 49 - 53.

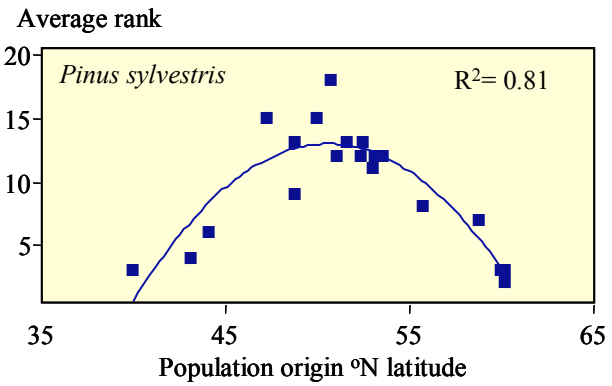


Figure 7-14. The relationship between average rank based on data from 7 (8) test localities and latitudinal origin of the tested Pinus sylvestris *populations. A high rank means good performance.*

As seen from the graphic illustration on ranks in Fig. 7-14, the northern and southern populations mostly performed poorly in the seven or eight test localities included in the evaluation. The number of test localities included in the evaluation differed in the paper. The authors were aware that this series gives limited clues to selection of populations in regions in which photoperiod plays a great role, such as Scandinavia. Provenance trials in north eastern Germany indicated that Polish and German populations showed superior growth.

The effect of longitude on performance was studied in two Russian field trials lat. 53.77ºN long. 82.33ºE, and lat. 50.67ºN long. 79.33ºE. The relationships between longitude and plant survival in these two trials were weak (R^2=0.06 and 0.17). The absence of any strong relationship between longitude and survival cannot be explained by the size of the trees. The critical phase in Sweden occurs at a stage when the trees are taller than the snow cover. The trees in these two trials must have passed this long ago since the mean heights were above eight meters and 2.6 meters, respectively. Both figures must be regarded as surprisingly good growth for the ages. There was no strong tendency to dependence on longtitude for height growth (Fig. 7-15).

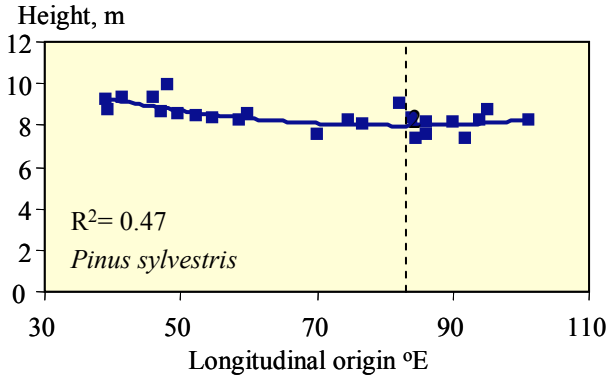


Figure 7-15. The relationship between longitudinal origin and tree height at age 17 in a Pinus sylvestris *trial located at* lat. 53.77ºN long. 82.33ºE. *The longitude of this trial is indicated by the dashed line.*

Picture 7-2. Rows of Picea abies *provenances showing large variation in budburst. Photograph Peter Krutzsch.*

Also for *Picea abies* there are provenance trials in Sweden over half a century old that have given us useful information about provenance variation as regards growth and yield. In Fig. 7-16 the results are summarised from one of the test plantations in the largest Norway spruce provenance series in Europe. Neither in this case does the local provenance give the best result with respect to survival or growth. In Sweden, Norway spruce should be transferred to the north to utilise the growth potential of this species to the full extent. The further to the north the shorter the transfers should be. The evolutionary explanation for the inferiority of the local provenance will be treated in the section *Darwinian fitness and domestic fitness* below.

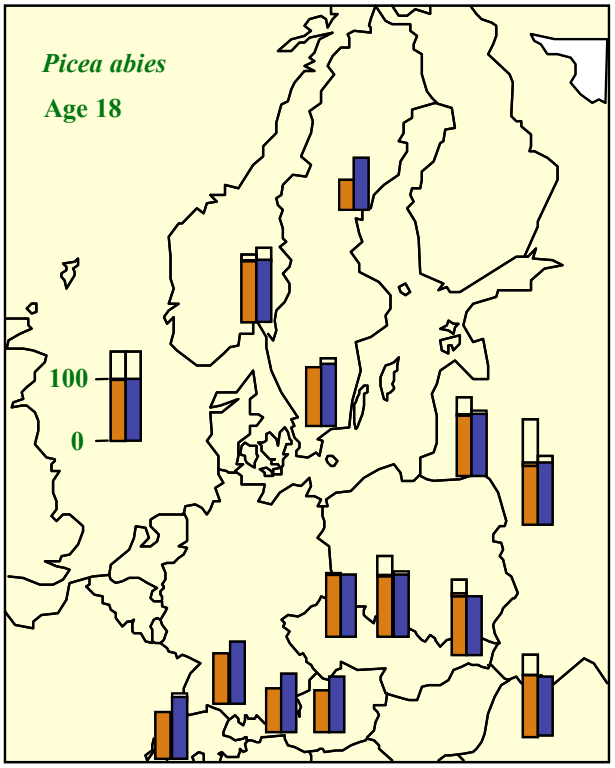


Figure 7-16. The relative stem volume and survival in a Picea abies *provenance trial with the experimental mean = 100. The size of the empty bars illustrates the superiority of a certain geographic region as compared to the experimental mean.*

Figure 7-17. The variation in number of days for bud burst of different Picea abies *provenances; the earliest flushing provenance was given the value 1. The green area shows provenances with at least 2 weeks later bud burst than the earliest. Results from a Swedish nursery trial at latitude 59.67°N 10 masl.*

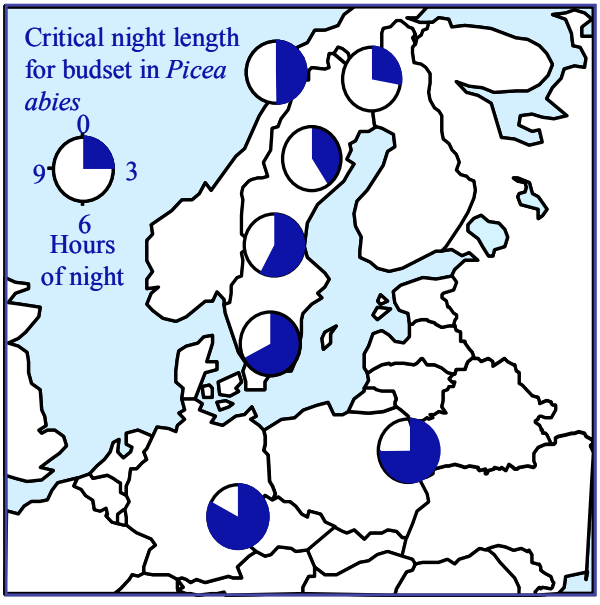


Figure 7-18. The critical night length for apical budset in Picea abies *provenances studied in growth chamber. Critical night length is the night length when 50% of the plants set buds.*

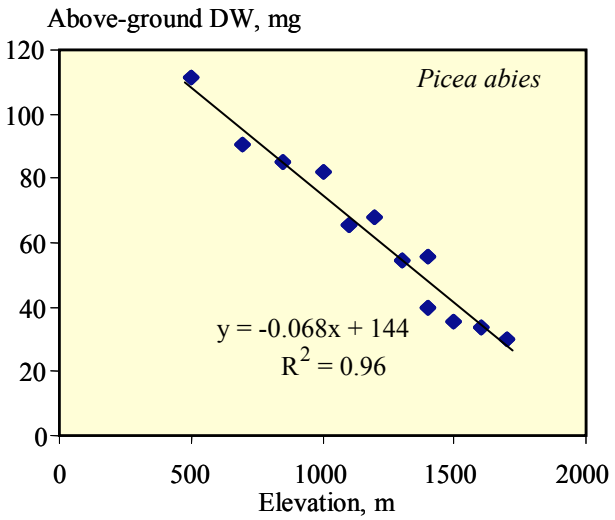


Figure 7-19. The relationship between Picea abies *population origin and above ground dry weight along an elevation transect in Austrian alps.*

For Norway spruce the timing of budburst in spring is extremely important for the adaptedness to the weather conditions at the reforestation site. For Norway spruce and many other tree species at high latitudes it is important that they do not start their growth too early during spring to avoid late spring frosts (Picture 7-2). For timing of budburst there is a large variation among provenances (Fig. 7-17). Budburst time is mainly regulated by temperature. Northern populations require a lower heat sum than southern populations for budburst It is also important to attain hardiness before the early autumn frosts appear. Apical budset is a trait fairly well correlated with hardiness and can be used to get an estimate of the degree of hardiness in a material. The onset of inwintering and thus building up of hardiness is mainly triggered by the night length. For this trait there are large differences among provenances. Northern populations require a shorter night length for onset of this process than southern populations (Fig. 7-18). Pronounced altitudinal clinal variation was observed for populations from the Alps (Fig. 7-19).

Both for budburst and inwintering it is evident that adaptation to the climatic conditions at the sites of origin has played a major role for the observed differences. For northern and high elevation populations it might be advantageous to respond rapidly to warm weather during spring to make use of the short summer conditions prevailing at high latitudes. To avoid early autumn frost exposure it is important that northern populations respond to short night lengths for building up of hardiness. Both budburst and the critical night length for budset display clinal variation from north to south in Scandinavia.

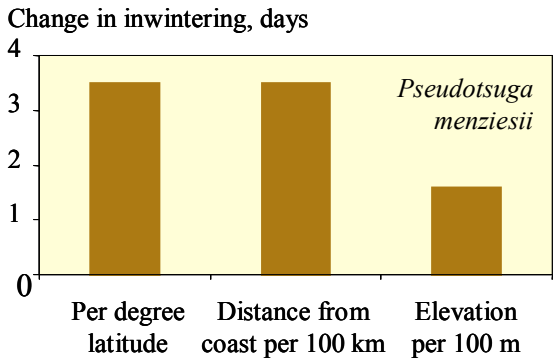


Figure 7-20. The impact on different geographic variables on inwintering in Pseudotsuga menziesii *studied in a German nursery.*

Provenance research in some other conifers

Most North American conifers show clinal variation in agreement with the observations for Scots pine and Norway spruce in northern Europe. *Pseudotsuga menziesii* (Mirb.) Franco, Douglas fir, and *Pinus contorta*, lodgepole pine, are of importance both in their native countries and Europe. Douglas-fir is well-known as being one of the most important timber trees in the world, often marketed as 'Oregon pine'.

Pseudotsuga menziesii is native to western North America and ranges from scattered populations at 19°N in Mexico to latitude 55°N in British Columbia. The longitudinal range is from 97°W to 128°W. Douglas-fir also occurs over a large variation in altitude, from sea level to 1,700 m in the coastal range and up to 3 300 m in the interior range.

The extremely broad distribution range of Douglas-fir is reflected in the large genetic variation. The main limiting factors for adaptation are temperature in the northern range and moisture in the southern.

One example of steep clinal variation is given in Fig. 7-20 from a range-wide study in a German nursery. The German study agreed well with the results presented above. Thus, there is a 3.5 days difference in inwintering for each degree of latitude or 100 km from the coast. The altitudinal cline was less pronounced, amounting to 1.6 days difference per 100 meters. These three geographic variables explained approximately 90% of the observed variation in inwintering. Moreover, the results from inwintering correlated strongly with the winter frost tolerance obtained from artificial freeze testing.

Below we have summarised some major results from the two varieties separately.

menziesii populations	Characteristics
High altitude populations	early budset, low frost damage
Northern populations	early budset, low frost damage
Inland populations from dry localities	early budset, low frost damage , high frequency of a second flush

glauca populations	Characteristics
High altitude populations	least autumn frost damage, early budburst and late budset
Inland populations from dry localities	high frequency of a second flush
Interior northern	infrequent second flushes

The results from Douglas fir reveal a more complex relationship with geographic variables than the simple relationship between latitude and growth rhythm traits in Scandinavia. In Scandinavia the climate mainly varies with latitude whereas the degree of continentality disturbs such a simple relationship between latitude and climate in North America.

Pinus contorta has played a prominent role in Swedish forestry during the last half of the 20th century. For this reason many provenance trials were established in Sweden north of latitude 60°. The results from these trials indicate large differences among populations, which are expected considering the wide distribution of lodgepole pine in North-America. One way to estimate the usefulness of a provenance is to multiply the percentage survival by a growth trait such as height or stem volume. This is particularly useful for areas in which survival of many provenances is unsatisfactory. In Fig. 7-21 the relationship between the product, *survival x mean stem volume,* and the latitudinal origin of the provenances is shown. In the lower part which deals with data from a southern test locality there is no problem with survival. The southern populations, which respond to a longer night for growth cessation than northern populations, give rise to taller trees. As we approach harsher conditions, the % survival becomes the most important component of the product and northern provenances are superior to southern since

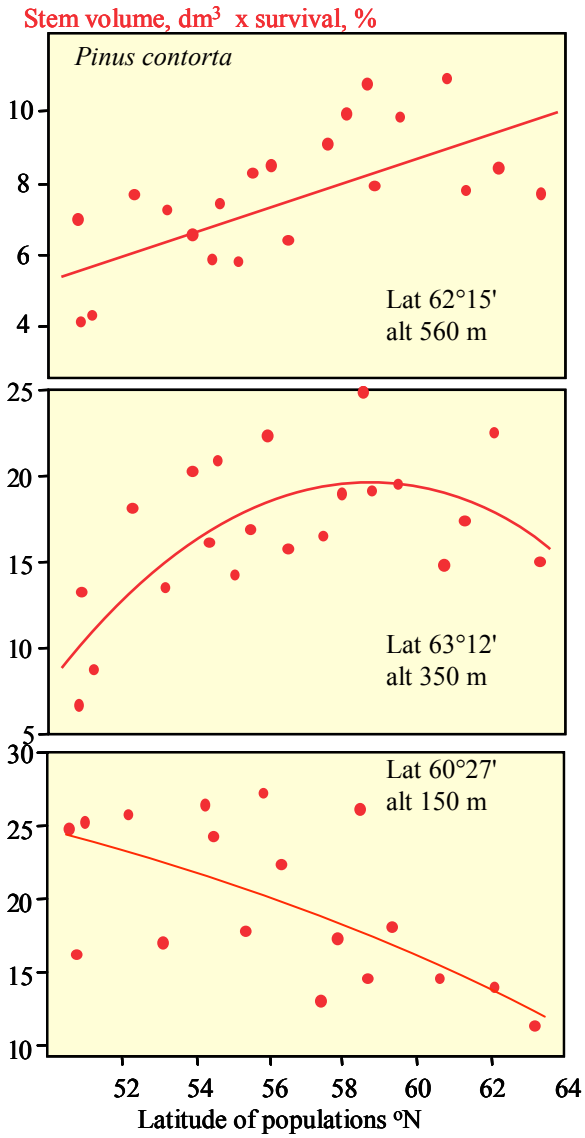


Figure 7-21. The relationship between the product – percentage survival x mean stem volume – and latitude of origin of Pinus contorta *provenances in three provenance trials with varying survival.*

the latter do not have a satisfactory survival. The results from lodgepole pine shown in Fig. 7-21 illustrate well that we have to weigh the survival against growth to reach an optimum yield per hectare. Moreover, the relative importance of these two traits changes from mild to harsh climatic conditions.

Sometimes freeze tests are used to assess the hardiness attained in a material during the process of inwintering. Plants are first grown under growth promoting conditions. After that a continuous night prolongation is applied. Freeze tests are then applied at certain intervals. If the freeze testing is carried out too early during the inwintering most plants will be severely damaged and if it is carried out too late, most plants have attained full hardiness. It is therefore of importance to carry out the freeze testing such that approximately 50 % of the plants

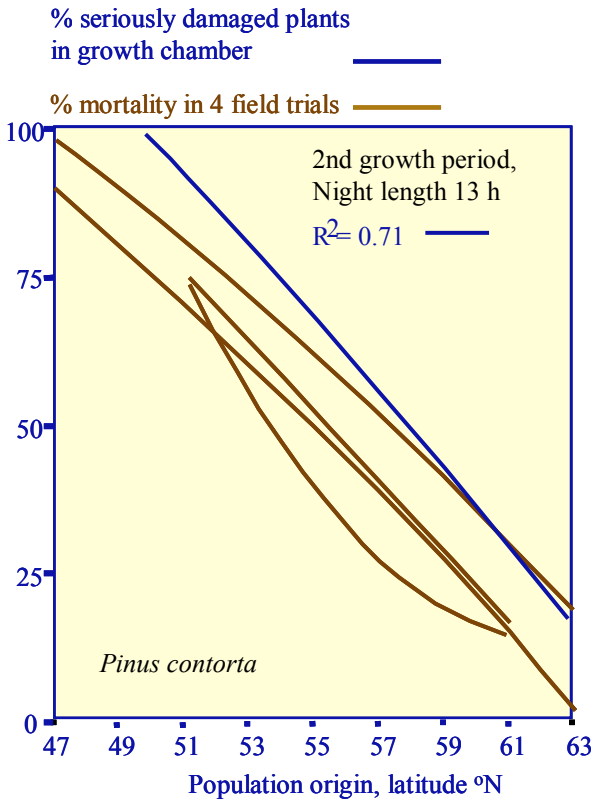


Figure 7-22. The relationship between severe frost damage and Pinus contorta *provenance origins after freeze testing in growth chamber as well as the relationship between tree mortality in four provenance trials in northern Sweden and* Pinus contorta *provenance origins. The relationships were estimated by regression technique.*

are severely damaged to obtain the best resolution with respect to hardiness in the material. Often the results are related to some environmental variable such as latitude or elevation. One example for *Pinus contorta* is shown in Fig.7-22, in which the percentage of severely damaged plants is plotted against the latitudinal origin of the provenances. Similar relationships between tree mortality in four field trials and latitudinal origin of provenances are also shown in this graph. The provenances included in the field trials and the freeze testing are not identical but they originate from the same latitudinal range in Canada. The agreement between the slopes of the five curves is good. This suggests that freeze testing well reflects the field mortality.

Another important issue is the susceptibility to pests and diseases of an introduced species such as lodgepole pine. Introduced species and populations are sometimes referred to as exotics. At the end of the 1980s there were certain weather conditions which caused severe attacks by the *Gremeniella abietina* fungus on lodgepole pine plantations in northern Sweden. Weather-conditioned damage has in many cases been the gateway for fungal attacks. To avoid attacks as much as possible it is important to use provenances with good hardiness.

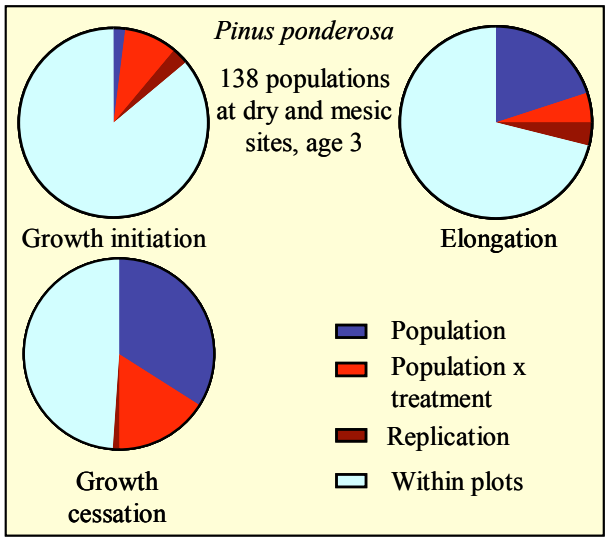


Figure 7-23. The population and population x test site variance components for growth initiation, growth cessation, and plant elongation in Pinus ponderosa *populations grown in two nurseries with differing water availability.*

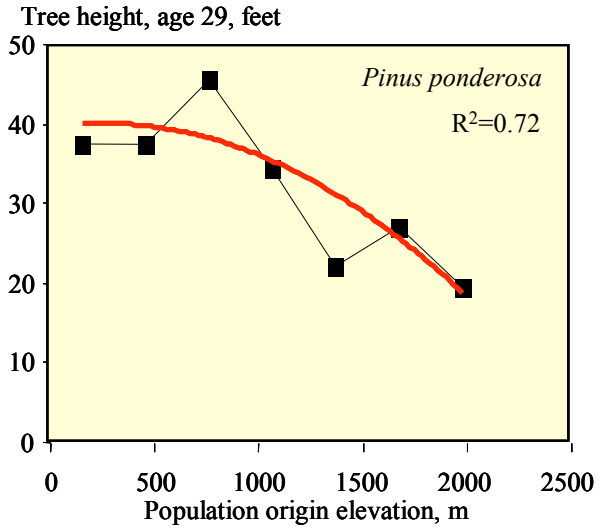


Figure 7-24. Tree height at age 29 of Pinus ponderosa *populations from a Californian elevation transect grown at a low elevation, 285 meters above sea level.*

Pinus ponderosa is an important tree species from western North America. In Fig. 7-23 the results from a study in two contrasting nurseries with a range-wide collection of populations are summarised. Thanks to the large number of populations it is possible to identify in an accurate way the importance of the origin - population effect - and of the interaction - population x site effect. As seen from Fig. 7-23 the population effect was stronger than the interaction for elongation and growth cessation in spite of the contrasting growth conditions in the two nurseries. The interpretation of these results is that there was strong natural selection for elongation and growth cessation during the past evolution of this species. In contrast, the growth initiation seems to have been less affected by natural selection in the past. In this respect *P. ponderosa* resembles *P. sylvestris*.

In Fig. 7-24 results on growth at 285 masl of *Pinus ponderosa* populations from an elevational transect in California are illustrated. As seen from this figure the same trend as in Scandinavia is observed, with poor performance of high elevation populations at a low test site.

Two north American species, *Pinus monticola* and *Pinus resinosa* differ from the general pattern of large provenance differences although they have a wide distribution area.

In *P. monticola* populations south and north of central Oregon in northwestern USA differ sharply. Except for this sharp border there is almost no genetic variation in growth and phenology either in the northern part or in the southern part of the distribution of this species. Very large phenotypic plasticity might be an explanation but the experts on this species have ruled this out. Another possible explanation is that the species occupies a specific habitat and is evidently outcompeted by other species in other habitats. In terms of selective environmental neighborhoods it would occupy two SENs (See Chapter 6), one south and one north of central Oregon. Less probable is that *P. monticola* experiences the environment as very variable over time. In consequence, natural selection has figuratively operated in varying directions during the evolution of the species. Evidently most other conifers with the same distribution area did not experience the environment as so variable as *P. monticola* did. Therefore, this explanation is less likely.

Pinus resinosa grows mainly in xeric habitats in a 700 kilometers wide band from eastern Manitoba and Minnesota to the Atlantic coast in the east. There are several climatic zones in this huge distribution area, which ought to have caused a population differentiation, but there is almost no differentiation. One hypothesis is that the species after the last glaciation has passed through several bottlenecks, *i.e.* the effective population size was low at several occasions. This might have eroded the genetic variation of the species. The low inbreeding depression observed in the species lends some support to this hypothesis. Another explanation could be that it only occupies xeric conditions and that the species for this reason experiences the environment as fairly homogeneous. The larger genetic variation in *Pinus strobus*, which has a similar distribution area, might be attributed to its occupation of a wider range of site conditions than *P. resinosa*.

Pinus caribaea is one of the most important planted species in tropical and subtropical countries. In one American series 48 provenance trials in Colombia (3 trials), Venezuela (11), and Brazil (34) stem volume at ages 3,5, and 8 years were measured. Occurrence of forking and fox tailing, which lead to poor quality of logs, was also

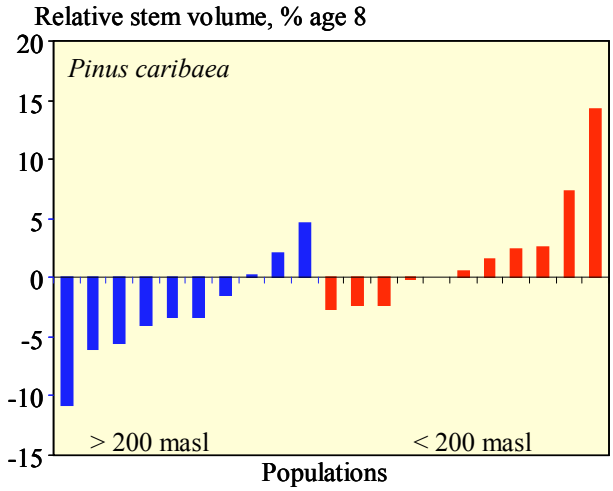


Figure 7-25. Population percentage superiority/inferiority of stem volume over mean stem volume at age 8 of 21 populations of Pinus caribea *in 48 provenance trials in Brazil, Colombia and Venezuela. One of the populations originated from Belize, two from Nicaragua and the 18 others from Honduras.*

assessed. The tree heights in the Brazilian and Venezuelan trials were around 12 metres at age 8 while it was only approximately 8 metres in the three Colombian trials. In Fig. 7-25 the percentage deviations from population mean of high and low-elevation populations in the 48 trials are given. Generally, the low-elevation populations (red columns) had a higher number of populations above the mean value than the high-elevation populations. However, the relationship between population elevation and relative performance was weak, R^2=0.23. The best performing population, Limon from Honduras, performed extremely well in Venezuela. Another Honduran population was the poorest performing; it originated from 560-600 masl.

The age-age correlations within the same trial were strong with one exception, the relationship between stem volume at years 3 and 8 (Fig. 7-26). This means that populations can be selected with good success at age 5 for growth and the two quality traits fox tailing and forking.

Araucaria angustifolia has a wide distribution in Southern Brazil and neighbouring countries. In 2009 it was estimated that only 3% of its former range remained owing

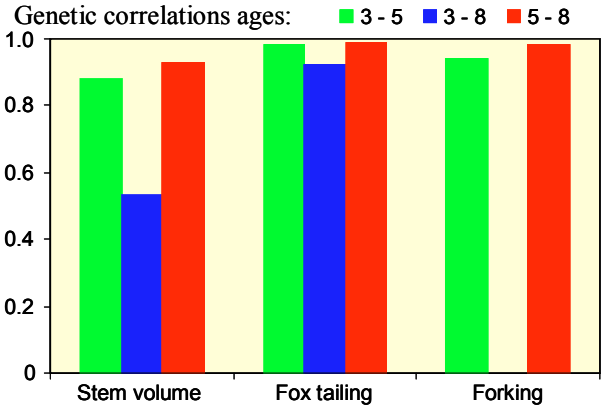


Figure 7-26. Mean age-age genetic correlations within the same trial of Pinus caribaea *for stem volume, fox tailing %, and forking % for ages 3, 5, and 8 years.*

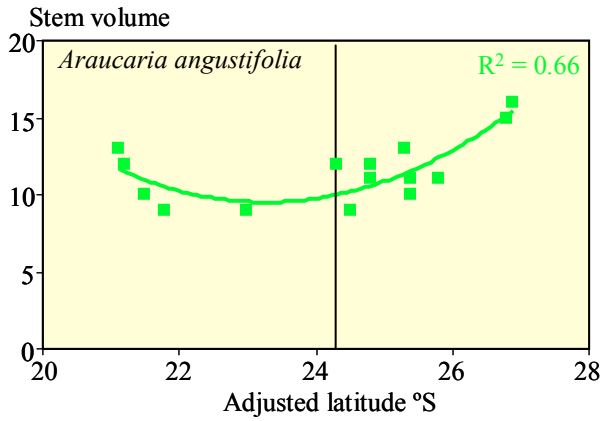


Figure 7-27. The relationship between adjusted latitude and stem volume at age 21 in a provenance trial with Auracaria angustifolia *at latitude 24.28ºS in Brazil. The adjustment is explained in the text.*

to various human activities. Fig. 7-27 reveals that also in this species geographic variation can be traced. The populations included in this trial originated from a wide elevational range, 675 - 1,800 masl. Therefore, an adjustment for elevation was made with 300 metres = one degree of latitude. This adjustment did not result in any large improvement of the relationship; from R^2=0.61 for the latitude - stem volume relationship to R^2=0.66 in the adjusted relationship.

Picture 7-3. Leaf colouring and leaf fall in Betula pubescens *in Uppsala. Trees from latitude 67° N (far left) are defoliated while the trees in front of the path from latitude 60° N still have green leaves. Photograph Gösta Eriksson*

Provenance research in some broadleaved tree species

Large provenance differences are not limited to northern conifers. Many broadleaved tree species also show large provenance differences. The relationship between growth and latitudinal transfer for a *Betula pendula* experiment is demonstrated in Fig. 7-28. The data in this graph originate from results at a test locality at latitude 64° close to sea level in Finland. The best growth is found in the provenances transferred slightly towards the north. Similar results were obtained for *Fraxinus americana* studied in Wisconsin, USA (Fig. 7-29). The reason for the poor growth of the southernmost provenances is that they do not build up hardiness in due time. Therefore, they become frost damaged most years.

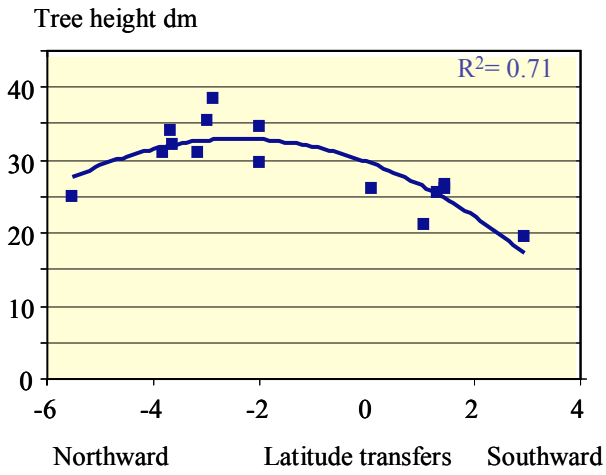


Figure 7-28. The relationship between latitudinal transfer and tree height at age 22 of B. pendula *populations in a Finnish field trial at 60.35°N. Minus means transfer to a northern locality and plus means a transfer to a southern locality.*

As stated before, oak species have played a great role in forest genetics studies in France. In Fig. 7-3 an example of estimated population differentiation in *Quercus petraea* was given for some important traits. The range of $\mathbf{Q_{ST}}$ estimates is given for the span of heritabilities observed for the different traits. The generally high minimum estimates for the growth rhythm traits and height indicate large population differentiation for these traits.

Good growth is a question of optimisation, since both too late an onset of growth during spring and too early growth cessation during the autumn will give rise to small plants and trees which will not set good seed. The latter means that they have a low fitness. One example of this problem of optimisation is taken from a

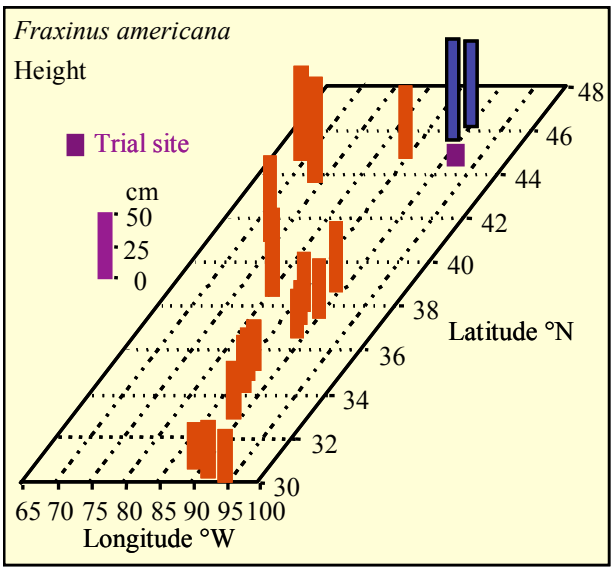


Figure 7-29. Plant height of Fraxinus americana *populations studied in a provenance trial in Wisconsin.*

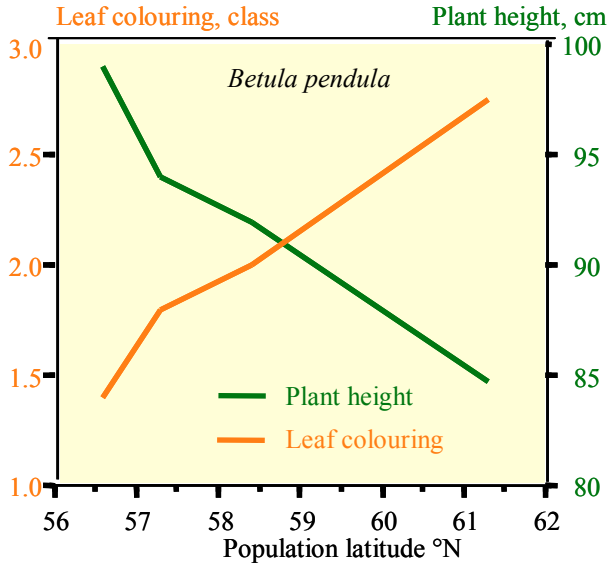


Figure 7-30. The relationship between origin and growth or leaf colouring at a certain date in Betula pendula *studied in a Finnish nursery trial at latitude 62°40'. The higher the value of leaf colouring the earlier the growth cessation.*

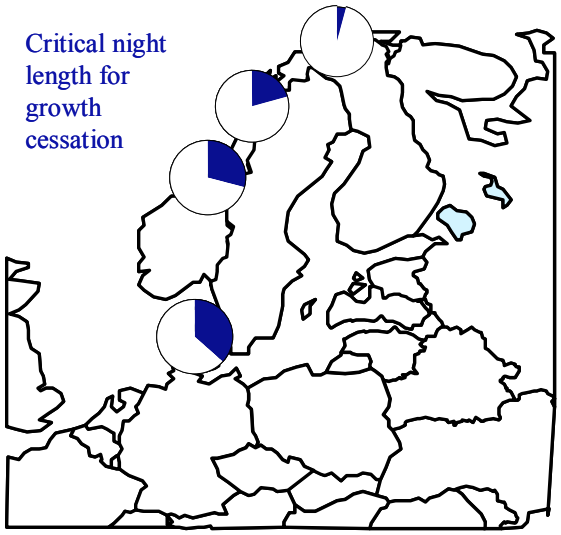


Figure 7-31. The critical night length for growth cessation of Scandinavian populations of broad-leaved tree species, Acer platanoides, Alnus glutinosa, Betula pendula, Hippophae rhamnoides, Sorbus aucuparia, Ulmus glabra *based on a Norwegian study under controlled conditions. The blue circle sector indicates the number of hours for bud-set; a filled circle = 12 hours.*

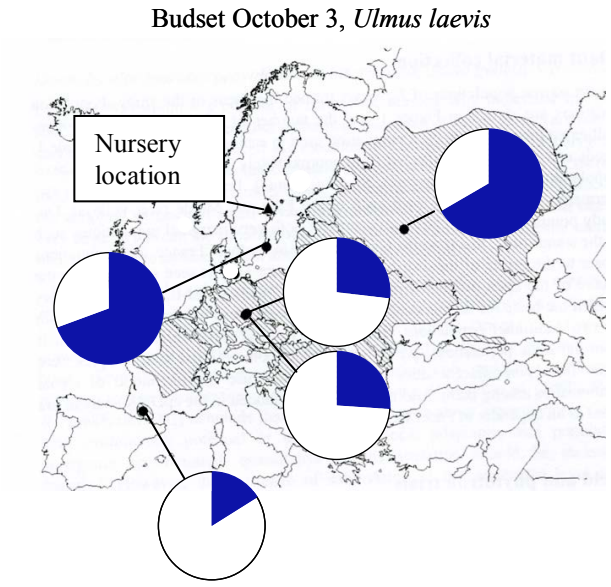


Figure 7-32. Budset in five Ulmus laevis *populations studied in a nursery in Uppsala, Sweden. The blue circle sector indicates the number of hours for bud-set; a filled circle = 12 hours.*

Finnish experiment with silver birch. As may be seen from Fig. 7-30, leaf colouring and growth are mirror images of each other. The plants with the advanced autumn colouring are smallest. The highest fitness at a certain locality will those trees have that show the best balance between onset of growth and growth cessation at this particular site. Since fitness is a relative concept it is valid for the trees in one population growing in one environment. The ranking with respect to fitness of the same trees might be totally different at another growth locality.

The general trend of earlier budburst and growth cessation in northern than in southern populations has been demonstrated for several tree species. Examples on growth cessation from studies in growth chambers and nursery are illustrated in Fig. 7-31 and 7-32.

The reason for the clinal variation from south to north in Scandinavia and from low elevation to high elevation is that the climate varies in a similar way. What we observe as provenance differences is a confirmation of what was illustrated in Fig. 6-6 that there is disruptive selection among provenances.

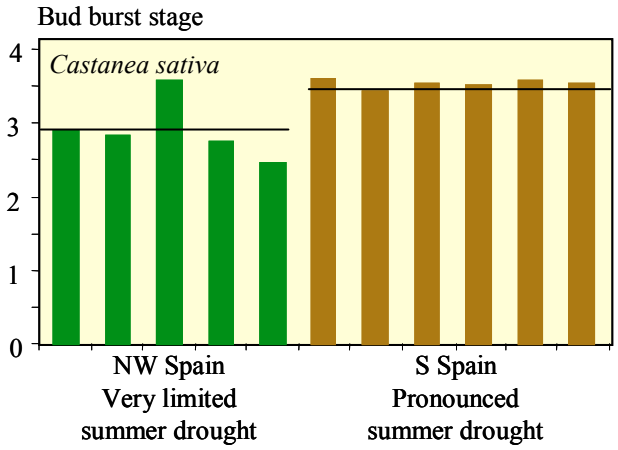


Figure 7-33. Bud flushing stage in Spanish Castanea sativa *populations studied in a Spanish nursery. The water availability during summer varied considerably at the site of origin in the two groups of populations.*

In countries outside Scandinavia in which the climate is not so much influenced by latitude there might be other relationships with geographic variables. Thus, in Spanish populations of *Castanea sativa* the southern populations had an earlier budburst than the northern ones (Fig. 7-33). The southern populations originate from localities with severe summer drought. Under such conditions it may be an advantage to have an early budburst to capitalise on the favourable growth conditions during the early part of the summer before the ambient conditions become too limiting for growth. Plants with a late budburst will have a shorter spring – early summer growth period, which in turn means that such plants will be shorter and less competitive than the early flushing plants. In some cases it might be the precipitation that is the most decisive environmental factor. In other cases climate changes with the distance from the coast.

One example of population differentiation from the Tropics will be given. In a provenance trial with *Tectona grandis* in Pah Nok Kao, Thailand, several traits were assessed at age 17. The populations covered a large part of the distribution area of *Tectona grandis*. The populations originated from the latitudinal range 6.50 - 19.38°N and longitudinal range 76.10 - 112.75°E. In addition, progenies from an Ivory coast seed orchard were included. Wood density was estimated as pilodyn penetration (Fig. 7-34). There were significant population differences for wood density and most of the other characters assessed in this trial. In contrast, the variation in isozymes was minor. It was concluded that regional differences were present but a considerable variation existed within regions as well.

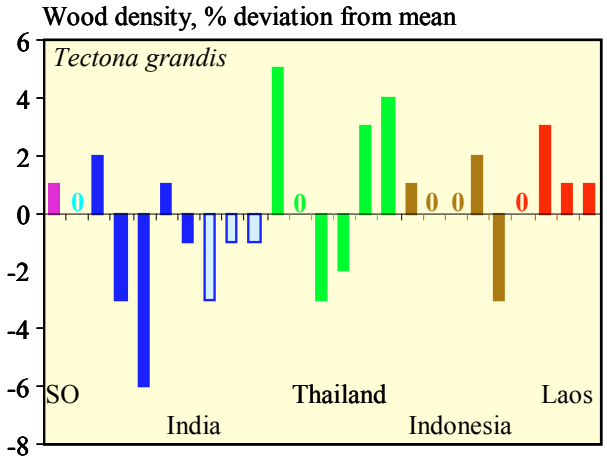


Figure 7-34. Percentage deviation from population mean for wood density of 25 Tectona grandis *populations. A positive value indicates a longer penetration and thus lower wood density. SO stands for progeny from an Ivory coast seed orchard. Second column from left is a northern Indian population. Blue (columns 3-7) and light blue (columns 8-10) are western and eastern Indian populations.*

Adaptation to edaphic conditions

Most examples presented above clearly indicate that there is a pronounced population differentiation for growth and growth rhythm traits that must be attributed to climatic differences over the range of distribution of species. There is limited information about the impact of edaphic conditions on population differentiation. At the species level there is a clear difference on preferences with respect to edaphic conditions. One clear example comes from the Pirin valley in Bulgaria with its two pine species, *Pinus peuce* and *P. Heldreichi*. Each species occupies just one of the two slopes. The two slopes differ with respect to soil conditions, *P. peuce* prefering silicate and *P. Heldreichi* limestone soil. Therefore, it might be speculated that such differences may be extended to populations within a species. There are few data available on well designed experiments to study adaptation to edaphic conditions. There is one example for *Fraxinus excelsior* studied in Germany. Two populations from dry and wet sites were included in an experimental series planted at three sites, one wet, one dry, and one intermediate locality. The tree height at age 10 is illustrated in Fig. 7-35, which shows that the populations from the wet origin outgrew the two populations from the dry sites at the wet test site. At the two other test sites there was no significant difference between populations from the two origins. Data from tree height at age 33 at the dry test site confirm the absence of population differences at age 10 for the dry test plantation.

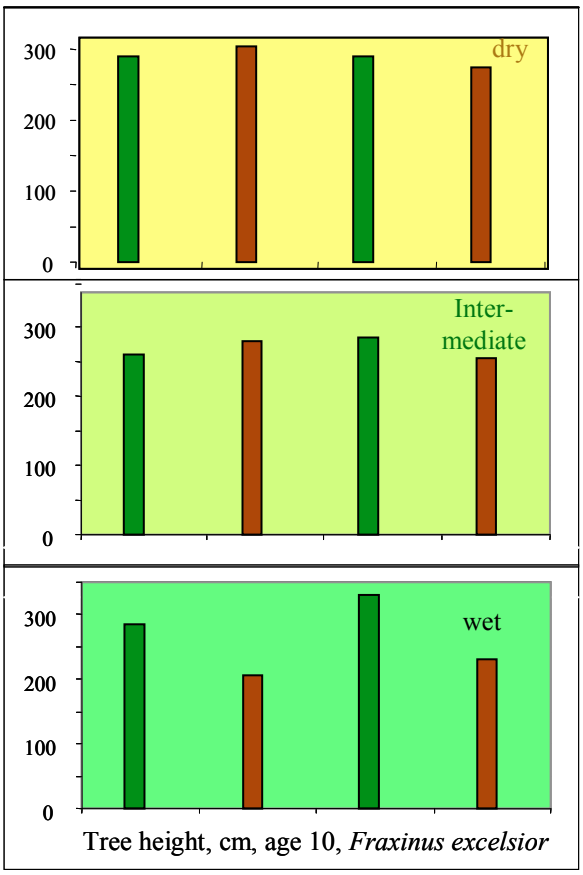


Figure 7-35. Tree height, cm, at age 10 of Fraxinus excelsior *populations from two types of origin, wet and dry sites, tested at three sites with varying water availability. Green bars refer to two populations from wet sites, brown bars refer to populations from dry sites.*

Utilization of provenance results

How can the results from provenance research be utilized in applied forestry? Continuous variation along ecological gradients has been demonstrated repeatedly. The question is: Over how large an area could a provenance be used without losing in production as we move from the optimum of this provenance? To elaborate on this, a hypothetical situation is shown in Fig. 7-36. In this figure the relative production of four provenances is shown graphically. If we accept a drop of production to 90 % of the maximum, these four provenances cover precisely the range in the figure. If we do not accept a greater loss than 5 % then we do not have any suitable provenance for areas I and II in this ecological gradient. The reason why we do not find a provenance that satisfies our requirements might be that the provenances tested so far have their origins too far apart from each other. In other words another provenance test with a denser net of provenances might give us the proper provenances for the entire ecological gradient under the requirement of no more than 5 % drop in production from the optimum.

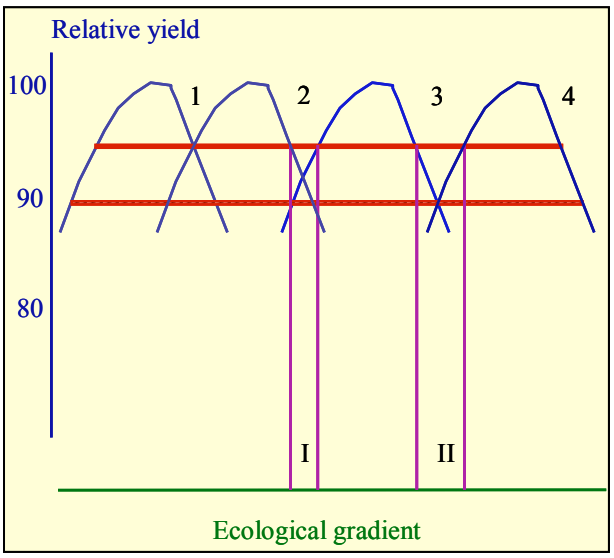


Figure 7-36. Schematic illustration of the relative yield in four provenances along an ecological gradient. For further explanation see the text.

In many countries seed transfer rules are based on results from provenance research. In Sweden the forestry act from 1994 says that local provenances should be used, which is in conflict with most provenance research. Optimum production will not be obtained if this recommendation is followed.

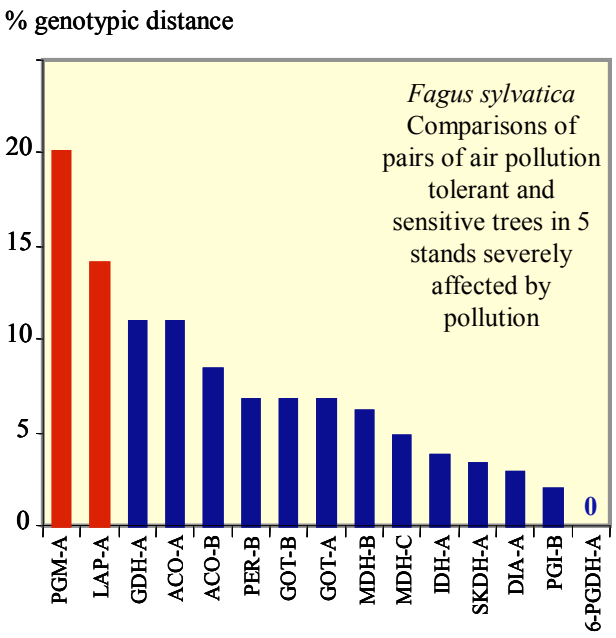


Figure 7-37. Estimates of the genetic distance of individual isozyme loci in German Fagus sylvatica *populations with trees tolerant and susceptible to air pollutants. A locus with large value suggests that it may be involved in air pollution tolerance.*

Markers

Most forest geneticists agree that the majority of isozymes are neutral markers and as such they do not contribute to fitness. However, it is evident that different ambient conditions influence the efficiency of certain isozymes. As a corollary it has also been assumed that different isozymes vary with respect to adaptedness. One way to estimate this is to compare the genetic distances of different loci in genetic entries varying widely in their response to the ambient conditions. One example of such a comparison is the characterisation of isozymes in German populations of *Fagus sylvatica* tolerant and susceptible to air pollution (Fig. 7-37). As seen from this figure the distances for loci PGM-A and LAP-A are much larger than for 6-PGDH-A and PGI-B loci. Therefore, it may be speculated that the two former loci may have been changed by natural selection while the latter are less affected by natural selection.

Another indication for non-neutrality of isozymes is the clinal variation in isozymes allele frequencies along environmental gradients. However, it should be noted that it takes several generations to level allele frequencies in different populations after a new mutation has arisen. This means that there is always a time lag until allele frequencies are levelled; the further apart the larger the difference in allele frequency between populations. Clinal variation of isozymes alleles may therefore be attributed to the time lag for levelling of allele frequencies. It should be noted that many population studies did not show clinal variation of the isozymes. An example of this is presented in the next paragraph.

In Fig. 7-38 the genetic distances estimated by isozymes between Swedish *Pinus sylvestris* populations are shown to the left. It should be noted that there is a limited genetic differentiation among these populations. The geographic origin of the populations is also indicated and it is clear that there is no geographic trend. The absence of geogra-

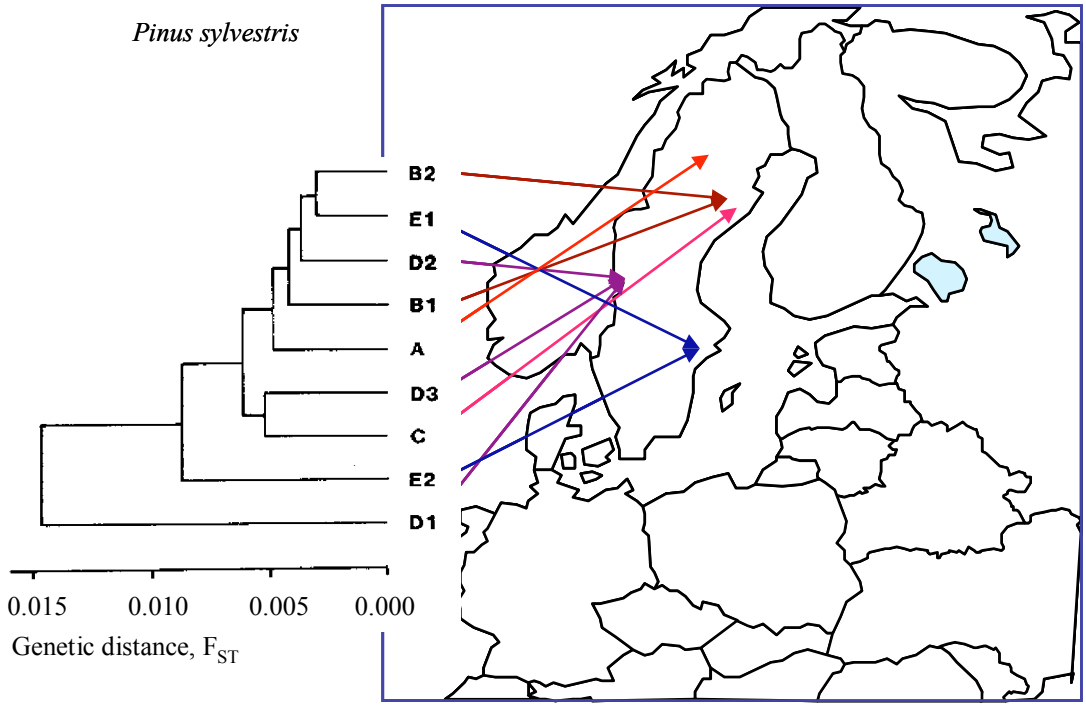


Figure 7-38. Origin of nine Pinus sylvestris *populations and genetic distances estimated by 11 isozyme loci.*

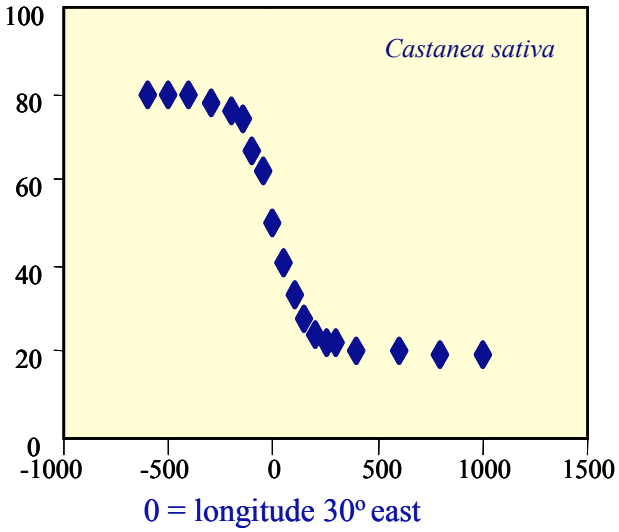


Figure 7-39. The percentage of alleles typical for the western populations of Castanea sativa *in west-east direction. There is a rapid change of allele frequencies in Bythinia in western Turkey.*

phic trends in studies involving isozymes is an indication that the isozymes studied are neutral. In this study it can be remarked that the E1 population was closest related to the B1 population and less related to its neighbour population E2. Both E populations would have limited or no survival at the B locality four degrees further north.

In *Castanea sativa* a conspicuous transition in allele frequencies was noted in Bithynia in western Turkey (Fig. 7-39). The transition zone was estimated at 324 km. The allele frequencies in the eastern and western Turkish populations were relatively uniform but different from each other. The estimated number of migrants per generation was higher within each of the three regions, western, Bithynian, and eastern, than between regions. One possible explanation is that the western and eastern populations developed in isolation from each other over several generations and recently came into contact with each other. The development in isolation should in this case have resulted in different allele frequencies in the two regions. Another alternative explanation is that the variation in allele frequency from east to west is a reflection of past natural selection. A closer look at the climatic conditions in the three regions shows that the climate does not vary much within the Bithynian region while it varied considerably in the eastern region. These facts speak against the selection interpretation of the results. Palynological data give some support to the first hypothesis of recent contacts of two previously isolated populations.

Table 7-2. Compilation of observed population differentiation in European broad-leaved tree species by aid of isozymes.

Species	$\mathbf{F_{ST}}$ or $\mathbf{G_{ST}}$ estimates
Acer platanoides	0.10
Alnus glutinosa	0.20
Betula pendula	0.03
Castanea sativa	0.11
Quercus petraea	0.02
Quercus robur	0.05
Sorbus aucuparia	0.06
Sorbus torminalis	0.15
Ulmus laevis marginal populations	0.33
Ulmus minor	0.18

The population differentiation estimates ($\mathbf{F_{ST}}$ or $\mathbf{G_{ST}}$) based on isozymes in most widespread conifers rarely exceed 0.05. This means that there is limited population differentiation with respect to isozymes markers and the most likely reason for this is strong gene flow among populations. In Table 7-2 a compilation of estimates of population differentiation is given for some broad-leaved tree species. It should be remarked that the estimates are dependent on how the population were selected and on the number of isozymes markers that was analysed. With these limitations in mind it is anyhow a tendency that widespread and wind-pollinated species such as *Betula pendula, Castanea sativa, Quercus petraea,* and *Quercus robur* show lower estimates than species with non-continuous populations such as *Acer platanoides, Alnus glutinosa,* and *Sorbus torminalis*. The highest estimate was noted for small, scattered, and marginal populations of *Ulmus laevis* at the northern margin of distribution in Finland. It is likely that genetic drift has played a great role during preceding generations of these small populations. *Pinus cembra* has relatively small and scattered populations in central European mountains. In spite of its scattered distribution, population differentiation was limited, $\mathbf{F_{ST}} = 0.047$. The most likely explanation for this is that the isolation of the populations occurred relatively recently. In this context 'recently' means a few generations ago.

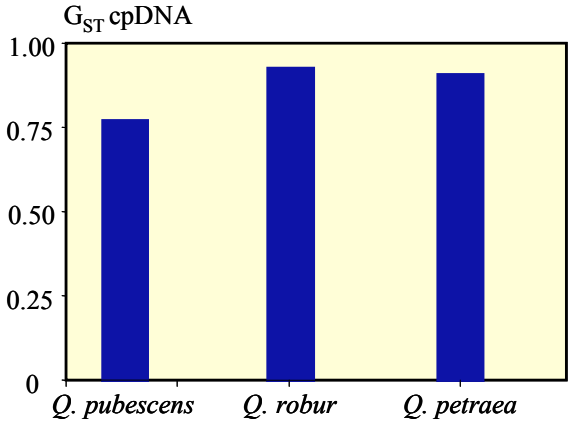


Figure 7-40. Genetic distance, G_{ST}, based on chloroplast DNA in Quercus pubescens, Q. robur *and* Q. petraea *populations.*

In Fig. 7-40 an example of a study including chloroplast DNA (cpDNA) markers in three oak species is illustrated. Such markers are frequently referred to as haplotypes. As seen from this figure there is a large population differentiation in all three species. The reason for this is that there are few markers and that several populations have just one marker. When several populations are monomorphic with respect to one marker and several other populations are monomorphic with respect to another marker a large differentiation is obtained. In such a case there is no discrimination between the monomorphic populations sharing the same cpDNA marker.

In Fig. 7-41 population differentiation based on isozymes and mitochondrial DNA (mtDNA) in some western American pine species is given. As seen from this figure the differentiation is much larger based on mtDNA than on isozymes. The estimates based on isozymes are relatively high, which may be attributed to scattered occurrence of these three species. As regards the differentiation by mtDNA the same explanation as for the oak species is relevant. Thus, most populations were monomorphic with only one haplotype.

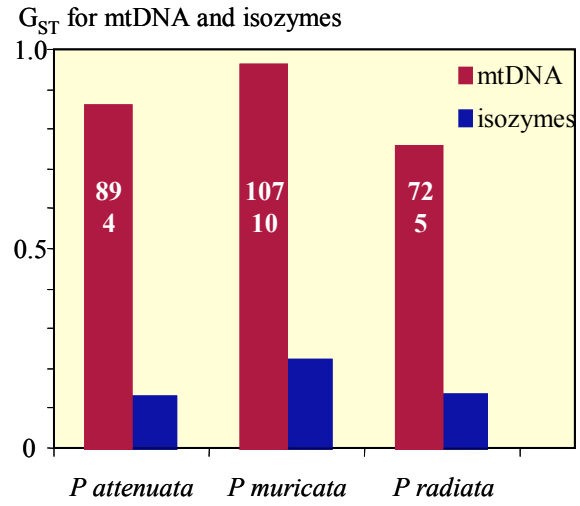


Figure 7-41. Genetic distances based on isozymes and mitochondrial DNA (mtDNA) in populations of the North American pine species Pinus attenuata, P. muricata, *and* Pinus radiata. *The figures in the bars refer to number of trees and populations included in the study.*

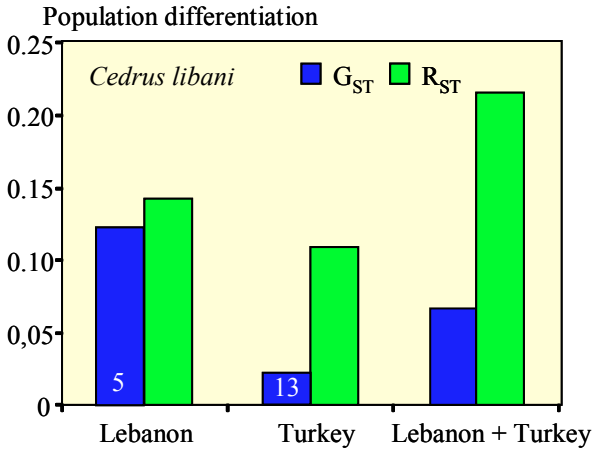


Figure 7-42. The differentiation between 5 Lebanon and 13 Turkish populations of Cedrus libani *esitmated by microsatellites. The R_{ST} estimates consider the change of sequences in DNA. When there is a difference between G_{ST} and R_{ST} estimates there is a geographical pattern.*

Microsatellites in chloroplast DNA were used to study the variation among 13 Turkish and five Lebanese populations of *Cedrus libani*. Besides traditional ways of estimating population differentiation (G_{ST}, Fig. 7-42 blue columns), variation was also estimated taking into account genetic distances between haplotypes (R_{ST}, Fig. 7-42 green columns). The latter builds on the assumption of a stepwise mutation model of haplotypes. As seen from Fig. 7-42 there is limited difference between G_{ST} and R_{ST} among the five Lebanese populations while there is a large variation among the 13 Turkish populations. R_{ST} enables a further differentiation than can be detected by G_{ST} estimates. When there is a difference between R_{ST} and G_{ST} as was the case for the Turkish populations, a geographic differentiation is indicated.

Estimates of R_{ST} also allow better possibilities to reveal any relationship between population differentiation and geographic origin than the traditional F_{ST} and G_{ST} estimates. One example of this is illustrated in Fig. 7-43. The pairwise R_{ST} estimates for eight Central American populations of *Swietenia macrophylla* (big-leaf mahagony) originating from Mexico in the north to Panama in the south was calculated. As seen from Fig. 7-43 the relationships labelled with red squares fit the curve well. The three blue populations deviated strongly from the general relationship. All of them included the only Panamanian population and all high R_{ST} estimates observed included the relationship with this Panamanian population. It may be speculated that the Panamanian population had developed in some isolation from the rest of the populations.

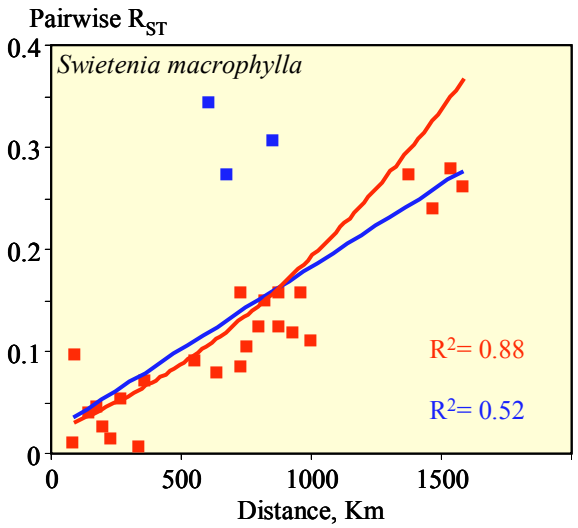


Figure 7-43. The relationship between pairwise R_{ST} and geographic distance between eight Swietenia macrophylla *populations in Central America. The blue line refers to the relationship between all populations and the red curve refers to the R_{ST}s with exclusion of the three blue deviating relationships.*

Fifteen microsatellites were used to study the differentiation (=F_{ST}) among 17 populations of *Tectona grandis*. Six populations from southern India and five populations from each of Thailand and Laos were included. In addition, one population from northern India was studied. The total differentiation as well as the differentiation in each of the three regions with five and six populations were estimated (Fig. 7-44). The total differentiation was 0.22. The within-region differentiation was low in the Laotian and southern India regions while it was larger in Thailand in spite of lesser geographic distribution than in Southern India. A possible explanation for the low differentiation of the southern India populations is great similarity in environmental conditions for the localities of the five populations from this region. However, the localities varied from very moist teak forest to dry teak forest. In conclusion four groups were distinguished:

Northern India
Southern India
Thailand + 2 Laotian populations
Three central Laotian populations

The limited diversity within some of the populations was attributed to inclusion of planted material.

Darwinian and domestic fitness

Geneticists sometimes distinguish between Darwinian fitness, *i.e.* the adaptedness in nature and domestic fitness, which is the ability of a genetic entry to produce biomass, high quality timber, shelter, or any other utility for us as human beings. These two types of fitness might coincide but usually this is not the case. In nature extremely good growth is in vain if this ability is not transferred to a progeny, which normally takes place via the seeds formed after sexual mating. Seed production can be an unnecessary and energy-demanding process for production of human utilities. This is especially pronounced for crops where vegetative propagation is applied, such as potatoes. The difference might be best understood when we realise how important regeneration is in nature. As regards cultivated plants man has taken over the responsibility for propagation and this ability is no longer decisive for the continued use of a species. Many ornamental plants would be out-

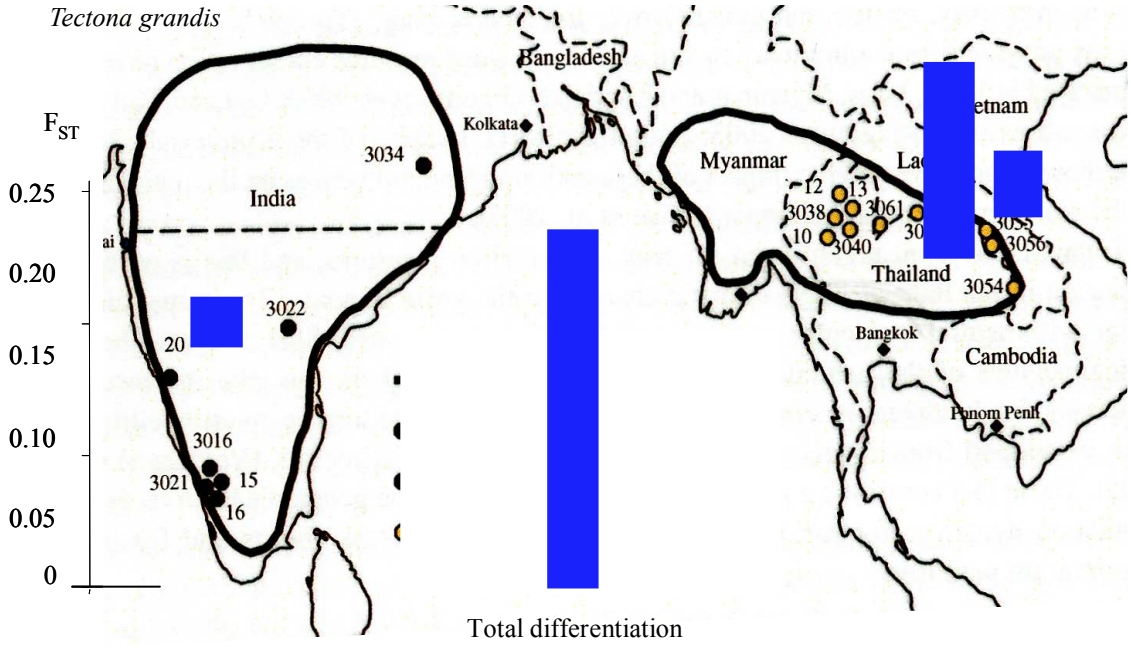


Figure 7-44. Population differentiation estimated by 15 microsatellites in 16 populations of Tectona grandis *from India, Thailand, and Laos. Separate estimates are given for the five populations in each of South India, Thailand, and Laos. Northern India was represented by one population.*

Box 7-2 Why is the local population of *Picea abies* not the best choice for reforestation of a clearcut area in southern Sweden?

	Comparison of the type of regeneration	
	Planting at a clearcut area	Natural regeneration
Day temperature	higher	lower
Night temperature	lower	higher
Budburst	earlier	later
Type of fitness required	domestic	Darwinian

The high day temperatures at a clearcut area induces an earlier budburst than in an opening in the forest. This means that the budburst in a clearcut area takes place at the time when the probability for frost exposure is higher than when budburst occurs in the opening in a stand. Since the local population has a lower heat demand for budburst than some exotic populations, *e.g.* Byelorussian populations, the latter are better than the local population.

At natural regeneration in small openings in the forest, an early budburst might be advantageous since such genotypes outcompete other late budbursting genotypes and are also competitive against other plant species.

competed if there was no human intervention. As human beings we cultivate them for their beauty and propagation is taken over by nurserymen, which means that their Darwinian fitness is obsolete. Below a few examples of domestic fitness are given.

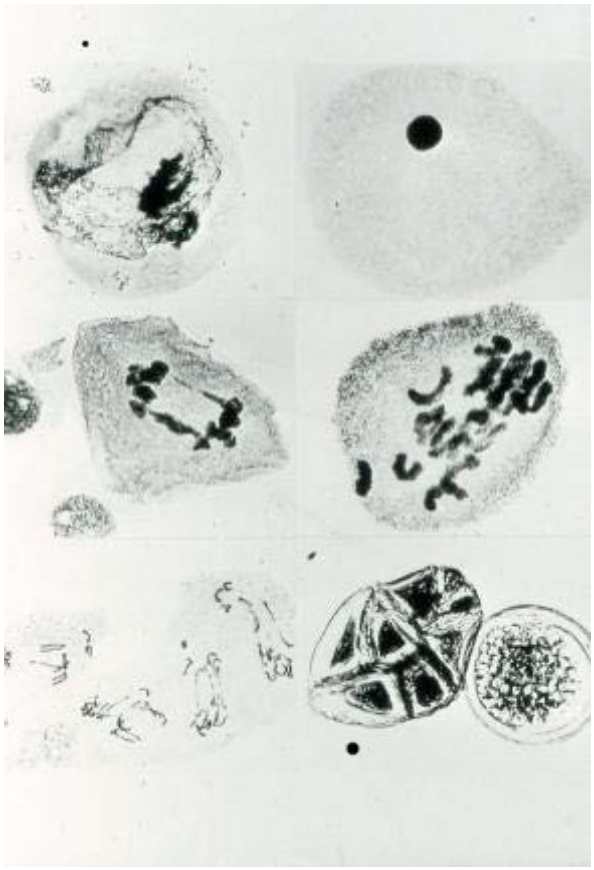
Byelorussian Norway spruce outgrows the domestic Norway spruce in southern Sweden. Most people have interpreted this as a consequence of the migration of Norway spruce into Sweden after the last glaciation. From the refuge in Russia Norway spruce migrated in a western direction and one branch migrated northwards in Finland and entered Sweden after passing to the north of the Gulf of Botnia. Finally it migrated southwards in Sweden. The passing of latitude 66° is assumed to have caused an enrichment of hardiness alleles at the cost of growth promoting alleles. This explanation is not valid since Norway spruce passed latitude 66° when the climate was warmer than today. The reason for the superiority of the Bylorussian Norway spruce is probably the type of reforestation used, planting after clear cutting. Norway spruce is not well adapted for regeneration on clear-cut areas since it has evolved under regeneration in small openings in forests.
The reason for the superiority of the Byelorussian Norway spruce is further outlined in Box 7-2. With respect to migration in Scandinavia it should be added that recent results (2012) indicate that there might have been a refugium of *Picea abies* in northern Norway during the last glaciation.

The later budburst is accompanied by later growth cessation, which might be harmful for building up of frost tolerance during the autumn. However, there are no signs of any problems of Byelorussian Norway spruce up to latitude 60° in Sweden. Therefore, it seems as if both the domestic and the Byelorussian Norway spruce start their inwintering too early and do not utilise the growth potential that the southern Swedish climate offers. If a population avoids frost damage it means that not only the survival is increased but also that stem defects are avoided and that the duration of the phase of establishment is reduced. The latter increases the productivity per year.

Why do we need to transfer Scots pine seeds from north to south in northern Sweden to get satisfactory survival? Once more it is a question of comparing self regeneration with planting. At planting the majority of the plants must survive while it might suffice with one plant per thousand under natural regeneration, since Scots pine has a profuse seed production. A Finnish scientist has estimated that one pine tree during its life time might produce one million seeds. To keep the range of distribution unchanged, it is theoretically sufficient that each pine tree gives rise to one new tree. One might question the need for such a waste of energy. This is further discussed in the next chapter.

In Chapter 1 details about the timing of meiosis in pollen mother cells of larch were presented. Several stages of the meiotic division are probably the most frost sensitive during the life cycle of an individual.

In southern and central Sweden, with its maritime climate, there are continual changes between cold and mild periods during the winter. During certain years dormancy was broken in the pollen mother cells of Siberian larch already during November - December. The limited heat



Picture 7-4. Examples of severe damage during meiosis in pollen mother cells of Larix. *Above are shown two cases of stickiness, one resulting in a total merger of all chromosomes into a spherical body. Centre. Severe disturbance of anaphase I. Below are shown an extra division at the tetrad stage resulting in eight instead of four microspores.*

during the following mild period was enough to induce a continuation of the meiotic divisions. If the period of mild weather is short and then followed by a frost period before the completion of the meiotic divisions, severe frost damage may be induced (Picture. 7-4). In certain years there was a total collapse of the meiotic divisions with temperature-induced pollen sterility as a consequence. Since pollen mother cells of European larch have a larger demand for chilling to break the dormancy, the pollen formation in this species did not show as much damage as the Siberian larch. Japanese larch takes an intermediate position between the other two species. How can these differences between the three larch species be evolutionarily explained? One plausible explanation is that European larch grows under less continental climatic conditions than the Siberian larch. This means that changes between mild and cold periods occur frequently. European larch genotypes that have a large chilling demand will have a higher fitness than those with a low chilling demand. In contrast, in Siberian larch there has not been any need for an increased chilling demand owing to the more stable cold winters in its distribution area. A large chilling demand probably did not contribute fitness to Siberian larch.

Summary

The genetic structure of a species is reflected in different ways by different traits. Generally isozymes show a limited among-population variation while growth and growth rhythm traits show major among-population variation. In most cases population variation in isozymes does not show any geographic structure. Tree species from the temperate and boreal zones, both conifers and deciduous tree species, show large clinal variation for traits of adaptive significance. Climatic conditions have played a major role for tree populations' adaptation to the ambient conditions. At high latitudes early inwintering is important. Similarly, the higher the elevation the earlier the inwintering. The growth cessation in these tree species is triggered by night length while growth initiation during spring in most cases is triggered by temperature sums. As a consequence of this, budburst varies over years. The commercially important tree species *Betula pendula, Quercus petraea, Picea abies, Pinus contorta, Pinus sylvestris,* and *Pseudotsuga menziesii* are examples of adaptation to climatic conditions. *Pinus resinosa* and *Pinus monticola* are exceptions to this pattern. The cause of this deviating pattern is still somewhat obscure.

Large among-population variation is not confined to boreal and temperate zones tree species but is revealed in commercially important tree species from subtropic and tropical regions of the world. At low latitudes where the photoperiodic conditions are less dramatic, other climatic elements such as drought have played a role in previous adaptation.

Large among-population differences have been reported for some molecular markers. This is particularly the case for chloroplast and mitochondrial DNA. The reason for this is that there are few markers and that several populations have just one marker. When several populations are monomorphic with respect to one marker and several other populations are monomorphic with respect to another marker a large differentiation is obtained.

Darwinian fitness is the ability of a genotype or a population to transfer its alleles to the progeny generation. Domestic fitness is the ability of a genotype or a population to produce some kind of utility for man. The latter is of great significance in all kinds of plant cultivation whether it is for biomass production or beautification. The improved yield per hectare brought about by southward transfers of *Pinus sylvestris* populations in northern Scandinavia is one example of this. Another example is the northwards transfers of *Picea abies* populations in Scandinavia. In both cases the reforestation conditions are decisive for the results.

Further reading

Eriksson G. 1968. Temperature response of pollen mother cells in *Larix* and its importance for pollen formation. Stud. For. Suec.63: 1-131.

Eriksson G, Andersson S. Eiche V. Ifver J. & Persson A. 1980.Severity index transfer effects on survival and volume production of *Pinus sylvestris* in Northern Sweden. Bestämning av ett hårdhetsindex för norra Sverige med hjälp av proveniensförsök med tall. Studia For Suec. 156. 132p.

E.K. Morgenstern 1996. Geographic variation in forest trees. UBC Press, Vancouver, Canada.

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

Variation among families

In this chapter we present observed variation within populations and genetic parameters derived from experiments with full-sib or half-sib progenies. Heritabilities and coefficients of additive genetic variation are presented for growth, growth rhythm traits, and disease tolerance. Additive genetic correlations and species hybridization are also presented.

More or less all early studies of variation within populations were connected to breeding programs. For this reason there was more information from tree species with high economic value such as *Picea abies, Pinus elliottii, Pinus sylvestris, Pinus taeda,* and *Pseudotsuga menziesii* than from other tree species. First we will present some observations of variation within populations and after that turn to estimates of genetic parameters.

Examples of variation among families for various traits

Large variation in timing of budburst of open-pollinated progenies of *Picea abies* in different populations from Slovakia and Poland was noted in a south Swedish nursery (Figure 8-1). In some of the populations the range in time is approximately two weeks. The variation in phenology has also consequences for growth; a short growth period usually means poor growth. Variation in juvenile growth of OP-families in a nursery test of several Norwegian and a few exotic populations of *Picea abies* is demonstrated in Fig 8-2. The variation of open-pollinated family means for age 3 heights is considerable. In a study of *Picea sitchensis* there was a large variation both within populations and between populations in tree height at age 10 (Fig. 8-3). In spite of the conspicuous difference between the families the heritability was as low as 0.07. This must be attributed to a large variation of the seedlings in a family both within and between replications.

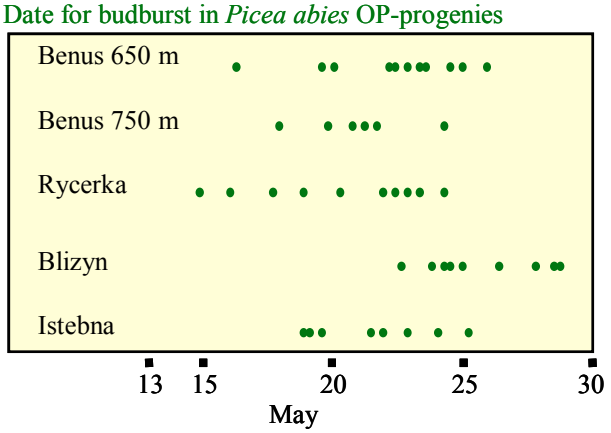


Figure 8-1. The variation in timing of budburst in open-pollinated progenies from individual parents in Polish and Slovak populations of Picea abies *studied in a nursery at 59°30'.*

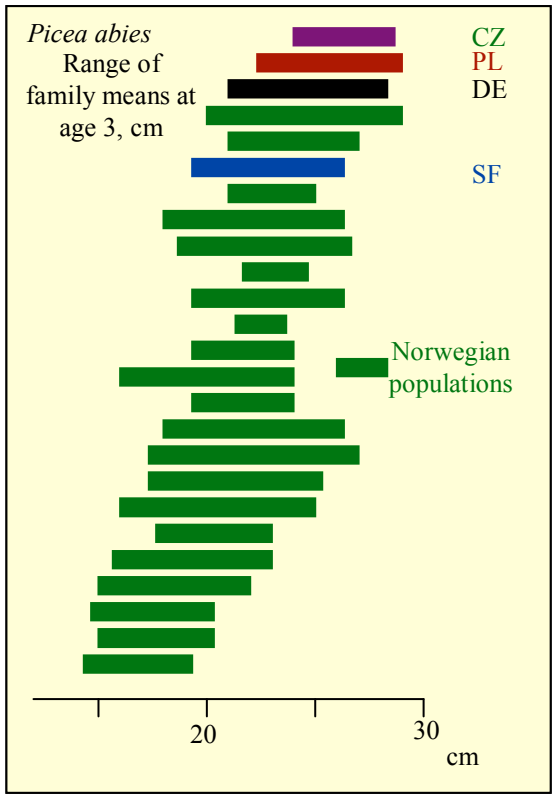


Figure 8-2. The range of family means of plant height at age 3 of Norwegian open-pollinated families of Picea abies *and a few exotic sources. CZ = Czech, DE = German, PL = Polish, and SF = Finnish populations, respectively studied in a Norwegian nursery.*

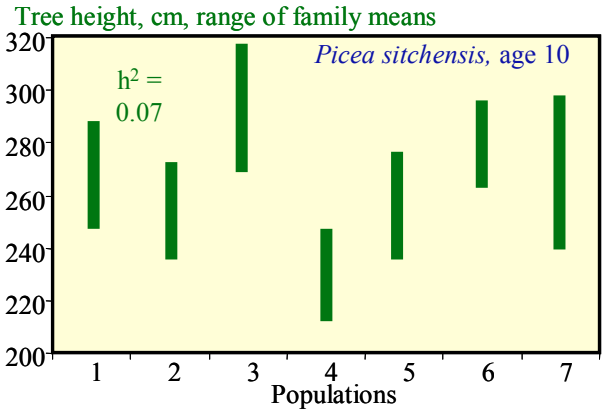


Figure 8-3. Range of family means for tree height at age ten of Picea sitchensis *open-pollinated families from seven populations.*

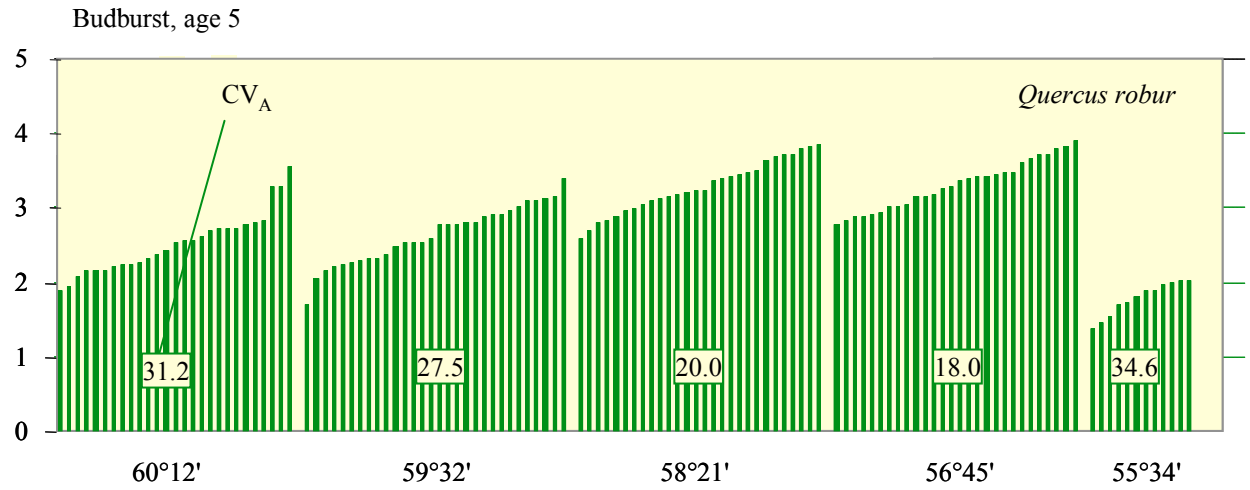


Figure 8-4. The variation in timing of budburst in open-pollinated progenies from individual parents in five Swedish Quercus robur *populations studied in a nursery at latitude 56°38'. The latitudinal origin of the populations is given. The coefficients of additive variation for budburst of individual populations are given.*

Figure 5-6 is another example of a large within-population variation of such an important trait as survival in *Pinus sylvestris* in northern Sweden. During the late part of the previous century increasing knowledge of within-population variation in broad-leaved tree species has accumulated. An example from *Quercus robur* illustrates this for budburst (Fig. 8-4). It should be stressed that too early or too late assessment will underestimate the genetic variation. Many investigations have been carried out with the economically important tropical tree species *Tectona grandis.* A considerable variation in breast height diameter among the 26 open-pollinated families was noted at an age of 8 years and 8 months (Fig. 8-5). The trial was established in Malaysia and the seeds of the OP-families were harvested in a seed orchard in Ivory Coast in Africa. Most of the clones originated from India.

Disease resistance or rather disease tolerance is of utmost

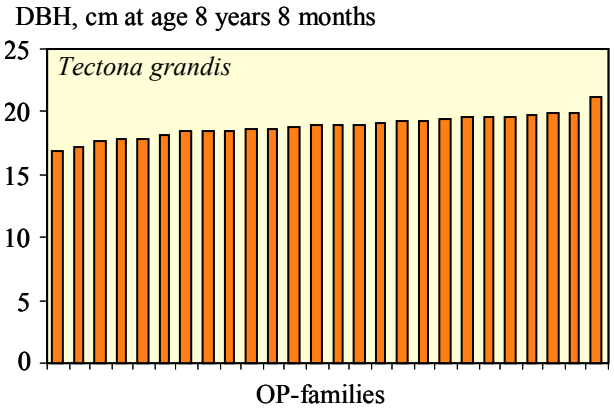


Figure 8-5. The variation in breast height diameter of 26 open-pollinated families of Tectona grandis L. *at 8 years and 8 months in a Malaysian progeny trial at lat. 4.97°N and 118.22°E. The progenies were collected in an Ivory Coast clonal seed orchard. Most of the seed orchard clones originated from India.*

Picture 8-1. A Pinus taeda *tree severely infected with fusiform rust. Photograph Gösta Eriksson.*

importance in several conifers. Fusiform rust, *Cronartium quercuum*, causes great losses for forest owners in South Eastern US owing to attacks on the important pine species, *Pinus elliottii* and *P. taeda* (Picture 8-1). There is a large variation in susceptibility as is illustrated in an experiment with 16 open-pollinated *P. taeda* families Fig. 8-6). Blister rust is another important disease affecting *Pinus strobus, P. lambertiana,* and *P. monticola*. In four experiments with over 200 open-pollinated families in each of the latter two species, the survival at age 5 after artificial inoculations with *Cronartium ribicola* the species means varied between 1.6 and 13.1%. In spite of this low field survival the family means varied from 0 to 54.8%.

Serious attacks of *Gremmeniella abietina* on *Pinus sylvestris* occurred in large parts of Sweden in 2001. This enabled estimates of genetic parameters for tolerance against the disease caused by this fungus. The results from five progeny trials and a seed orchard are presented in Fig. 8-7. As seen from this figure the needle loss and

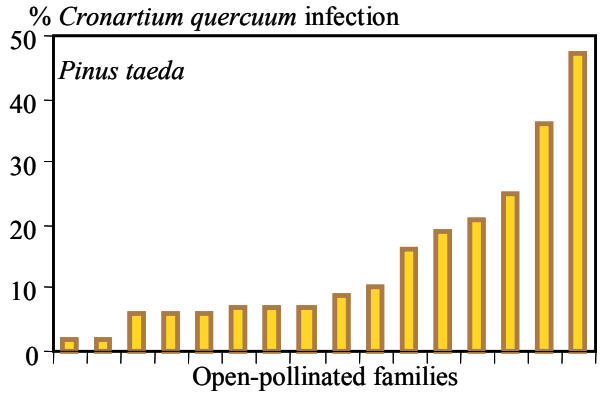


Figure 8-6. Variation in fusiform rust tolerance in Pinus taeda *families.*

new damage were considerable in two of the trials. They were accompanied by high heritabilities while low estimates were noted in the two trials with the lowest damage. The high heritabilities indicate that breeding for resistance against *G. abietina* may be successful.

Nineteen micropropagated clones of *Betula pendula* from a population at latitude 61.78°N in Finland were analysed chemically and exposed to mountain hare (*Lepus timidus*) feeding. The focus here will be on palatability. There was a significant difference in palatability with a large variation among the clones (Fig. 8-8). The most eaten clone was the first preference of the hares. There was a significant difference in height among the clones but not for basal diameter or stem volume.

Dutch elm disease caused by *Ophiostoma novo-ulmi* and chestnut blight caused by *Cryphonectria parasitica* seem to be the most spectacular diseases in European and North American deciduous tree species. Great efforts have been devoted to identify tolerant material and a few elm cultivars have been released. The success in obtaining disease tolerance in *Castanea dentata* is meagre. The limited success in elms and American chestnut suggests that there is limited variation in disease tolerance in these two species.

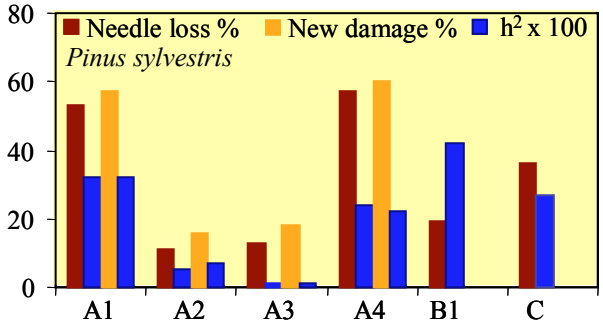


Figure 8-7. Needle loss and new damage in a series of 4 Swedish Pinus sylvestris *progeny trials, A1-A4; needle loss in a progeny trial, B1; and needle loss in a clonal seed orchard, C owing to* Gremmeniella abietina *attacks. The heritabilities x 100 of each trait are shown in blue to the right of the traits. For C it is broad sense heritability.*

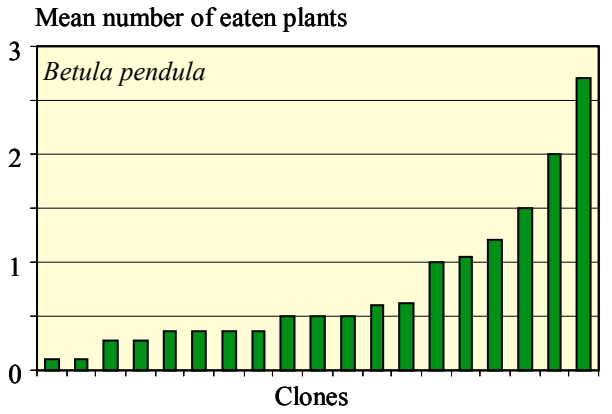


Figure 8-8. The variation in palatability estimated as mean number of eaten plants of 19 Betula pendula *clones in a trial in southern Finland at latitude 61.78°N.*

Interspecific hybrids

There has long been an interest in production of species hybrids in order to combine good characteristics from the two parental species. An anticipation of finding heterosis effects was also a reason for species hybridization. There are many examples of species hybrids in genera *Populus, Larix*, and *Pinus*. Examples of interspecific hybrid performances in these three genera are given below.

Several trials with *Pinus caribaea* and *P. elliottii* hybrids were established in the subtropical climate of Queensland, Australia. The results from a series with four trials are presented in Fig. 8-9. With one exception each type of progeny was represented by 36 full-sib families from a 6 x 6 factorial mating. The F_1 was the exception, which was represented by a 12 x 12 factorial mating. The mean stem volume in the trials varied from 307 to 500 dm^3. Therefore, we calculated the deviations from the mean in each trial (Fig. 8-9). As seen from this figure there was no superiority of the F_1 hybrid over *P. caribaea*. Interestingly, the back cross of the F_1 with *P. caribaea* was outstanding in this series of trials. As regards basic density *P. elliottii* was superior to F_1, F_2, and particularly to *P. caribaea*.

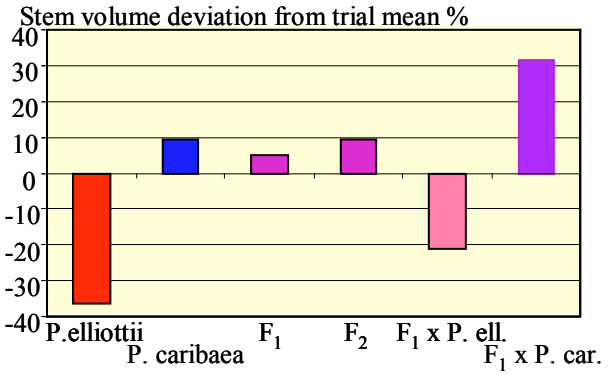


Figure 8-9. Mean percentage deviation from trial mean in stem volume of P. elliottii, P. caribaea, *their F_1 and F_2 hybrids, and the back crosses of F_1 with the pure species at age 20 in four trials in Queensland, Australia.*

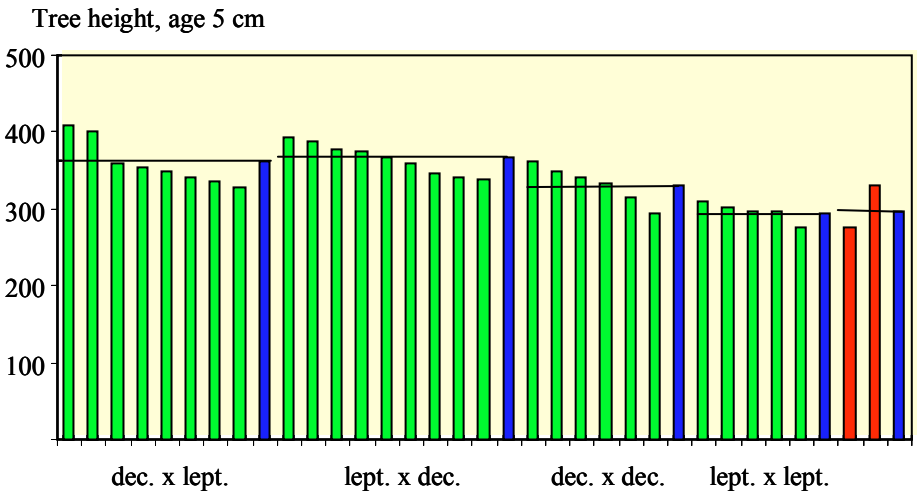


Figure 8-10. Mean tree height at age 5 of intraspecific and interspecific families of Larix decidua *and* L. leptolepis *in a progeny trial at latitude 45.50°N in Maine, USA. Two* L. decidua *check lots are shown in red. The mean height for each type of material is shown in blue.*

An experiment with intraspecific and interspecific crosses of *Larix decidua* and *Larix leptolepis* was established in Maine at latitude 45.50°N. The height at age 5 of the families and the mean heights of the four types of mating show that the interspecific hybrids have a higher mean than the intraspecific families (Fig. 8-10). However, some of the intraspecific *L. decidua* families outgrew several of the interspecific hybrid families while the intraspecific *L. leptolepis* families belonged to the poorest growing families. None of the two commercial *L. decidua* check lots reached the level of the hybrids. The statistical analysis revealed significant differences between families and types of mating. Four of the interspecific crosses between the two larch species had their reciprocal crosses included in the trial. This gives the best information on differences among the interspecific families. From Fig. 8-11 it is seen that the cross *L. leptolepis* x *L. decidua* outperformed the reciprocal *L. decidua* x *L. leptolepis* in all four cases.

An interspecific crossing experiment with *Populus tremuloides* (Pts) and *Populus tremula* (Pta) was designed with four types of mating, Pts x Pts, Pts x Pta, Pta x Pts, and Pta x Pta. The progeny trial was established near Rhinelander in northern Wisconsin, USA. For each type of cross a 4 x 4 mating design was aimed at. Only one family was obtained from the Pta x Pta crosses. Therefore, only the results from the other three types of mating were reported. Figure 8-12 reveals that the two interspecific hybrids showed a superior height and diameter growth compared to the mean of the 16 Pts x Pts families. The stem volumes of the two interspecific hybrids were 5.2 and 6.6 times higher than the intraspecific crosses. Even if the Pta x Pta crosses failed it is most likely that the cross *P. tremuloides* x *P. tremula* leads to hybrid vigor.

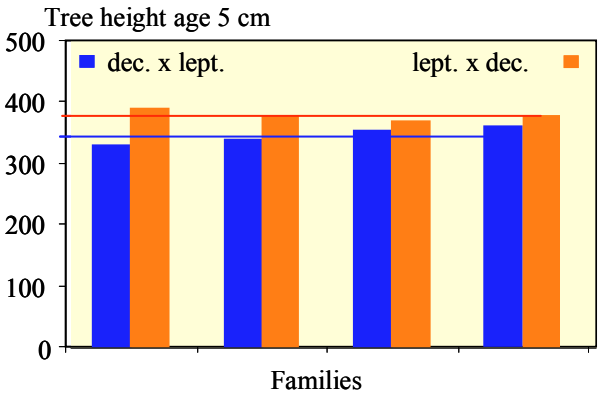


Figure 8-11. The tree height of interspecific crosses with Larix decidua x Larix leptolepis *and their reciprocal crosses at age 5 in a progeny trial in Maine at latitude 45.50°N. The means for the two types of hybrid are given.*

These examples show that hybrid vigour may occur following interspecific matings. However, it is unlikely that all interspecific hybrid families will outgrow the corresponding intraspecific families (cf. Fig. 8-10).

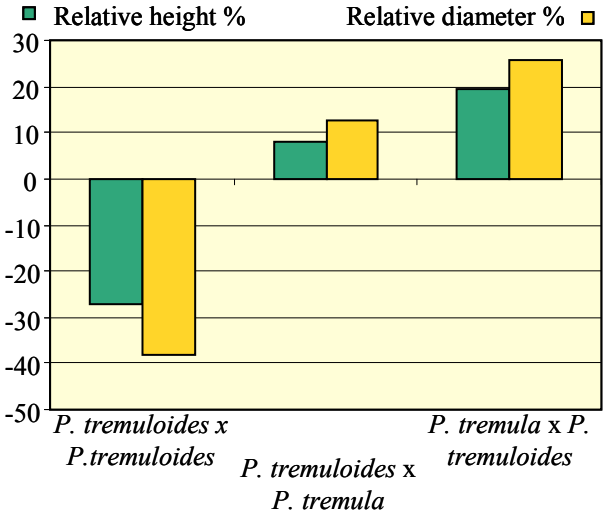


Figure 8-12. Mean relative height and diameter at age 3 of two interspecific crosses between P. tremuloides *and* P. tremula *as well as* P. tremuloides *crosses. The trial was established in Rhinelander, Wisconsin, USA; latitude 45.67°N and 89.42°W. The tallest mean height was 156 cm and the widest diameter was 21 mm.*

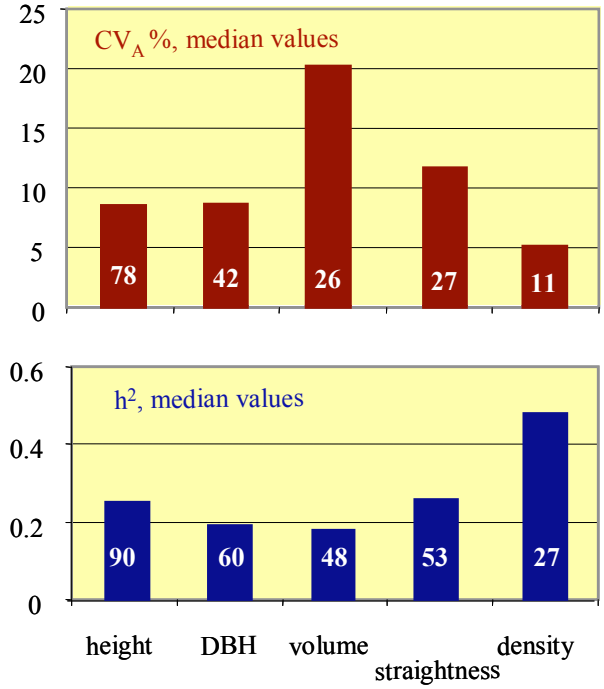


Figure 8-13. A summary of estimates of heritability and coefficients of additive variation in tree species based on a literature review by Cornelius from 1994. The figures in the bars indicate the number of studies behind each trait and parameter.

Heritabilities and coefficients of additive variation

In breeding programs estimates of heritability, h^2, have taken a prominent role and more recently estimates of the coefficient of additive variance, CV_A, have been published. It should be noted that in most breeding programs estimates of h^2 and CV_A were based on a phenotypically limited part of the entire populations. Therefore, the estimates may be lower than they would be if there had been a representative selection of parents in the tested populations. In other cases the estimates are based on plus trees from different populations and if they are of a wide origin, the estimates may be inflated with a strong population effect and thus exaggerate the true estimate for a single population.

In a review article from the early 1990s Cornelius summarized published data on h^2 and CV_A for tree species

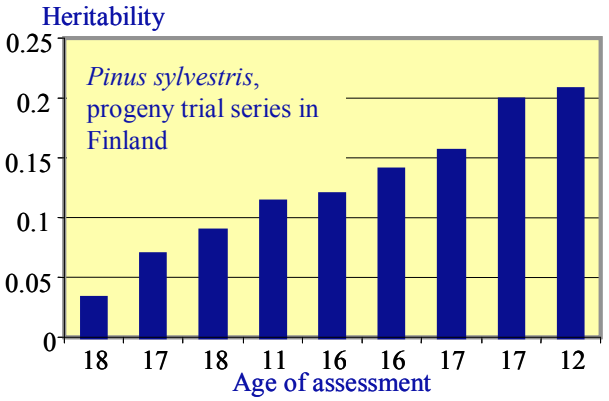


Figure 8-14. Tree height heritability estimates from progeny trial series with Pinus sylvestris *in Finland. The age of assessment is given for each series. Based on Matti Haapanen's thesis from 2002.*

(Fig. 8-13). From this figure it is seen that the mean values for heritability of the growth traits were approximately 0.20 while it was about twice as high for wood density. It should once more be stressed that heritability is valid for the population under study as well as the ambient conditions prevailing at the test sites. This figure also reveals that the growth traits have at least twice as large CV_A estimates as density. This means that the prospects for genetic gain in growth are generally higher than for gain in density even if the heritability is twice as large for density as for growth.

Finland is a country with a large number of *Pinus sylvestris* progeny trials. In a thesis from 2002 results for tree height measurements from several series of Finnish progeny trials were summarized (Fig. 8-14). The assessments took place at different ages, 11-18 years. The heritability estimates varied between 0.033 and 0.21. The variation could to some extent be attributed to the relative impact of G x E interaction. Thus the lowest heritability estimate was noted for the series with the highest ratio of family x site interaction variance to family variance, and conversely the highest heritability estimate was noted for the series with one of the lowest values for this ratio. Within the same series of trials, heritability estimates were obtained at various ages, only the heritabilities for the latest assessments being shown in Fig. 8-14. There was no clear age trend in the heritability estimates. In some cases the heritability increased with age, in others it decreased.

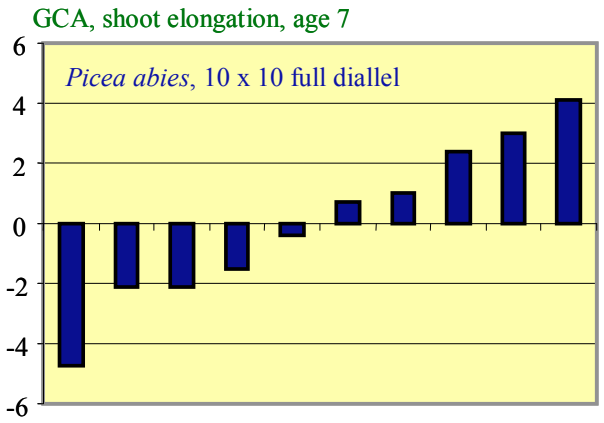


Figure *8-15. General combining abilities for the obtained plant elongation at a certain date at age 7 of the 10 parents in a full-diallel mating of* Picea abies.

In Norway Jon Dietrichson initiated a study of variation in three domestic populations of *Picea abies* by carrying out all possible crosses between 10 trees in each population. In Fig. 8-15 an example of results for the percentage of plant elongation at a certain date during the 7th growth period is illustrated. As seen from this figure the difference in breeding values was not extremely large, but still statistically significant. An early growth cessation means that the growth period is not fully utilized. One of the conclusions from this Norwegian study was that the variation within individual populations for growth rhythm traits was larger than the variation among populations.

A few examples from warmer climates will be given to illustrate that estimates of genetic parameters are not only limited to tree species from boreal and temperate forests.

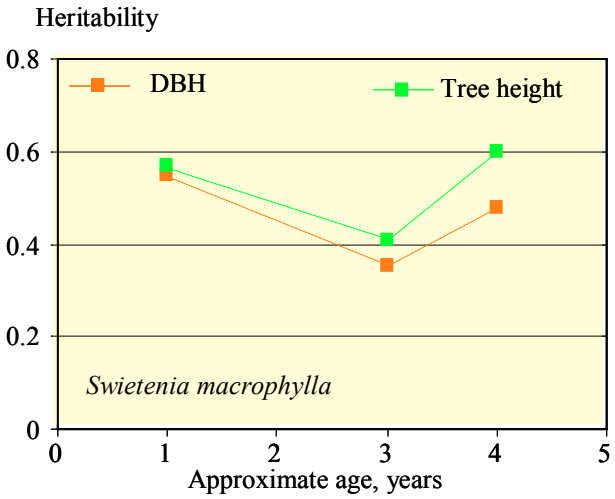


Figure 8-16. The development of the heritabilities for tree height and breast height diameter at different ages in a Costa Rican progeny trial (Lat. 10.95°N, 84.70°W) with 91 open-pollinated families Swietenia macrophylla *originating from Central America in the latitudinal range 7.33-19.45°N.*

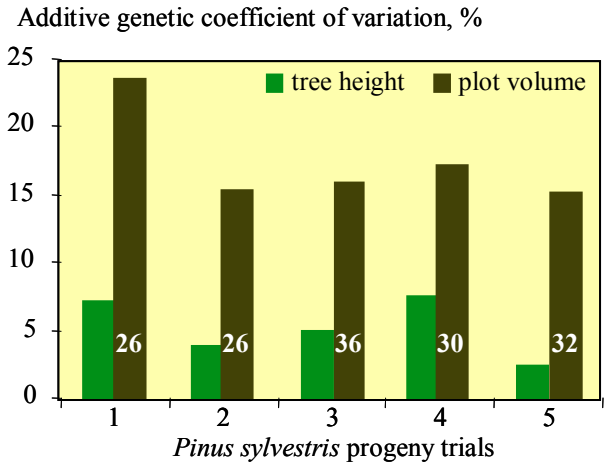


Figure 8-17. The additive genetic coefficient of variation for tree height and volume in 6x6-tree plots at latest assessment in five progeny trials with Pinus sylvestris *in southern Sweden. Ages of assessment are indicated in white.*

In a progeny test with 96 OP-families of *Pinus caribaea* in Brazil (Lat. 20.3°S 370 masl) high heritability estimates for growth traits at age 14 were reported; 0.44, 0.28, and 0.43 for height, DBH, and stem volume.

In a Costa Rican progeny test with 91 open-pollinated families of *Swietenia macrophylla*, mahagony, high heritability estimates were reported for growth traits as illustrated in Fig. 8-16. The OP-families originated from Central America in the latitudinal range 7.33-19.45°N. The annual precipitation varied strongly, 1200-3500 mm. The large heritabilities may partly be attributed to the broad range of climatic conditions at the growth places of the female trees. Another contributing factor was the observation that progenies from isolated trees had poorer growth. This was attributed to inbreeding in such trees.

In Sweden some of the *Pinus sylvestris* progeny trials have 6x6-tree plots. In five such trials the additive genetic coefficient of variation was estimated for tree height and plot volume at ages 26-36 (Fig. 8-17). As seen from this figure the CV_As were much higher for plot volume than for tree height suggesting that breeding for increased plot volume would be rewarding. It should be added that there was a slight trend for decrease of CV_A over time (7-36 years) both for tree height and plot volume.

The number of studies with estimates of parameters for quality traits is limited owing to the low age of most trials. An example from a trial in which heritabilities both for growth and quality traits were estimated, is shown in Fig. 8-18. Some of the exterior quality traits had low heritabilities while the tracheid length at ages 11 and 31 showed high heritabilities.

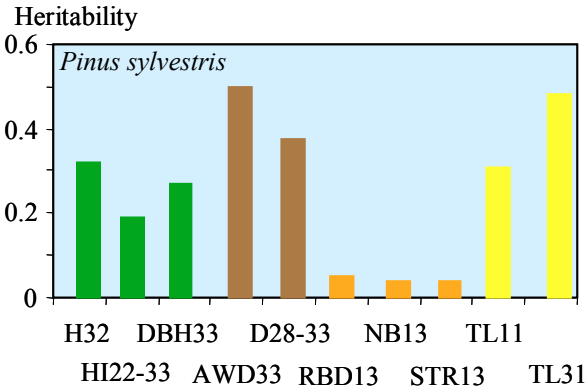


Figure 8-18. Heritability of some growth and quality traits at different ages. H = tree height, DBH = breast height diameter, HI = height increment, AWD = area-weighted wood density, D = wood density, RBD = diameter of largest branch in whorl 5/diameter of stem just below this whorl 5, NB = number of branches in whorls 4 and 5, STR = straightness, TL = tracheid length.

An example of estimates of parameters for frost tolerance is given in Fig. 8-19, which is based on freeze testing of *Pinus sylvestris* full-sib families from a partial diallel mating design with twelve parents. The large effect of the general combining ability is conspicuous while the specific combining ability was of limited importance.

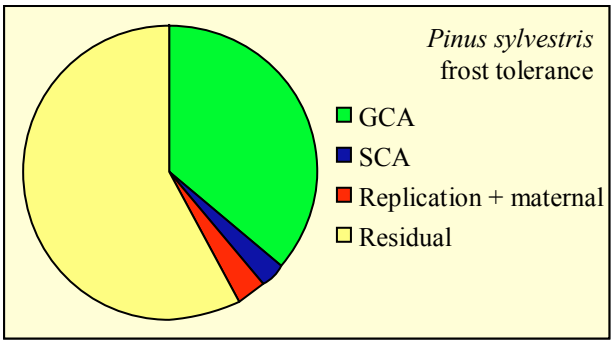


Figure 8-19. Frost tolerance percentage variance components for general combining ability, GCA, specific combining ability, SCA, residual and pooled variance components for replication and maternal effects.

Several studies in the border area genetics-physiology were carried out in growth chambers in Uppsala, Sweden (Pictures 8-2 - 8-4). In growth chambers, plants can be exposed to various photoperiods (day/night length) temperatures, nutrient and watering regimes. Plant and tree growth is a complex trait that may be decomposed into several components (cf. Fig. 8-20). At high latitudes there are two major components, growth rate and duration of the growth period. Growth rate may be split into nutrient efficiency, water use efficiency, and photosynthetic efficiency.

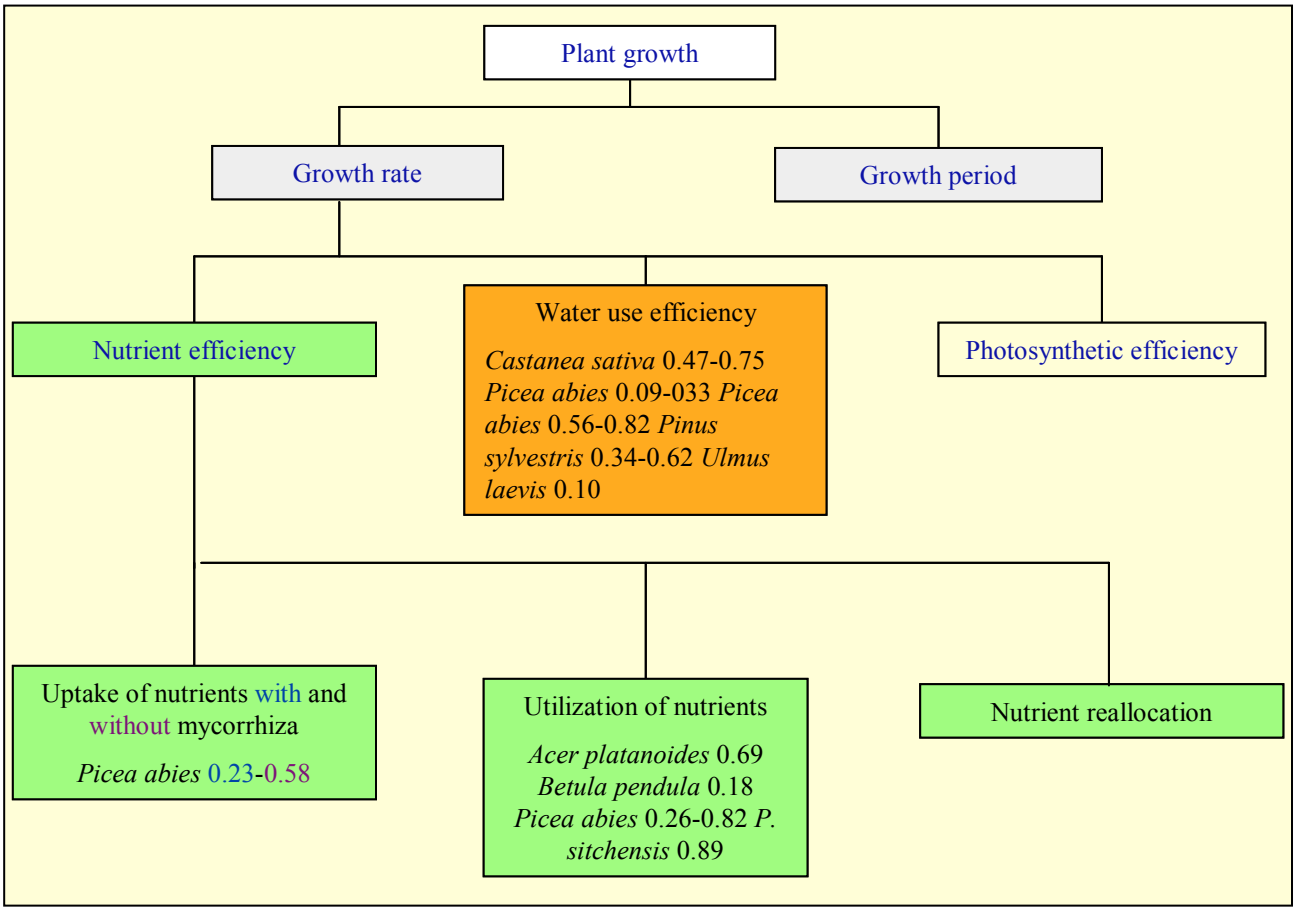


Figure 8-20. A compilation of heritabilities for water use efficiency, uptake of nutrients, and nutrient utilization based on studies in the Uppsala phytotron with various tree species.

Free access Restricted access

Picture 8-2. Two-year old seedlings of Norway spruce exposed to free access of a balanced nutrient solution and restricted access of nutrients pipetted daily during the growth period. Photograph Per Lindén.

High N High N+M Low N +M Low N

Picture 8-3. Plant growth after 5 weeks in a study of variation in uptake of nitrogen in Norway spruce seedlings. Treatments from left to right, free access of nitrogen, free access of nitrogen + mycorrhiza Laccaria bicolor, *strongly restricted access of nitrogen + mycorrhiza* Laccaria bicolor, *strongly restricted access of nitrogen. Photograph Per Lindén.*

Nutrient efficiency may be further sub-divided into uptake of nutrients, utilization of nutrients once the nutrients are inside the plant, and reallocation of nutrients. Separate estimates for each nutrient element may be obtained for each component of nutrient efficiency. Several of these components were studied by our group in the Uppsala phytotron and the range of heritability estimates for these components were obtained for several tree species. In many cases the estimates of heritability (Fig. 8-20) and CV_A were high. The Norway spruce families that had the poorest growth at low nitrogen level in the treatment without mycorrhiza benefitted most from mycorrhizal association. This explains the lower heritability in the treatment with mycorrhiza. It should be noted that only juvenile plants can be studied in growth chambers. If these components are regulated by different sets of genes it may be possible in breeding to combine several of these components in one progeny. Even combinations that have never existed can be obtained in breeding if the sets of genes regulating different growth components are identified. In agreement with results for *Pinus contorta* the heritability estimates in many cases reached much higher levels than ever reported for growth traits from field experiments. It should be noted that the estimates in Fig. 8-20 (previous page) were not inflated by any population effect. An example of an experiment with two nutrient regimes, free access and restricted access, are illustrated in Fig. 8-21. All *Pinus contorta* populations originated from

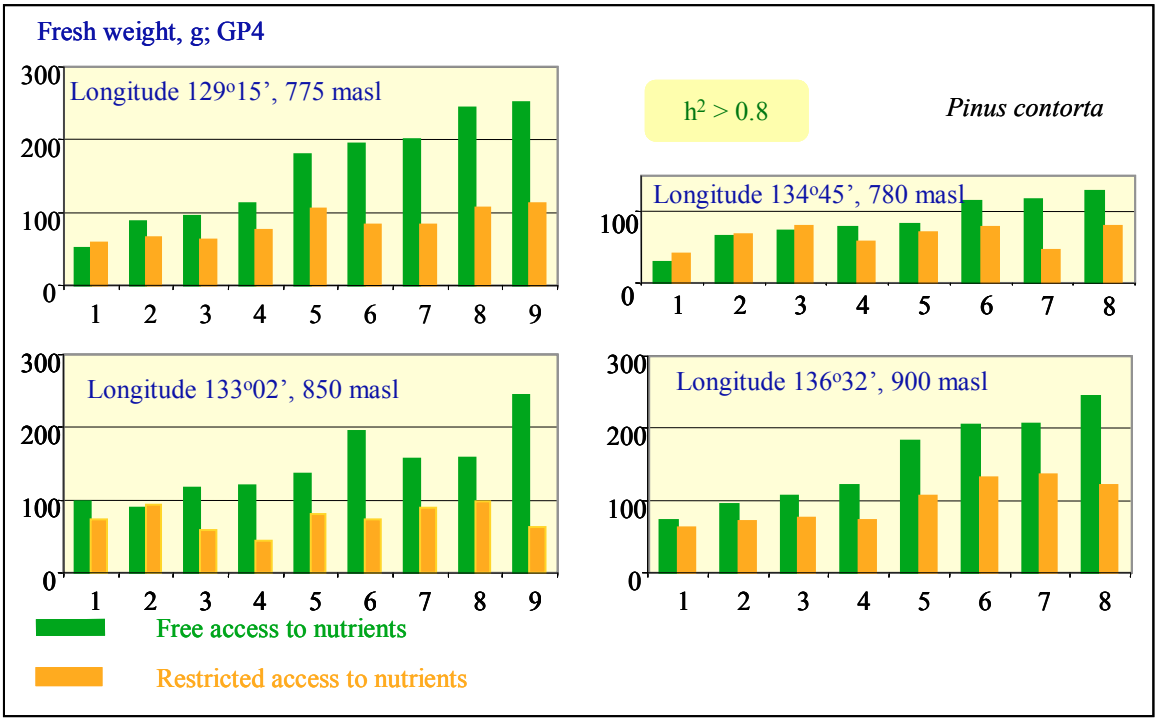
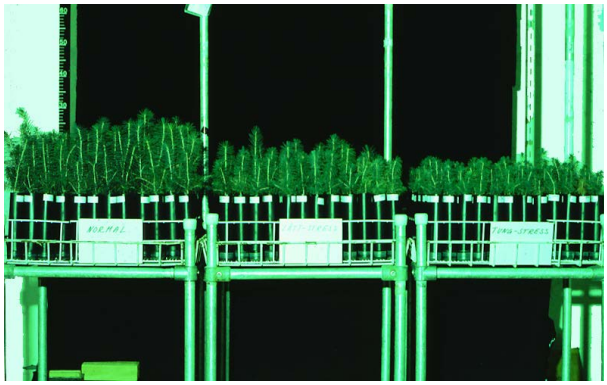


Figure 8-21. Within-population variation in fresh weight after growth period 4 (GP4) in four Pinus contorta *populations from the same latitude, 60°N, but varying longitudes in Canada. Two treatments were applied, free access and restricted access of nutrients.*



Picture 8-4. An example of early evaluation of drought tolerance of young Norway spruce seedlings. Plants on the left truck had free access of water, the plants on the centre truck were exposed to moderate drought while the plants to the right experienced a severe drought. Photograph Per Lindén.

approximately the same latitude and the same elevation. There was a strong response to free access of nutrients in all populations. One reason for the comparatively poor growth of the population from longitude 134°45' may be attributed to some inbreeding owing to its isolated occurrence and small size, 2 hectares. In spite of the low number of progenies per population the heritability estimates were high, > 0.80. The heritability of plant height at age 6 in field trials with the same plant material was ten times lower, 0.08. Heritability estimates are usually

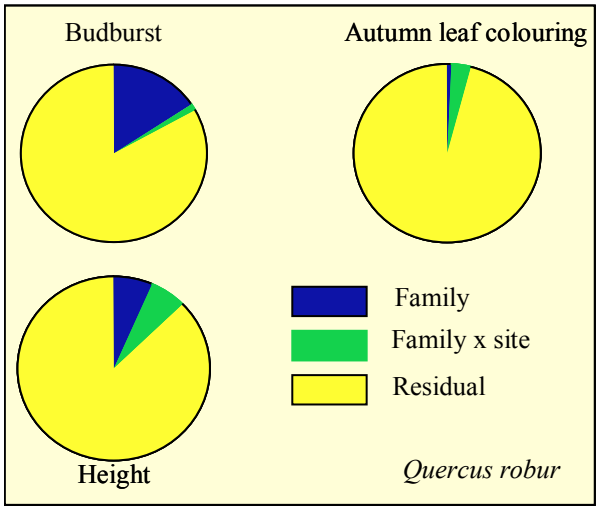


Figure 8-22. Variance components for family and family x site effects for budburst, autumn leaf colouring, and height growth in Lithuanian Quercus robur *populations.*

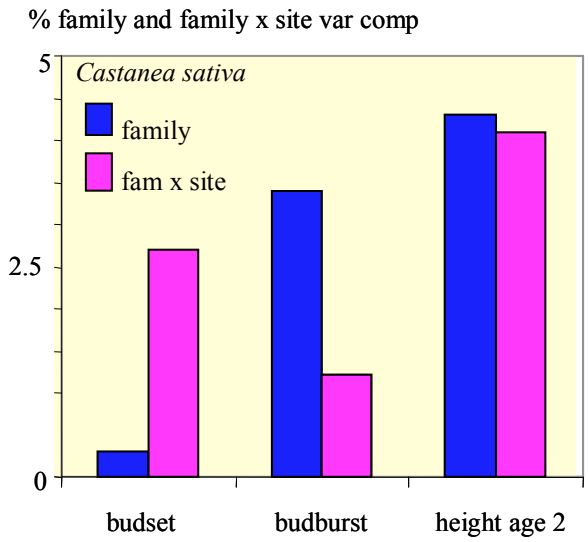


Figure 8-23. Family and family x site interaction variance components for budset, budburst , and plant height in juvenile material of Castanea sativa.

high for material tested under controlled conditions. This must be attributed to the uniform conditions in controlled environments that lead to low phenotypic variance. Since this variance is the denominator of the heritability it explains the high estimates of heritability under these uniform conditions.

In a Lithuanian series of progeny trials with *Quercus robur* juvenile growth, budburst, and autumn leaf coloring were studied. As seen from Fig. 8-22 the family variance component for budburst was several times larger than the component for family x test site, which means that there are good possibilities for these oak populations to respond to changes in climate by a change in time of budburst. There are limited possibilities for change of autumn coloring of leaves in these populations. Autumn coloring is correlated with building up of frost tolerance during autumn.

The results from a series of progeny trials with *Castanea sativa* in Spain, Italy, and Greece are similar to the oak results (Fig. 8-23). Thus the ratio *family variance component/family x test site variance component* was highest for budburst. It is noteworthy that the plant height family variance component and the family x site interaction variance component were of equal size. Usually there is a negative relationship between these two components. Such a negative relationship is clearly indicated for budset.

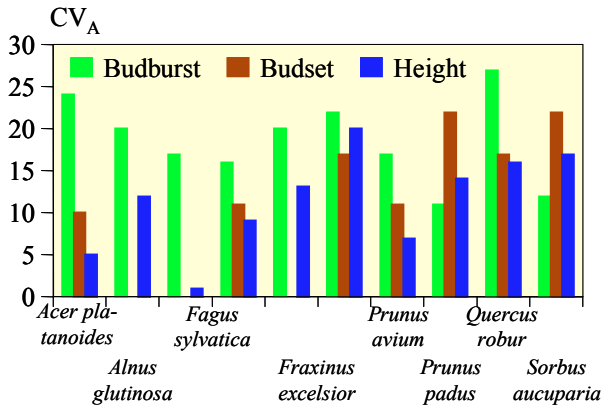


Figure 8-24. Coefficients of additive variation for budburst, budset, and plant height of deciduous tree species with varying combinations of life history traits. Based on the thesis by Virgilijus Baliuckas.

Broad-leaved tree species with different combinations of life history traits were studied with respect to budburst, budset, and juvenile height in nursery experiments by Virgilijus Baliuckas. Distribution; continuous or scattered, wide or limited; pollen vector; and stage in ecosystem are examples of life history traits. These studies were conducted to test if life history traits influence the variation within and between populations. According to the hypothesis species with a continuous distribution with wind pollination over large distances, such as *Quercus robur,* are expected to have larger within-population variation than species with contrasting life history traits, *i.e.* scattered distribution and a pollen vector transporting the pollen over short distances, such as *Acer platanoides*. The results as regards the coefficient for additive genetic variation are compiled in Fig. 8-24. A CV_A of 20 must be regarded as promising for breeders to change the trait by selection. Similarly, such a value is beneficial for future adaptation in nature if changes occur in the environment. In many of the species the highest CV_A values were noted for budburst while CV_A for height never reached 20. There is no clear tendency that the hypothesis outlined above is true. It ought to be remarked that a comparison of the species is not totally straightforward since the populations studied were represented with different numbers of trees and the distribution of collection localities was different. Thus, *Fagus sylvatica* is limited to the mildest climate in Sweden while *Alnus glutinosa* has a much wider distribution, which means that *F. sylvatica* had to be sampled from a much smaller climatic range than *A. glutinosa*.

Genetic correlations

Estimates of genetic correlations are of great significance for breeding. For northern Scandinavia the relationship between frost tolerance and growth traits is crucial for breeding success. An example for *Pinus sylvestris* in northern Sweden is given in Fig. 8-25. Contrary to the

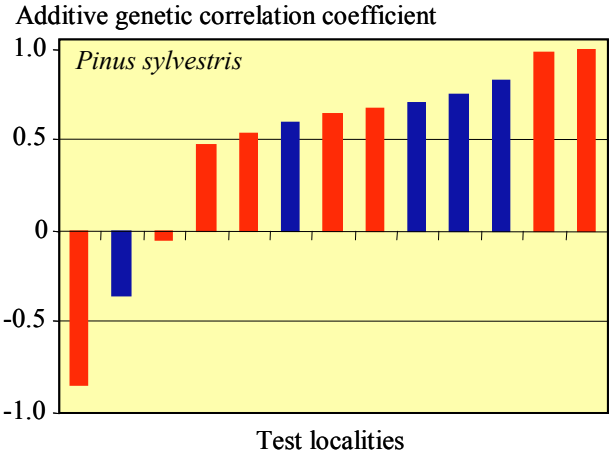


Fig. 8-25. The family genetic correlation coefficients for the relationship between tree condition and tree height in test localities belonging to four different series of trials. Test localities in northern Sweden with a temperature sum > 700 degree days are shown in red and localities with a temperature sum < 700 are blue.

negative correlations at the population level most correlations at the family level were positive. The damage caused by the fungus *Phasidium infestans*, which only hits trees under snow cover, may explain the positive relationship. Trees with good growth should thus contribute to increased survival.

Much attention has been focused on the possibilities to predict field survival from freeze testing under controlled conditions. As seen from Fig. 8-26 there were only three of the nine correlations that exceeded 0.50. The *a priori* expectation is that there should be stronger relationships at low survival than at high survival. At high survival the death of trees is more due to random events rather than genetic factors. However, there was no trend that the correlations should decline with increasing survival.

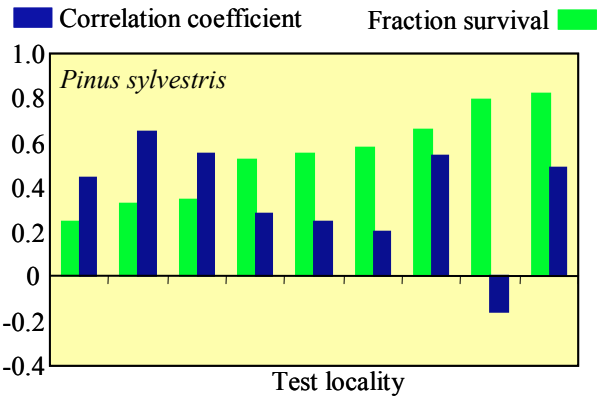
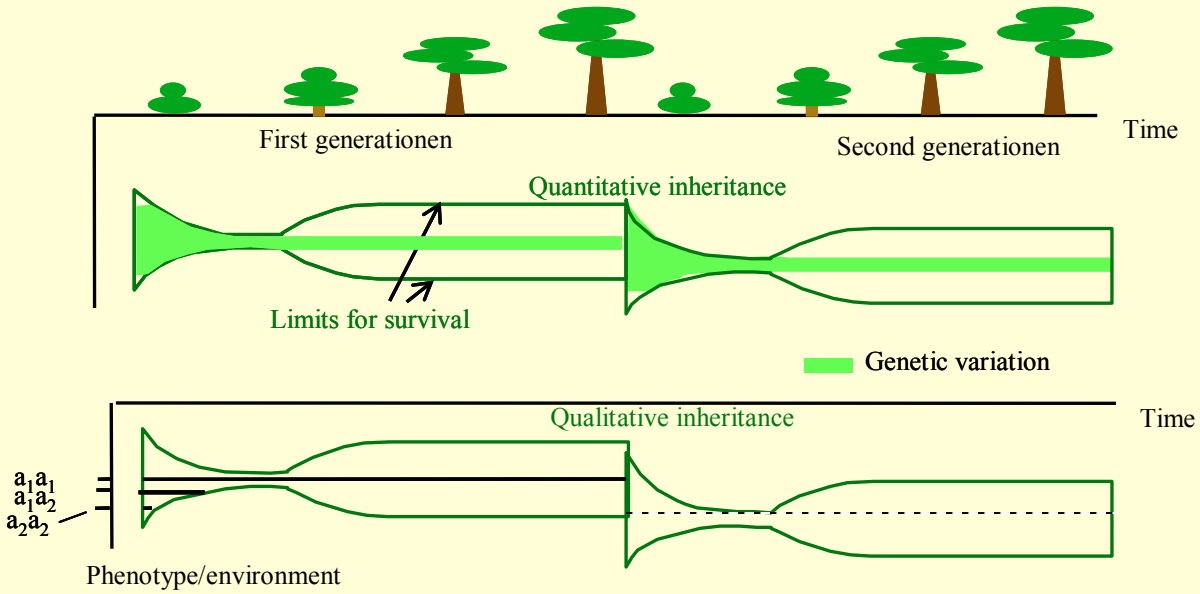


Figure 8-26. The fraction surviving trees in nine Pinus sylvestris *field trials and genetic correlation coefficients for the relationship between field survival and frost damage after freeze testing.*

Box 8-1. Advantage with quantitative inheritance in traits with high fitness values



Naturally regenerated material of Scots pine in northern interior Sweden has a large genetic variation. In this part of Sweden the plants are exposed to extreme strains during late winter when they have reached a size of approximately one meter. Once the trees have emerged from the snow cover during late winter the large amplitude in temperature between day and night may be harmful to plants, which respond rapidly with physiological activities upon the high day temperatures. Depending on the ambient weather conditions the bottleneck will take different positions along the vertical environmental scale. During these critical years the genetic variation will be narrowed considerably. When the plants have developed into trees the environmental conditions no longer constitute a strain to them. Plants which were culled during the phase of establishment would if they had survived be able to grow and even outcompete some of the trees that passed the bottleneck unharmed.

At the next occasion for regeneration the segregation of genotypes is different and so is the position of the bottleneck. Thanks to the broad segregation there are plants able to pass the new bottleneck.

What would happen if alleles at just one locus had been responsible for the survival, *i.e.* survival showed qualitative inheritance? To illustrate this we have assumed that the three genotypes in one locus take different positions on the environmental scale. In order to be able to pass the second bottleneck it is required that either a_1a_2 or a_2a_2 pass the first bottleneck to have the required a_2a_2 genotype for the second bottleneck. Since neither a_1a_2 nor a_2a_2 passed the first bottleneck the population would not give rise to a second generation and thus become extinct. If the inheritance is quantitative there is a large segregation and some individuals would guarantee the continued survival of the population

Why is there such a large within-population variation in *Picea abies* and *Pinus sylvestris* and many other tree species?

The large within-population variation described above seems to reflect poor adaptedness of the populations studied. Unique for many tree species is the long generation time. This means that a tree during its lifetime will experience large annual fluctuations in weather conditions and even climatic changes. For these reasons it might be an advantage to have a large variation around a mean value such that there are always some genotypes well-adapted to the conditions prevailing at the time of regeneration. Expressed in another way, there is a trade off between high adaptedness in the short-time perspective and the potential for response to changes in a long-time perspective. A prerequisite for a large segregation is that the traits of adaptive value are quantitative. Quantitative inheritance per se might be of adaptive significance in long-lived tree species. Natural selection changes allele frequencies in different directions depending on the ambient conditions, which promotes large within-population variation (Box 8-1 and the discussion on stabilizing selection in Chapter 6).

Summary

Large within- population genetic variation for many traits of adaptive significance occurs in most tree species. The estimates of heritability and coefficient of additive variance, CV_A, for growth traits, including plot volume, vary from low to moderate in field trials. The heritability for wood density was in many cases high but the CV_A was low. In one artificial freeze testing comprising full-sib families from twelve parents the general combining ability was several times higher than the specific combining ability. The heritability in growth chamber studies was usually a few times higher than in field trials, which must be attributed to the uniform conditions in growth chambers. For most traits of adaptive significance there are good prospects for genetic change via natural selection or breeding. The American chestnut, *Castanea dentata*, does not seem to have any tolerance against chestnut blight, *Cryphonectria parasitica*. Most *Ulmus glabra* families are highly susceptible to Dutch elm disease, *Ophiostoma novo-ulmi*, which is a great constraint to improvement of tolerance against this disease. Encouraging correlations between field tree condition and growth were noted for some field trials. Correlations between freeze testing damage and field survival were weak to moderately high.

Further reading

Baliuckas, V. 2002. Life history traits and broadleaved tree genetics. Silvestria 258. Acta Universitatis Agriculturae Sueciae.

Cornelius, J. 1994. Heritability and additive genetic coefficient of variation in forest trees. Can. J. For. Res 24:372-379.

Eriksson, G. and Jonsson, A. 2005. Adaptability to nutrient availability, water availability, and temperature in seven tree species - cultivation under strict control in growth chambers. In Recent Res. Dev. Genet. Breed. 2:25-43.

Haapanen, M. 2002. Evaluation of options for use in efficient genetic field testing of Pinus sylvestris (L.). Finn. For. Res Inst. 826. METLA.

Forest tree breeding

General questions related to forest tree breeding are first presented. Then selection of species and the principles of long-term forest tree breeding are discussed. Finally, operative aspects of selection of plus trees, seed orchards, mating design and observed gains in tree breeding are presented.

What should be considered before the start of a breeding programme?

Several aspects, both genetic and non-genetic, must be considered before a breeding programme is established. First of all the objective(s) of the tree breeding programme must be identified. In many countries it might seem self evident that the objective is to produce the raw material for saw mills and the pulp and paper industry. Less evident is that breeding might be focused on production of material for amenity forests. In Iceland, which was once covered with much larger forests than today, there is a great interest in extending the forests to non-forest land. The use of forests for prevention of erosion is another breeding objective. Related to this is the use of forests as lee plantations. Christmas tree cultivation and street tree improvement are of economic importance in several countries (Pictures 9-1 and 9-2). As a consequence of the varying objectives of forest plantations, different selection critera must be used to build up breeding populations that will meet the different objectives.

Of greatest importance is of course the economic value of the products obtained from the tree species included in tree breeding activities. This value must be weighed against the investment in staff and materials that are required. If it is assumed that the species even on a long-term basis will have a considerable economic value it is motivated to plan for long-term breeding. All around the world there are many long-term breeding programmes.

In Fig. 9-1 different intensities in the improvement of ornamental trees and shrubs in Sweden are presented. At the lowest intensity only identification of good seed stands takes place. For Norway maple, which is of great importance for various urban plantations and landscaping, there is an economic incentive for the establishment of seed orchards.

Of primary interest in any breeding programme is to decide which traits should be improved. The more traits that are included in the improvement programme, the harder the breeding activity. If we assume that one tree per 100 is carrying a trait and the traits are uncorrelated, one million trees (1/100 x 1/100 x 1/100) are required to find one tree with the desired combination of all three traits. If the traits are positively correlated, the tree with the desired traits might be found among a lower number of trees.

Picture 9-1. A Christmas tree plantation of Pinus virginiana *in Texas, USA. Photograph Gösta Eriksson.*

Picture 9-2. Beautification of cities. Street trees in Raleigh, NC. USA. Photograph Gösta Eriksson.

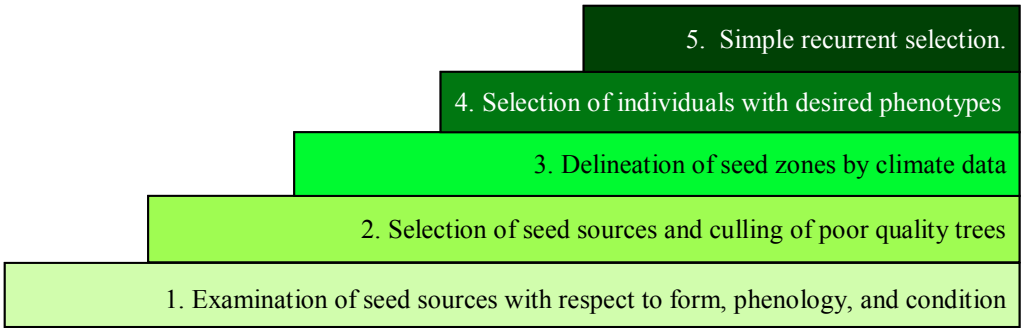


Figure 9-1. Different levels of improvement for ornamental trees and shrubs used in various types of landscaping plantations in Sweden.

When the traits for improvement have been identified, it is important to estimate the genetic variation in these traits and the mode of inheritance of each trait. Estimates of the additive variance or the additive coefficient of variation are important, since the additive variance can be exploited in mass selection. When the additive variance is known, we can calculate possible genetic gains. If the proportion of non-additive genetic variance is considerable, the breeding becomes more complex.

Knowledge of flowering biology is a prerequisite for a successful breeding. Without flowering no breeding can be carried out, and it is important to know the conditions that promote flowering. This is probably easier to trace in the boreal and temperate zones with their seasonal change.

Flowering phenology, *i.e.* the occurrence of different phases of flower development over time, should also be determined in order to be able to predict the probability for matings within seed orchards or in other plantations aimed for seed production. Pollen dispersal is a factor of great significance for predictions of matings with pollen from unbred forests in the surroundings of the seed orchard. Contamination with unbred pollen generally reduces the genetic gain in the seed produced in proportion to the amount of contamination (Fig. 9-2). In certain cases it is more serious, as will be discussed later on in this chapter.

Norway spruce, Scots pine and many other conifers carry both female and male strobili on the same tree. Other species such as ash and aspen are monoecious and usually carry one sex only. Theoretically, selfing may occur in many tree species. As is evident from Chapter 5 selfing is mostly accompanied by a pronounced inbreeding depression. The North-American red pine and yellow cedar are exceptions to this. Certain species such as the birches have self-sterility alleles which prevent selfing. A tree with the self-sterility alleles s_1 and s_2 does not form any seeds if the pollen grain contains either of the alleles s_1 and s_2. It does not matter whether the pollen originates from the same tree or another tree; the female tissue prevents fertilization with pollen containing these alleles. Conifers do not seem to have self-sterility alleles. Instead they have varying numbers of lethal alleles. Besides, polyembryonic embryos are frequently formed in conifers. This means that there is frequently competition among embryos such that only one forms a viable embryo in each seed.

If a decision is taken that the breeding should be of long-term character it is important that there is a stable tree breeding organisation that lasts for decades. Without such stability there is a high risk that short-term problems are given priority at the expense of long-term and perhaps less glamorous tasks.

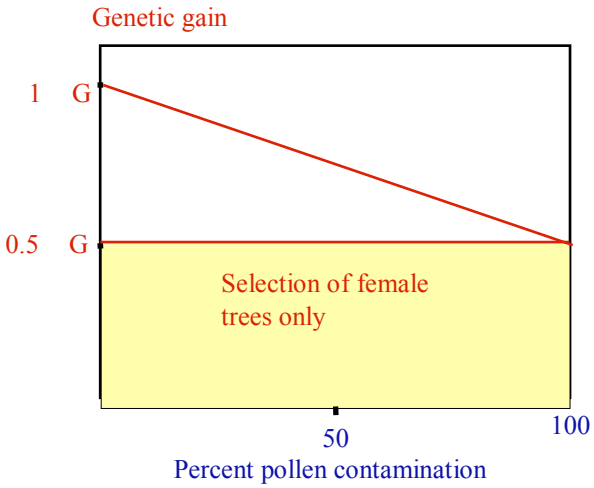


Figure 9-2. The relationship between genetic gain, ΔG, and the percentage pollen contamination. At 100% contamination, only the gain obtained from selection of female trees remains.

Various types of tree breeding

Forest tree breeding may be structured in many ways, one of them being shown below.

Selection
- species level
- provenance level
- population - stand level
- ividual tree level

Breeding to combine desired traits
Polyploidy breeding
Breeding using mutations, molecular markers, and genetic engineering

Generally, breeding aims at combining useful traits from different parents via matings among them. This is followed by selection of the best performing trees in the progeny. Selection at either levels without crossing can hardly be regarded as breeding in a strict sense. In spite of this we treat introduction of exotic species in this chapter. Provenance research was extensively treated in the Chapter 7. As was stressed in that chapter, forest tree breeders hardly distinguish between populations and provenances when seed is collected in natural forests. A major focus in the rest of the chapter is on selection of ividuals or plus trees, *i.e.* trees with desirable phenotypic characteristics (Picture 9-3), and how matings among plus trees should be done to improve breeding. Before coming into species and plus tree selection and their breeding we will briefly comment on polyploidy breeding and mutation breeding.

Polyploidy occurs frequently in higher plants and has played an important role in agricultural plant breeding. In several cases the polyploids in a genus are larger than the diploid species of that genus. Polyploidy also had a leading role for establishment of the Swedish forest tree breeding. In 1935 the famous wheat breeder, Herman Nilsson-Ehle, detected a giant aspen tree in a forest in southern Sweden. It proved to be a triploid. Nilsson-Ehle envisaged polyploidy breeding of Norway spruce and Scots pine in Sweden to obtain giant trees of these species. He convinced influential foresters that Sweden ought to have an organised tree breeding, and there has been such an organization since 1936. One of its first tasks was to produce polyploids of Norway spruce and Scots pine. Triploid trees of these two species did not grow into giants but rather they were dwarfs. Different genera have different ploidy optima. Certain grass species have their optima as hexaploids while the optimum for Norway spruce and Scots pine is evidently at the diploid level.

Picture 9-3. An excellent plus tree of Eucalyptus grandis *growing in Australia. Photograph Gösta Eriksson*

Mutation breeding raised great expectations during the 1950s and 1960s. These expectations were mainly linked to the hope that certain chemicals would bring about mutations at particular loci. Mutation breeding has the best prospects in highly bred crops, in which breeders might be interested in a change at one locus. If this could be achieved the breeders do not need to use the labour demanding back crossing over 7 - 8 generations to transfer one specific allele into the crop. Back crossing means that the original parent is used as one mating partner over several generations and selection for the desired trait takes place in each generation (Fig. 9-3). Owing to the long generation time of forest trees the back crossing technique is hardly possible. Mutation breeding is of little or no value for most forest trees, though it has had some importance in changing flower colours in ornamental plants. In many respects mutation breeding and allele transfer via gene technology are similar. One difference is that a modern molecular geneticist knows which allele he/she transfers to a recipient whereas the induction of mutations is brought about blindly.

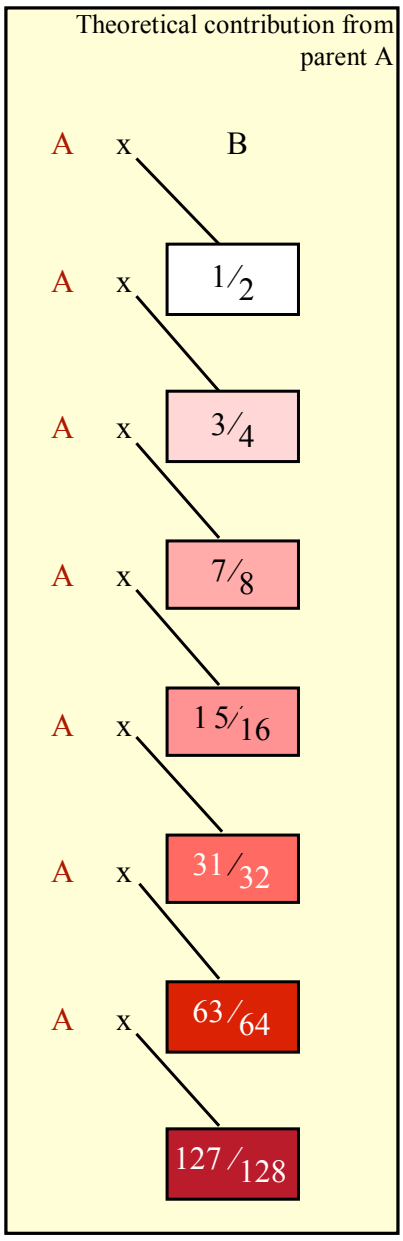


Figure 9-3. The theoretical contribution of the A parent to the offspring over several generations in a back crossing programme.

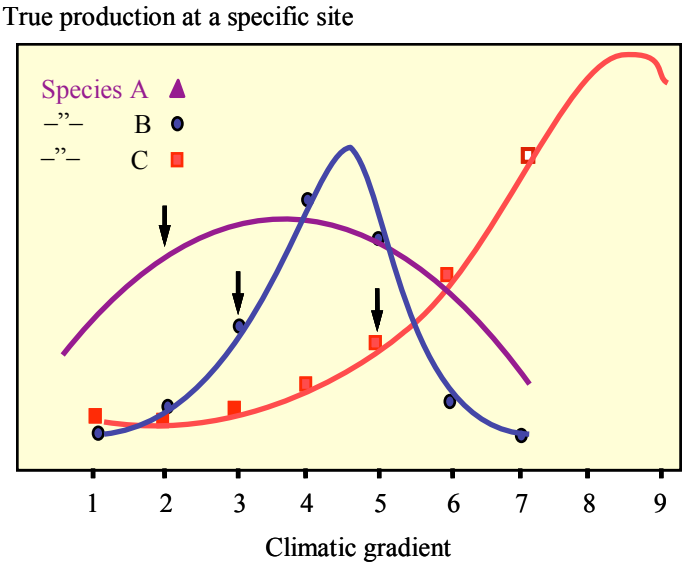


Figure 9-4. Illustration of the importance of having several provenances of each tree species in species trials. For further information see the text.

Species selection

In Scandinavia the flora is poor owing to the short time since the last glaciation. This is pronounced for forest trees. In consequence we may not have the tree species that would give the best yield. It is motivated to compare the performance of domestic trees with the performance of exotic tree species. At the start of tree cultivation in developing countries it is useful to evaluate which species should be included in breeding programmes. For this information, species trials are required.

When establishing species trials it is urgent to carry out a careful selection of the provenances that should be included. An idle selection of provenances may cause misleading results as illustrated in Fig. 9-4. In this graph the true production of different provenances of species A, B, and C is given over an environmental gradient. In Fig. 9-4 the provenances tested are shown as filled symbols. Among the tested provenances, the one coming from test locality 4 belonging to species B gives the best test result. Since we know the true production we also know that the best production can be brought about by species C. However, the proper provenance of species C was not tested. If there had been only the three provenances marked with arrows in the species trial, the result would have been still further away from the truth.

The conclusion that might be drawn from Fig. 9-4 is that species trials must have several provenances of each species to be meaningful. If a species has not shown a maximum such as is the case for species C in the graph it may be questioned whether we have complete information on the ranking of the species. From the previous chapter it is evident that Norway spruce and Scots pine show pronounced clines, which is why we expect that introduced species originating from climatic conditions similar to the Scandinavian also show clinal variation. If the knowledge about provenance differences in a domestic species is as good as it is for Norway spruce and Scots pine in Scandinavia it is easy to select the provenances of a domestic species for species trials. In such situations one or two provenances might be sufficient. A larger number of provenances of the exotic species that should be tested ought to be selected. They should be selected from areas with similar climate and edaphic conditions to those of the test area.

In summary, species trials require large test plantations at more than one locality. The researcher requires great intuition and skill to select the proper test localities and provenances to be included in the experiment. Only then can we expect to get accurate information about the potential of different species.

Large test plantations mostly mean that it will be hard to find sufficiently uniform localities. One way to overcome this problem is to employ a two-step strategy. In the first step, growth rhythm studies are carried out in greenhouses or nurseries. After evaluation of data from the first step there ought to be information on the provenances that have potential for a certain test locality. Such tests are of particular value when frost damage significantly interferes with growth. In Scandinavia frost damage occurs frequently. Large savings may be achieved by running a first step species trial in a nursery before the costly field trials are established. Thanks to this the test plantations do not need to be as large as they would have been without this pretesting. The probability of finding small homogeneous test plantations is much larger than of finding large ones. Thus, there are several advantages with this two-step strategy.

History

The history of the Swedish forest tree breeding will be used to illustrate some of the thinking in many breeding organisations during the early stages of tree breeding. Even if an organised tree breeding was established in 1936 it took until the mid 1940s before large scale selections of plus trees took place. Scions were collected from the plus trees and grafting was done. The grafts were later planted in seed orchards for commercial production of seed. For further improvements, crosses were carried out between the plus trees in the seed orchards. The progenies were raised and planted in progeny trials. Normally such a trial contains several progenies. Progenies are frequently referred to as full-sib or half-sib families depending on the type of cross used to create the progeny. One major objective of the progeny trials is to estimate the genetic value of the parental trees. Thus, the parental tree genetic quality is revealed by its offspring in well designed experiments. Parents are selected for new seed orchards based on the evaluation of the progeny trials. Such a selection cannot be carried out more than once or twice since we soon reach a situation where there are no more parents to select among and the number of trees in the breeding population would not reach a satisfactorily large N_e (Fig. 9-5). Gradually it became evident that the best trees in the best families had to be selected. Scions are then collected from these trees for establishment of the second generation seed orchards. When progeny-tested parents are used for establishment of new seed orchards, American forest tree breeders call them one and a half generation seed orchards.

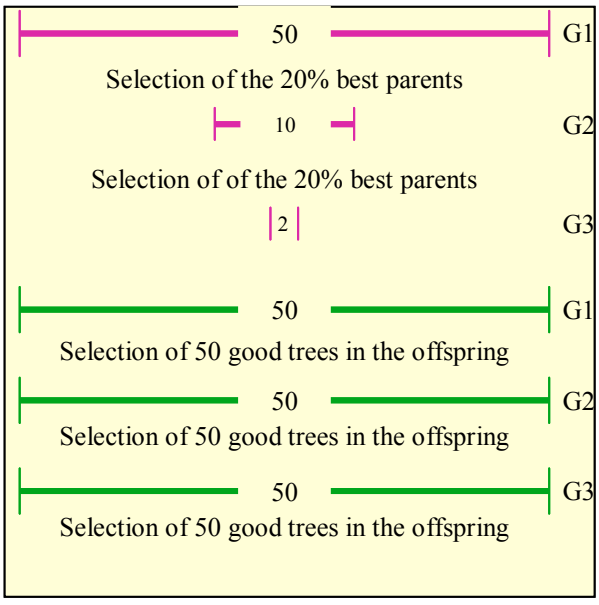


Figure 9-5. Recurrent selection of a certain fraction of parents makes additional selections after a couple of generations impossible. To have satisfactory size of the population, selection must be carried out in the progeny.

Another way of mitigating the reduction in number of trees in the breeding population is to select plus trees in unbred native populations. However, this means that the gain achieved from earlier selection will be lost to a certain extent (see Fig. 9-6). The loss is proportional to the portion of unbred material that is inserted into the breeding population. If only 50 % of the trees have passed previous selection and breeding, the gain will drop to half of what is possible if the most advanced bred material is used. Insertions from the wild become less attractive the higher the degree of breeding. The same can be said about pollen contamination from the wild.

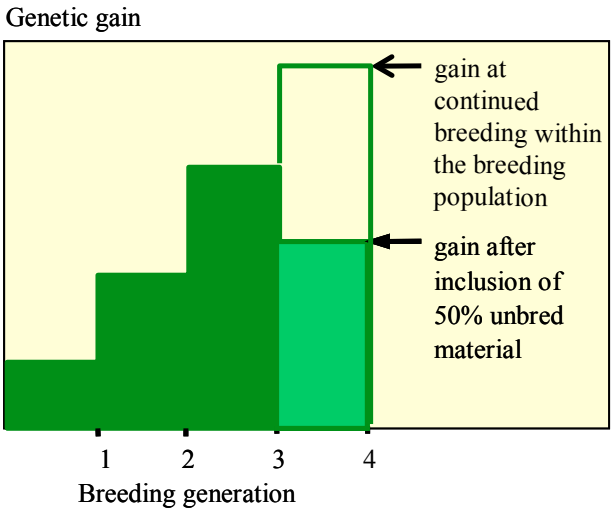


Figure 9-6. Inclusion of unbred material in the breeding population after a few generations of breeding causes a drastic reduction of the genetic gain.

Long-term breeding

Long-term breeding might be envisaged as a cyclic course of events, in which crossings, establishment and evaluation of progeny trials, selection of trees/plants for the next generation of the breeding population based on the evaluation, and planting of grafts of the selected trees are the components of this circle (see the left part of Figure 9-7). For each completed cycle the material for cultivation has been improved. Theoretically we have three options for exploitation of the improvement in the breeding population. We may establish seed orchards for seed production, establish clonal archives for production of cuttings, or produce plants via tissue culture.

Population functions

To enable an understanding of breeding and its consequences for genetic erosion it is important to clarify the function of different populations that might be distinguished in Fig. 9-7 (See Box 9-1). The core of a breeding activity is the breeding population which is to be found in the cyclic part of Fig. 9-7. Seeds from seed orchards or vegetativly propagated plants from clonal collections constitute the production population, *i.e.* they are the starting material for wood-producing forests, if the production of wood is the breeding objective. Generally the production population is the population that should produce human utilities whether it is biomass or beautification. The starting material for the production population is the propagule population. Seed orchards, clonal archives, and plant material for tissue culture propagation are all components of the propagule population. It should be noted that one and the same seed orchard simultaneously might function as a breeding population and a propagule population. In the former case the seed orchard is used for crossings, the resulting seeds giving rise to seedlings, which are established in progeny trials. The role of seed orchards as propagule populations is fullfilled when seeds are produced for sowing in nurseries or for direct seeding in forests.

Box 9-1 Functional types of populations

Breeding population: the collection of trees that will carry the advancement of breeding into future generations

Gene resource population: the seeds, acorns , nuts, plants, or trees that are included in the gene conservation

Production population: the trees that will produce human utilities

Propagule population: the trees or plants utilized in sexual or vegetative propagation

To guarantee a sustainable gain in the breeding work a high additive variance is required in the breeding population. In Box 9-2 it is illustrated schematically why in a long-term perspective it might be a disadvantage to have few trees in the breeding population. We can have a lower genetic variation in the production population without loss of cultivation security.

Recurrent selection

In multiple generation breeding of crop plants three dif-

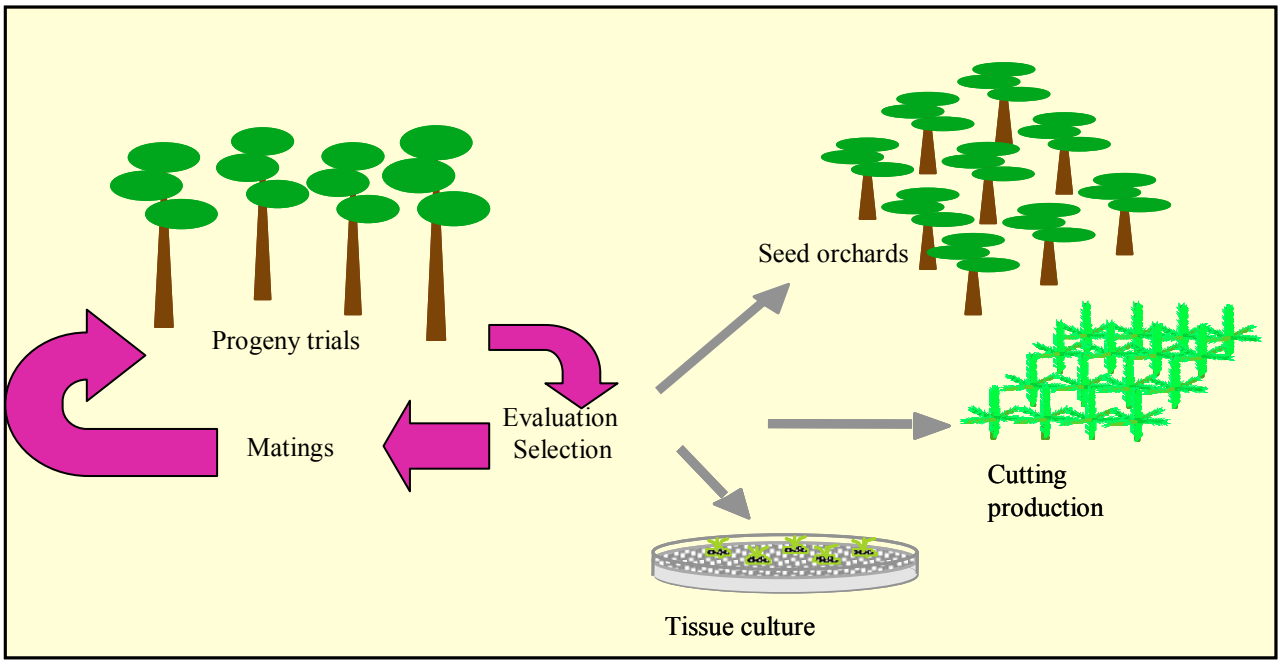


Figure 9-7. The principle of forest tree breeding with estimation of breeding values, selection, matings and progeny trials in a cycle as well as generation of material in three different ways for the production forests. (Somewhat modified from an idea by Öje Danell.)

Box 9-2 The need for a large material

Tree	Chromosome 1	2	3	4	5	6	Sum of + signs
A	-/-	-/-	+/+	+/+	+/+	-/-	6
B	+/-	-/-	+/+	+/-	-/-	-/-	5
C	+/-	-/-	+/+	+/+	+/-	-/-	5
D	+/-	-/-	+/+	+/-	+/-	+/-	5
E	+/-	-/-	+/-	-/-	+/-	-/-	4
F	+/-	-/-	+/+	+/-	-/-	-/-	4
G	-/+	+/-	+/-	-/-	-/-	-/-	3
H	-/-	-/-	-/-	-/-	+/-	+/-	2

Many sexually propagated tree species are diploid and thus have two chromosomes of each kind. In the hypothetical example there are six pairs of chromosomes in 8 individuals, A to H. Alleles affecting the trait in a positive way are given + signs while alleles affecting the trait in a negative way are given – signs. For each locus there are three possibilities: ++, +-, and --. For simplicity we assume that the effect of + and – signs are the same in all loci.

On these assumptions the trees with the highest number of + signs grow tallest or have the best stem quality or any other trait that the + sign represent. In our case tree A have most + signs and it may be designated as a plus tree. The unfortunate situation is that it is homozygous ++ in three loci. The long-term breeding aim of ++ in all six loci (although the breeder will not be able to detect that) can only be accomplished in our case by inclusion of trees D and G, which complement each other with respect to + signs in all 6 loci. The final aim of 12 + signs will obviously take several generations.

The example tells us the following:

•The plus trees are not necessarily the best for long-term breeding

•Many trees are required to enable an enrichment of all positive alleles

Based on an idea by Gene Namkoong

ferent types of recurrent selections have been applied: simple recurrent selection, recurrent selection for general combining ability, and reciprocal recurrent selection. Recurrent means in our case that something is repeated over and over again in a cyclic way as is illustrated in Figure 9-7. For the most complex recurrent selection it is difficult to illustrate the different components in a cyclic way. Therefore, we prefer to show all three types of recurrent selection as linear flow charts to facilitate comparisons among them (Figs. 9-8 and 9-9).

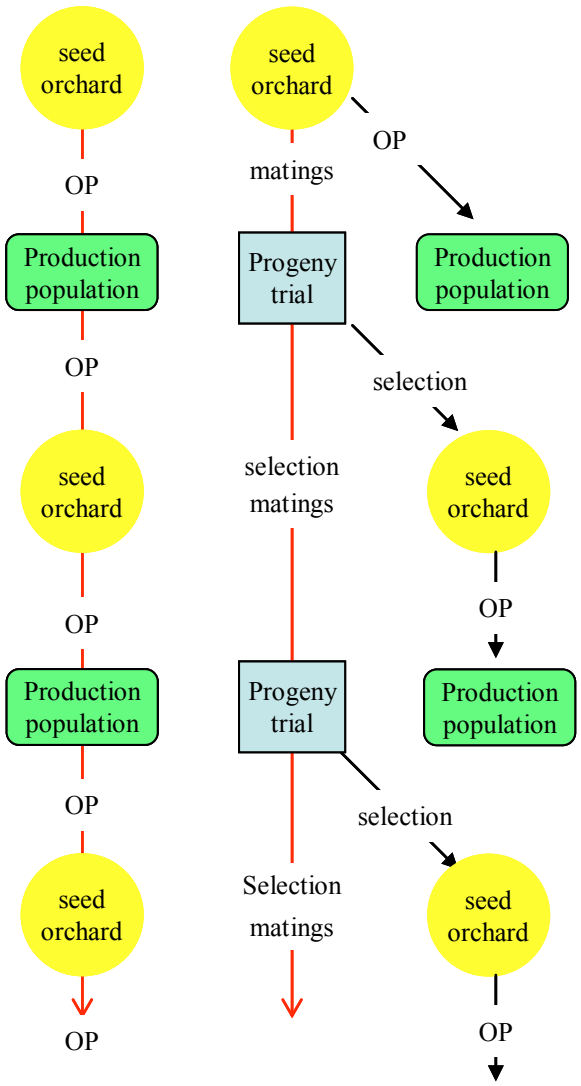


Figure 9-8. The principles of simple recurrent selection to the left and recurrent selection for general combining ability to the right. OP = open-pollination.

Simple recurrent selection is not any intensive type of breeding (Figure 9-8). The seed from seed orchards is used to raise seedlings in nurseries or for direct seeding in forests to establish a production population without any pedigree. The best trees in the production population are selected for establishment of a new generation of seed orchards and the process is repeated again. When funding for breeding is limited this is one option that can be used.

In recurrent selection for GCA, matings are carried out for establishment of progeny trials. Open-pollinated seed is used to establish a production population. The progeny trials are evaluated and the best trees in the best families are selected and crosses among them are carried out. The offspring is planted in progeny trials. The selection is also used for establishment of a new seed orchard with genetically improved material. Open-pollinated seed from such seed orchards is used for establishment of a new generation of the production population. Many intensive breeding programmes use recurrent selection for GCA.

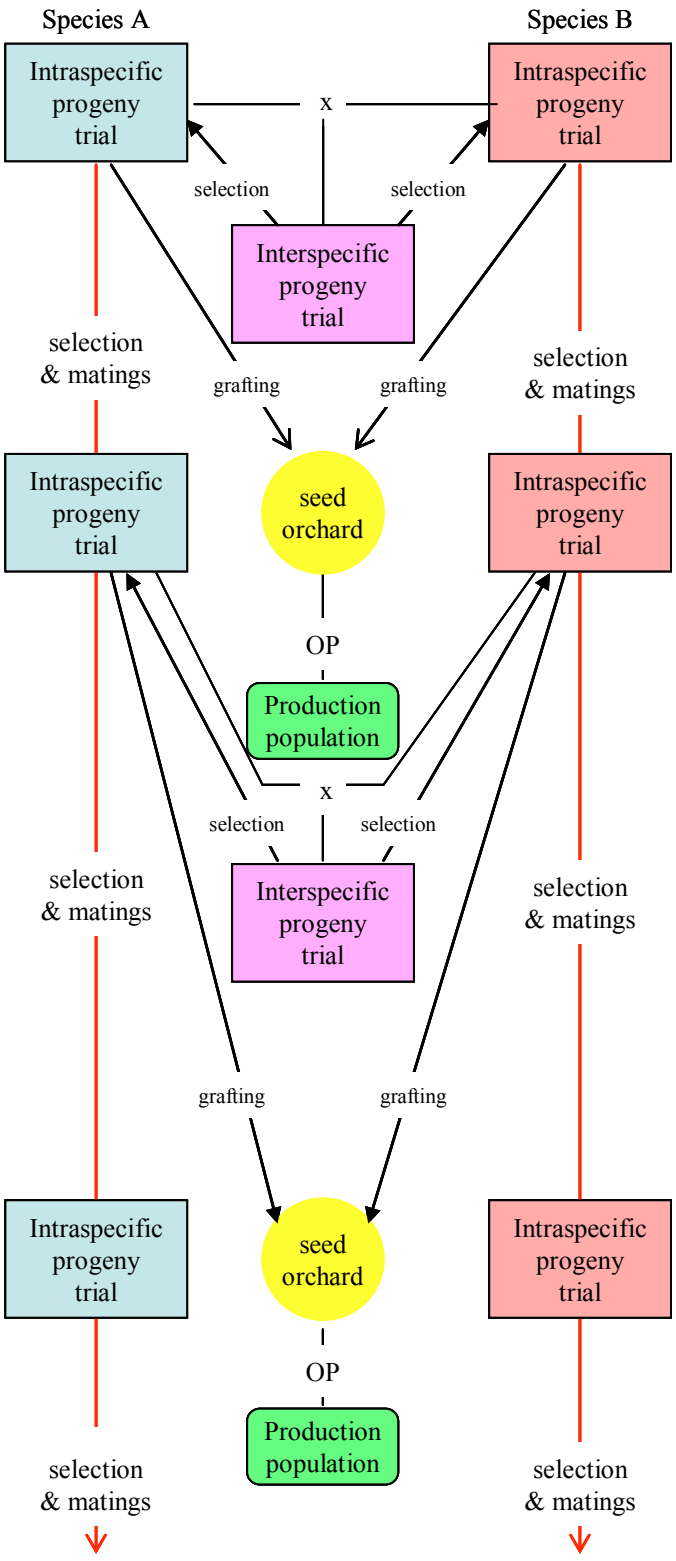


Figure 9-9. Reciprocal recurrent selection; for further explanation see text.

Reciprocal recurrent selection is the most complex form of recurrent selection. Since it is mostly used in species hybridization we have illustrated it for such a case (Fig. 9-9). Based on the evaluation of progeny trials of the two species, trees are selected for interspecific matings. The hybrids obtained are used for establishment of interspecific progeny trials. The data from this type of trial are used for selection of parents that give good interspecific hybrids. Thus selected trees are used for establishment of the seed orchard that should produce the seed for the production population. The selected parents are also used for intraspecific matings to generate material for intraspecific progeny trials. Selection of parents is then carried out in these intraspecific progeny trials. These parents are used for interspecific mating and the process is repeated again. As may be seen from Fig. 9-9 in this type of recurrent selection it takes two generations to obtain the seed for the production population. For this reason it is not much used in forestry. In South Korea two north American pines, *Pinus rigida* and *Pinus taeda*, were introduced for hybridization. The former species is hardy but has a bad stem form. The latter species does not have a satisfactory hardiness for this part of the world but has an acceptable stem form. Therefore, efforts are taken to combine hardiness and growth form in the interspecific hybrids. The breeding programme for this interspecific hybridization is carried out according to reciprocal recurrent selection. This is a typical case of species hybridization used to combine two good traits from each of the parental species.

It should be stressed that Figs. 9-8 and 9-9 show the principles of the three types of recurrent selection. In practice modifications of them occur.

Multiple Population Breeding System

One of the major problems in breeding is that the high priority breeding objectives of today may be of limited value when it is time to harvest the gains from tree breeding. Another factor of great uncertainty is that the environmental conditions may change dramatically over a rotation time of 50 - 150 years. Changes of the reforestation and silvicultural methods will take place with high probability over such a period. To this must be added the environmental change, which to some extent is beyond human control. Today when climatic change is probably a fact, the forest tree breeder faces great problems. Unlike the cultivation of cereals there is no possibility to change cultivars every or every second year. An effective forest tree breeding programme ought to be designed such that it matches the future changes in breeding objectives and environmental change. The American forest geneticist, Gene Namkoong, developed a breeding concept that essentially fulfils these requirements. His concept means that the breeding population is subdivided into smaller subpopulations instead of being kept as one big breeding population. His concept of breeding is called the Multiple Population Breeding System (MPBS). The subpopulations

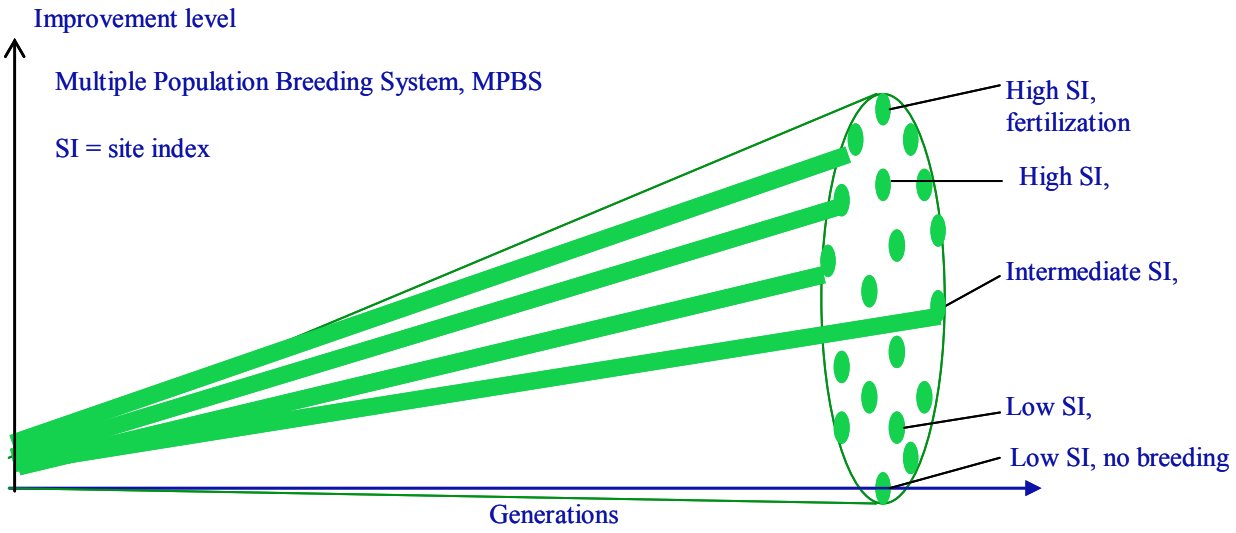


Figure 9-10. Schematic illustration of the splitting of the breeding population into some 20 subpopulations according to the Multiple Population Breeding System concept.

are preferentially planted over a broad span of site conditions (cf Fig. 9-10). The target trait might be the same in all subpopulations or a stronger emphasis might be given to stem quality rather than biomass production in some of the subpopulations. The MPBS means that disruptive selection takes place among subpopulations. The MPBS concept is adopted in the Swedish breeding programmes for silver birch, Lodgepole pine, Norway spruce, and Scots pine. As is seen from Figure 9-11 different subpopulations will be distributed to various combinations of temperature and photoperiodic conditions. A world-wide inventory during 1999 showed that the MPBS concept is adopted in many breeding programmes.

Each subpopulation should have 50 trees, which may seem a low number. If the entire breeding population has 20 subpopulations the N_e becomes much larger than 50. At such an effective population size there are few alleles lost for random reasons unless they are extremely rare. In Fig. 9-12 the minimum number of trees required to save one rare allele per locus is illustrated for three different cases; one rare allele in each of 10, 50, or 100 loci. As is seen from this figure the allele frequency plays a greater role for the minimum number of individuals that ought to be saved than the number of loci with rare alleles. To be sure that alleles at a frequency of 0.01 and higher will be saved only a few hundred trees are required.

The inbreeding that may take place at N_e = 50 trees amounts to 1 % ($F = 1/2N_e$) and will not cause any inbreeding depression of importance. In various breeding programmes with other organisms than forest trees sustainable gains over 50 generations have been obtained at population sizes lower than 50. If the selection of plus trees considers the adaptation that might have taken

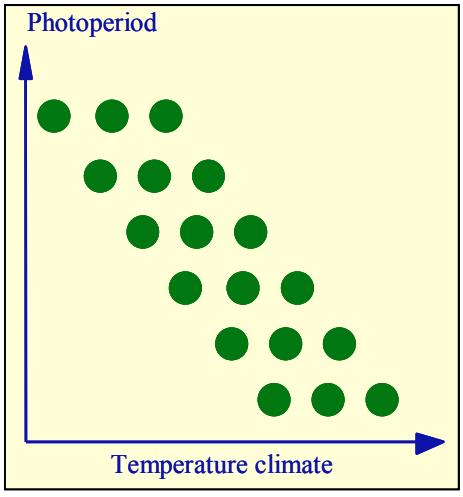


Figure 9-11. Principle illustration of the design of the Swedish tree breeding with subpopulations distributed under different temperature and photoperiodic conditions.

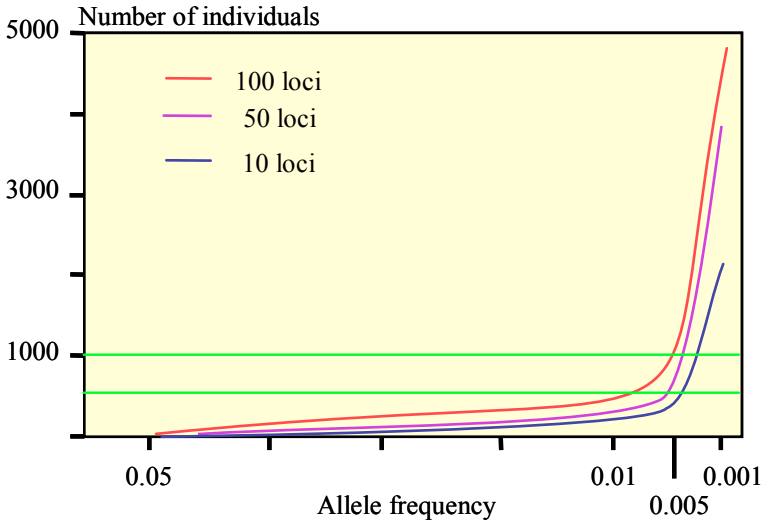


Figure 9-12. The minimum number of individuals required to save one rare allele at each of 10, 50, or 100 loci.

place under different site conditions, the probability of including rare alleles increases. An allele might be rare at species level but be more frequent in a subpopulation of the species thanks to its contribution to fitness in this subpopulation.

With subdivision of the breeding population it is no longer the case that only *one* population passes through the circle in Fig. 9-7, rather each subpopulation passes through the circle. The speed with which the subpopulations pass through the circle will probably vary depending on the site conditions or the breeding goals of the individual subpopulations. In all subpopulations the larger part of their additive variance will be kept while the additive variance among subpopulations will increase. This is an ingenious system which in its simplicity guarantees an increased additive variance and which simultaneously offers possibilities for changes of breeding goals. Finally, recurrent selection for general combining ability is mostly used within each subpopulation.

In summary the main advantage of the MPBS is that it combines the capture of the total existing genetic variation with a satisfactory variation within each subpopulation and that it allows the target populations to adapt to the prevailing environmental conditions. Another advantage is that the speed of evolution might be faster in a population of 50 trees than in a large population containing thousands of trees.

Sublining

The above described MPBS should not be confused with sublining which is also a subdivision of the breeding population but in this case it is targeted for one breeding goal. The purpose of sublining is to avoid inbreeding in the production population. This is accomplished by selection of one clone from each subline for establishment of seed orchards for production of commercial seed. Inbreeding is in this case permitted in each subpopulation. The reason for launching this concept was that it was feared that it would not be possible to avoid inbreeding in the breeding population in a long-term perspective. This fear is probably exaggerated, at least in breeding populations with several hundred trees. It should be noted that sublining does not aim at an increase of the among-population additive variance which is in contrast to the MPBS concept.

Dag Lindgren has developed the concept of status number, which can be interpreted as the size of a population comprised of unrelated trees. Status number has given breeders a possibility to estimate the unrelatedness in the breeding population.

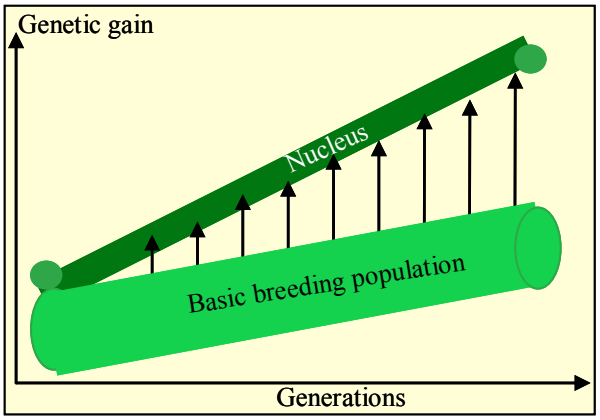


Figure 9-13. Schematic illustration of the genetic gain that can be obtained from the two populations according to the nucleus breeding concept.

Nucleus breeding

Another system for long-term breeding which has been applied in certain programmes is to split the breeding population into subpopulations of unequal size. The smaller nucleus contains 30 - 50 trees while the larger part keeps 300 - 400 trees. The most intensive breeding occurs in the nucleus, which has given the name nucleus breeding to this system (Figure 9-13). The objective is that gene conservation and long-term gain will be guaranteed in the larger subpopulation while the breeder profits from the larger gain that may be obtained in the smaller subpopulation. As is evident from Figure 9-13, the difference between the two subpopulations will increase over the generations and it will be tempting to concentrate all breeding efforts to the nucleus only. In some programmes which apply this system a transfer of material from the larger subpopulation to the nucleus is envisaged. The fear for inbreeding is also in this case the reason for the latter suggestion. However, it should be remembered that genetic gain is lost when material is taken from a lower level of breeding to a higher (Figure 9-6). As is the case for the MPBS method recurrent selection for general combining ability is mostly used within the two populations.

Short-term breeding

Whichever type of breeding that is selected it may be complemented with intensive breeding under a few generations to identify clones for elite tree seed orchards. Figuratively it can be seen as a means to skim the cream off the milk at the cost of narrowing down the additive variance. In principle it differs from long-term breeding in the number of trees included in the breeding operation and in that there is no long-term intention in this operation. The latter is a contrast to the nucleus breeding in which the elite part of the population is aimed for long-term breeding.

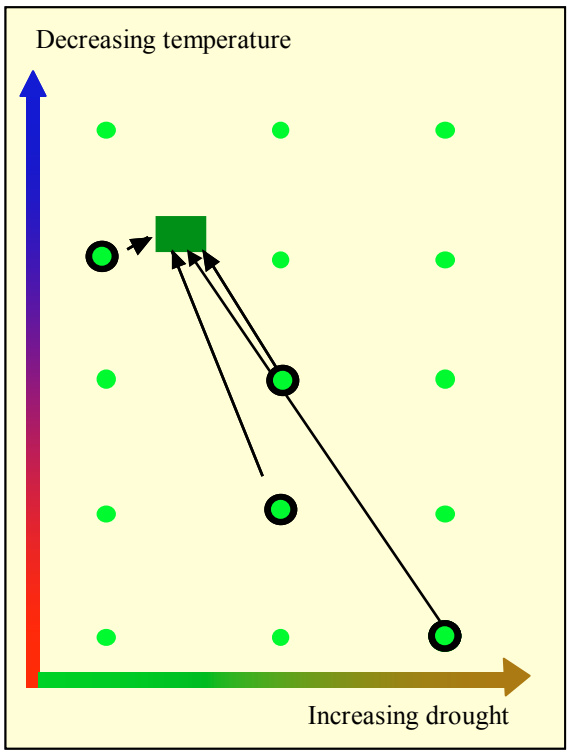


Figure 9-14. The principle for selection of populations for progeny testing in case of increased temperature and drought in future. Light green circles are populations. The dark green rectangle is a test locality and the encircled populations will be represented by single-tree progenies.

Mitigation of global change

One example how such a mitigation might be achieved is presented in Fig. 9-14. Acorns were collected from more than 20 individual trees in each of 30 populations for establishment of a series of combined provenance and progeny trials of cork oak all over the cork oak distribution area in the Mediterranean basin. Planting all 600 single-tree progenies would demand too large experimental test localities and was thus not feasible. Therefore, the combined trial was designed according to the principle illustrated in Fig. 9-14. Only in four of the populations were single-tree progenies included. Since we do not know the strength of drought and temperature change in future, populations were selected to include a few probable changes in these ambient factors. After evaluation, the progenies in the best performing population(s) can be utilized as a source for acorn production.

A concrete example of a breeding strategy

In reality breeding programmes may be more complex than the pure forms indicated in the above sections. In the breeding cooperative in New Zealand a new breeding strategy for *Pinus radiata* was launched in the late 1990s. It has elements of MPBS, nucleus breeding and sublining. It consists of two sublines, which are referred to as superlines. Each superline consists of a nucleus with a large main population and 7 subpopulations with separate breeding goals in accordance with the MPBS concept. The breeding goal of the main population is general improvement of growth and quality traits.
The breeding goals in 6 of the 7 subpopulations are:

- High wood density
- Structural timber, *i.e.* strong stiff, stable timber with small knots and low spiral-grain angle
- Clear cuttings, *i.e.* “clear wood from unpruned trees”
- Long internodes, good growth rate
- Good growth rate, low *Dothistroma* infection
- Excellent growth and form

The 7th subpopulation consists of introduced material from the Guadeloupe island. The breeding goal of this subpopulation comprises most of the goals in the other 6 subpopulations.

As seen from the list above, some subpopulations have breeding goals mostly related to timber quality, while others are related to good growth or resistance to *Dothistroma*, which is a serious disease in certain parts of the country.

To make the story still more complex, breeding in each subpopulation might be regarded as short-term breeding.

The main population is seen as a ”genetic insurance”. In addition to this there are specific gene resource plantings in New Zealand of the five existing provenances as well as a large number of clones in archives.

Selection of plus trees

In most intensive breeding programmes the scrutiny of plus tree candidates was rigorous during the original selection of plus trees. One problem was that the selection mostly took place in stands originating from self regeneration. In such a stand, the quality development is dif-

Picture 9-4. A plus tree of Scots pine from the complementary selection around 1980. The selection took place at about one third of the rotation age in a planted stand. SkogForsk archive.

ferent from the development in a planted stand, in which the bred material will grow. Planted stands usually have a much wider spacing than in naturally regenerated forests. Another problem with selection of plus trees in stands close to the end of the rotation time is that imperfections in the most valuable part of the stem might be hidden inside the trunk. In connection with a new selection of plus trees in Sweden during the 1980s it was decided that the new selections should preferably take place in planted stands which had reached a third of the rotation time (Picture 9-4). Thereby it was possible to carry out stem quality selection based on the economically most important part of the stem.

During the first plus tree selection, the growth of the plus trees was compared with the growth of the tallest trees in the same stand. Wood cores were taken to enable a correction for different ages of these comparison trees and the age of the plus tree. In spite of this it is difficult to carry out an unbiased selection in uneven-aged stands.

Picture 9-5. A seed orchard of Norway spruce with isolation bags. Isolation is taking place with isolation paper bags. Photograph Inger Ekberg.

Seed orchards

Seed orchards may be classified in different ways. One way is to distinguish between seedling seed orchards and clonal seed orchards. Another classification takes into account the type of material included in the seed orchard. There may be clones from two species, interspecific seed orchards, from two provenances, interprovenance seed orchards, or finally the clones may originate from one provenance, intraprovenance seed orchards.

Seedling seed orchard

This type of seed orchard is usually established as a progeny trial with seedlings raised from open pollination or controlled crosses. The aim is to use the best trees in the best families as seed producers. One disadvantage with this type of seed orchard is that progeny trials rarely stimulate abundant flowering. In many cases seed orchards are located in warmer climates to stimulate flowering. If the progeny trials are located in another climate this may result in selection of wrong clones owing to genotype x environment interaction. If this interaction occurs the best trees in the best families in the progeny trial are not identical with the best trees in the best families in the climatic zone in which the seed should be used.

Another disadvantage with seedling seed orchards is that flowering usually starts later in this type of seed orchard than in grafts in clonal seed orchards. Therefore, this type of seed orchard is most suitable for tree species with an early flowering. For species in which there are problems with union of the scion and the root stock it may be necessary to use seedling seed orchards. This kind of problem is designated as grafting incompatibility.

For sanitary reasons, seedling seed orchards of *Pinus contorta* were established since imports of scions of this species to Sweden is not permitted. In order to have an

Table 9-1. Different types of clonal seed orchards and their application in Scandinavia

Type of seed orchard	Application in Scandinavia	Disadvantages
Interspecific	For production of hybrid larch *Larix decidua x L. leptolepis L. decidua x L. sibirica.* Usually one clone of one of the species and several of the other species.	Species frequently have non-overlapping receptivity and pollen dispersal
Inter-provenance	Most old *Picea abies* seed orchards were of this type with varying number of Scandinavian and continental clones	The objective is to obtain provenance hybrids. Under the most favourable conditions 50% hybrids may be obtained, while the others are the result of matings among clones within each of the two provenances
Intra-provenance	This is the dominating type of seed orchard for *Picea abies* and *Pinus sylvestris*	Only the general combining ability can be exploited
Biclonal	Exists only for research purpose	Isolation is a strong prerequisite to avoid contamination from surrounding stands
Monoclonal*	Does not exist	Successful mass pollination without preceding isolation of female strobili is a prerequisite

* When such a seed orchard is pruned to a maximum height of 3 metres it is known as a hedge seed orchard

approximately even spacing after thinning, progenies from one female are planted in groups with a denser spacing within groups than between groups. The intention is to save one tree per group based on phenotypic examination. To stimulate flowering, the *Pinus contorta* seed orchards are located south of the climatic zone in which the seed should be used. Parallel to the establishment of seed orchards, progeny trials were established in the zone in which the seed should be used. This guarantees that the best families are selected while the selection within family has to be carried out in the seedling seed orchard outside the zone of cultivation.

Clonal seed orchards

In Table 9-1 clonal seed orchards are classified and characterized. Whether or not they are applied in Scandinavia is also indicated in the table.

Biclonal or monoclonal seed orchards are of interest only for progeny-tested clones. If the disadvantages mentioned in Table 9-1 can be avoided, the largest gains may be obtained from these two types of seed orchard. At the turn of the century (1999/2000) only a few biclonal seed orchards existed in Scandinavia.

The early breeders were aware of the problem that not only interspecific or interprovenance hybrids were obtained in interspecific and interprovenance seed orchards, respectively. In the early days of tree breeding the labour cost was several times lower than now and the breeders counted on some manual seedling classification in nurseries with culling of all non-hybrid seedlings. This does not seem to be possible either from a biological or economic point of view. In some instances a species or a population was represented by one single clone. Such a clone is used as a female parent and cones are harvested from this clone only.

Intraprovenance seed orchards are the most suitable type of seed orchard for newly selected plus trees. At planting of the grafts the breeders aim at a maximum distance between grafts of the same clone. This is done to reduce the probability for selfing. Another condition is that the best possibilities for random mating in the orchard should exist. Biclonal and monoclonal seed orchards are interesting alternatives for future, intensive breeding with artificial mass pollination.

Scots pine seed orchards have generally been successful with respect to their role as propagule population, *i.e.* to produce seed for production populations. Many conventional seed orchards have limitations, which means that the gains that ought to be obtained from a theoretical point of view are not obtained. Many Norway spruce seed orchards in Scandinavia were not properly located, resulting in reduced flowering. To obtain good flowering in Norway spruce high temperatures are required at the time of bud initiation, which takes place one year before flowering. To have the Norway spruce seed orchards as far away as possible from Norway spruce stands, farm land was preferred for location of seed orchards in Sweden. The reason for this was to avoid pollen contamination from stands as much as possible. However, the occurrence of cool winds makes the local climate unsuitable for flower induction.

Picture 9-6. Female strobili of a Norway spruce graft. Note the apical location of the strobili. Photograph Kjell Lännerholm.

The female strobili of Norway spruce appear in the apical part of a twig which prevents further vegetative development of the apical part of a strobilus-carrying twig (Picture 9-6). This means that there are no possibilities to have abundant flowering in the same Norway spruce tree in two consecutive years. The flowering in Norway spruce seems to be cyclic (Figure 9-15) with a good flowering year followed by one or a few poor flowering years.

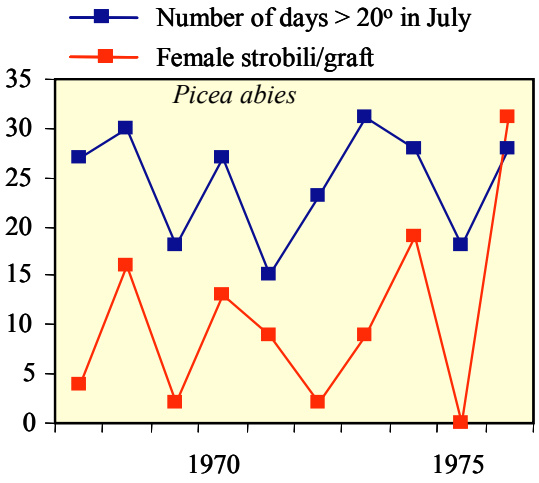


Figure 9-15. The number of days with a temperature > 20°C during flower initiation and mean flowering next year in a clonal trial of Norway spruce at latitude 59°30'.

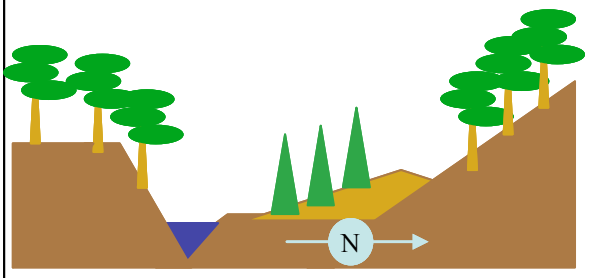
Based on a detailed analysis in a large number of seed orchards in Scandinavia, the ideal location of a seed orchard was developed (Box 9-3). Before locating a seed orchard it is urgent to clarify the local climate of the candidate locality in order to get flowering at all.

After effects

During the early 1980s worrying reports on the poor hardiness of seed orchard progenies were published in Norway. The seed material from clones growing a few degrees of latitude south of their origin had a longer growth period and reduced hardiness compared to the progenies from the same clones in the original stands. This phenomenon was called after effects. An earlier term for this phenomenon is preconditioning.

An example of after effects is illustrated in Figure 9-16. The material illustrated in this figure was collected from individual trees in southern and central Norway at low and intermediate elevation, <350 masl and at 350-450 masl, respectively. In each region, trees of Norwegian origin and trees of central European origin (Harz, Germany or Tirol, Austria) growing for one generation in

Box 9-3 Ideal seed orchard location



Local climate

Topography

- Such that cold air or fog do not remain in the seed orchard or run through the orchard
- Somewhat elevated position that reduces the cold air stream

Aspect

- Protection against dominating winds from north or southwest
- Good light conditions, open to sun radiation, preferably on a southwest slope

Soil conditions

- Light river sediments with a satisfactory fraction of fine mineral (25% fine sand or finer)
- Good drainage owing to the elevated location and the light sediment

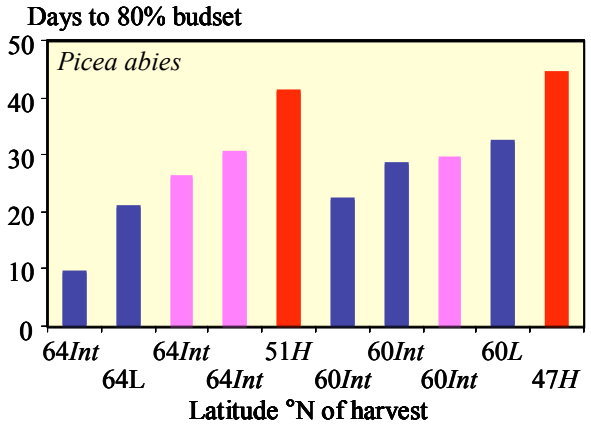


Figure 9-16. Days to budset from July 1st in materials of different origin; approximate latitudes are given with indication of elevation masl, L < 350, Int 350-450, H > 700. Blue = Norwegian families; red = German/Austrian families; lilac = German (left) or Austrian origin (right) but harvested in Norway.

Norway were harvested individually. Open-pollinated seeds from individual trees were also obtained from Harz (700 masl) in Germany and Tirol (900 masl) in Austria. In Fig 9-16 the mean number of days for reaching 80% budset of the different genetic entries are shown. The offspring from the Harz origin trees but growing in central Norway showed a significant earlier budset than the entries harvested in Harz in Germany. The same pattern was shown for the offspring of Austrian origin. It was assumed that the Harz and Tirol progenies from stands in Norway mainly consist of hybrids between central European origins and Norwegian origins. Especially the Tirol x Norway progenies have a performance close to the purely Norwegian progenies, which is a good illustration of a true after effect.

Great efforts have been devoted to this phenomenon of after effects in Norwegian forest genetics research. A systematic testing of temperature and photoperiodic conditions has indicated that it is the temperature conditions from the proembryo stage to the mature seeds that is critical for the change of the growth rhythm. The explanation may be that signals from the environment give an imprint on the female genome such that certain genes are expressed. A signal at a southern locality would thus cause a southern behaviour of the progenies produced at a southern locality and conversely a northern locality would cause a northern behaviour. The mechanism of these effects and the role of epigenetics (see Chapter 2) are under investigation.

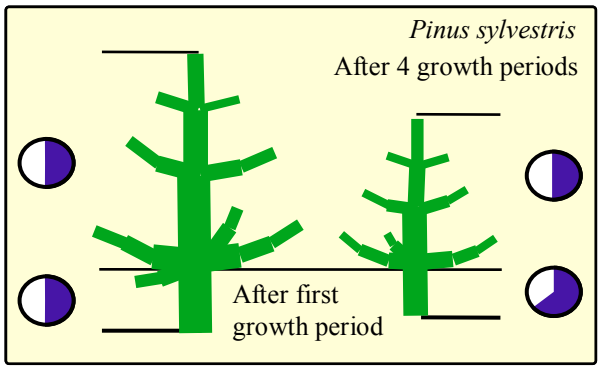


Figure 9-17. Illustration of the effect of the photoperiod during the first growth period on growth in three consecutive growth periods in Scots pine.

One explanation for the ”memory” in trees is that much of the growth for the next season is programmed in the bud. The growth that we observe is actually an elongation of already formed stem units. In the light of this the lower hardiness of the southern progenies might be explaned by the longer time for formation of stem units at the southern locality. In consequence their elongation takes a longer time than is the case for the northern material. As a corollary of this, budset and hardiness take place later during the season in material matured at southern than at northern localities.

After effects may be due to a purely physiological effect. Such an explanation is based on the fact that growth in trees and shrubs is dependent on the current conditions as well as conditions during previous years. One example of this is illustrated in Figure 9-17. As is evident from this illustration the plant to the right is smallest owing to the longer nights it was exposed to during its first growth period. The right plant continued to grow less than the sister plant that had the shorter night during the first growth period. It should be noted that the plants had the same photoperiodic conditions during growth periods 2-4. The influence from the first growth period remained even during growth period 6. The plants seemingly had a memory mechanism.

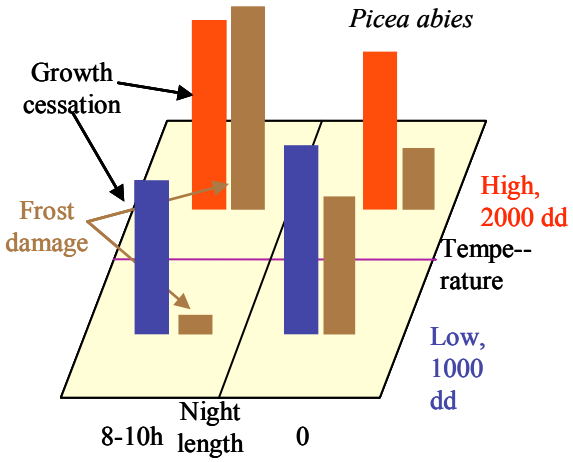


Figure 9-18. The effect of temperature and photoperiod during seed maturation on growth rhythm and frost damage during the second growth period in Norway spruce seedlings. The larger the bar the later the growth cessation and the larger the frost damage, respectively. The night length and temperature sum, degree days dd, are given.

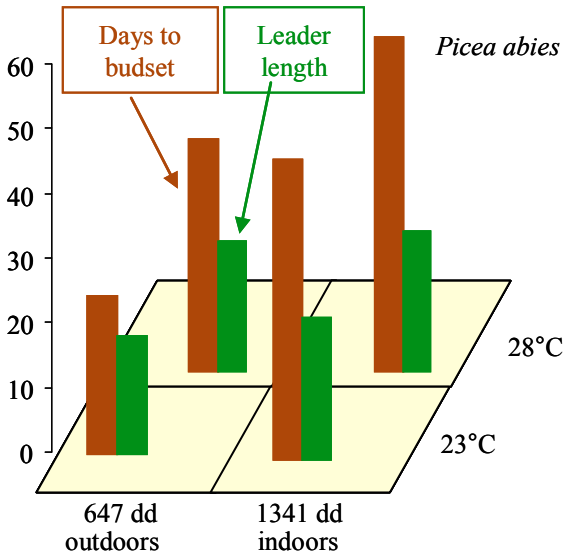


Figure 9-19. Number of days to budset from July 1st, and leader length in somatic embryo plants generated at 23 or 28°C and originating from zygotic embryos generated indoors or outdoors at temperature sums 647 and 1341 degree days, respectively.

Another example from Norway spruce of such a memory is presented in Fig. 9-18. The different combinations of photoperiod (continuous light and 8-10 hours of night, respectively) and temperature (heat sum in degree days, dd, 1000 and 2000, respectively) during seed maturation resulted in variation in growth rhythm. As seen from Fig. 9-18 the combinations high temperature + long night (upper left) and low temperature + continuous light (lower right) had later growth cessation than the two other combinations. The late growth cessation was accompanied by the largest frost damage.

A new aspect on after effects was studied in somatic embryo plants in another Norwegian investigation. In this experiment somatic embryogenesis was initiated at two temperatures, +23°C and +28°C, in material generated from different temperature conditions during crossings. This experiment can reveal if the memory from the crossing environment remains in somatic embryo plants. They were generated from vegetative tissues in zygotic embryos of several seeds. It was shown that there was a strong effect of the crossing temperature on budset in plants obtained from somatic embryogenesis (Fig. 9-19). The clonal effect was significant too. A less pronounced effect from the crossing environment was noted for leader length. The difference between the combinations 647dd + 23°C and 1341dd + 28°C was significant. The corresponding difference for plants generated indoors at 1341dd was minute and not significant. Also for leader length it was stated that *the clones displayed a very large variability in the growth response to temperature (data not shown)*. It should be added that there was no effect of the crossing environment on plant survival and bud flushing after transfer to soil. One of the most significant results in this investigation is that differences in temperature during the development of the somatic embryos also resulted in different performances of the embryogenic plants. Thus, after effects are not only initiated during seed maturation but also during the process of somatic embryogenesis independent of the seed. Another more far-reaching conclusion by the authors was that the major part of the variability in budset and tree growth among natural populations was attributed to the temperature during zygotic embryogenesis.

Of greatest significance for understanding the phenomenon of after effects is to study the progenies of the material with changed behaviour, *i.e.* northern behaviour of southern origin and vice versa. A final proof of the nature of after effects will not be obtained until progenies are raised from the trees that had unexpected behaviour as young seedlings. If their progeny keep the character it will be a proof.

To determine which explanation is true is not of purely academic interest but of great practical significance. Especially in Scandinavia, seed orchards were located south of the area in which their progenies will grow. This was

Picture 9-7. Above. Clonal rows of cuttings in a nursery in Escherode in Germany. Photograph Gösta Eriksson.

done to stimulate flowering and secure good seed development as touched upon previously. If after effects are of genetic nature the seed from seed orchards located far outside the zone of cultivation cannot be used as intended. If after effects are of physiological nature it is fairly simple in modern nurseries to programme the cultivation conditions such that the problem with long growth period and late hardening is overcome.

Vegetative propagation and clonal forestry

All cuttings produced from one donor plant (= ortet) as well as all cuttings (ramets) produced from them belong to a clone (Picture 9-7). All plants/trees of a clone are genetically identical. If a tree breeder has identified a supertree it is tempting to multiply it vegetatively on a large scale and market it. Many ornamental plants, berries, and fruit trees are vegetatively propagated and are marketed as individual clones. From a genetic perspective, vegetative propagation means that not only the additive variance is exploited but also the non-additive variance

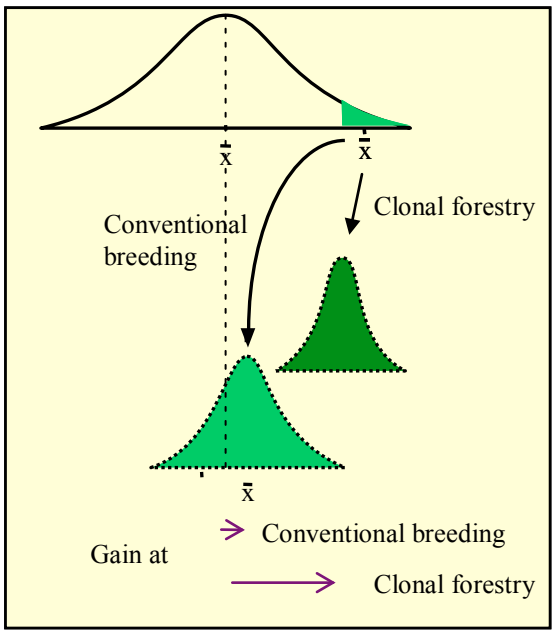
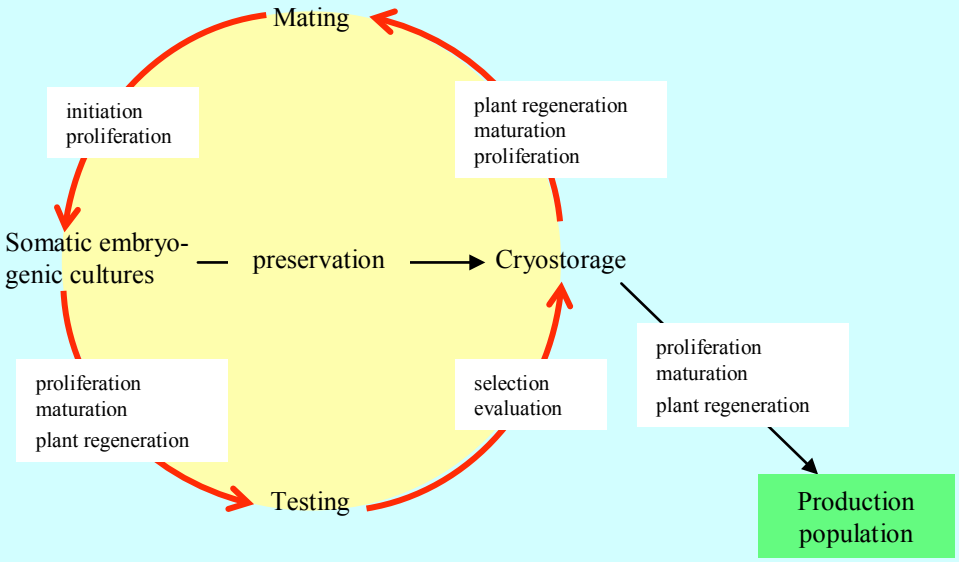


Figure 9-20. Schematic illustration of the genetic gain from conventional breeding and from clonal forestry.

(Fig. 9-20). The higher the broad-sense heritability the higher the gain from vewgetative propagationThe great majority of results from conifers suggest that there is not much non-additive variance to exploit in traits of interest to improve. However, exceptions do occur.

Another reason for breeders to use vegetative propagation is for mass propagation of valuable families. In species such as Norway spruce with its irregular flowering it may be useful to propagate the plants of families obtained from crosses between parents with high breeding values. Artificial crosses are also motivated owing to the high degree of pollen contamination (see page 159) in seed orchards. Contaminations reduce the gain considerably in conventional seed orchard seed.

Box 9-4. Somatic embryogenesis in breeding



Following mating, the mature or immature embryos are induced by the hormones cytokinin and auxin to produce somatic embryogenic cultures. From each genotype, some of the embryogenic cultures are cryostored in liquid nitrogen. Others are further treated with the hormone auxin in order to produce mature embryos. From these embryos, plants are regenerated for testing in the nursery and field. After evaluation and selection of the best genotypes, new plants are regenerated from the cryostored embryogenic cultures for establishing production populations in the forest. Plants are also regenerated for use in a new breeding cycle.

Still another reason for vegetative propagation is to use the material in the evaluation of parents (Box 9-4). A great advantage in this case is that one genotype can be tested under several different environmental conditions. For species which are easy to propagate vegetatively, such tests are in operation in some breeding programmes. Simulations have shown that the gain might be increased considerably by clonal testing compared to ordinary progeny testing with sexually propagated material.

There is a general public fear that clonal forestry is risky since clones might be attacked by pests or diseases. Several theoretical analyses have been carried out. They all show that 30 - 40 clones give the same or better cultivation security than much larger numbers of clones.

Even if there are no attacks from pests or diseases we might expect that a clone that grows very well under certain site conditions may perform poorly under other site conditions. The reverse may be the case for another clone. Therefore, clones or clonal mixtures should require more rigorous testing to avoid losses in commercial plantations than is required for ordinary seed lots from stands. The latter are assumed to be buffered by their broader genetic variation. The results from young field trials are somewhat contradictory. One series of trials indicated significant clone x site interaction while another did not. Calculation of ecovalence values is a statistical method to estimate the percentage contribution of a clone to the clone x site interaction. The larger the ecovalence of a clone the more it contributes to the interaction. In Fig. 9-21 the distribution of ecovalence values for one of the series of clone trials mentioned above is presented. This series has 11 field trials in Denmark and southern Sweden and it has 96 clones from four provenances. Although there was a strong clone x site interaction none of the clones contributed more than 3 % to the interaction (Fig. 9-21).

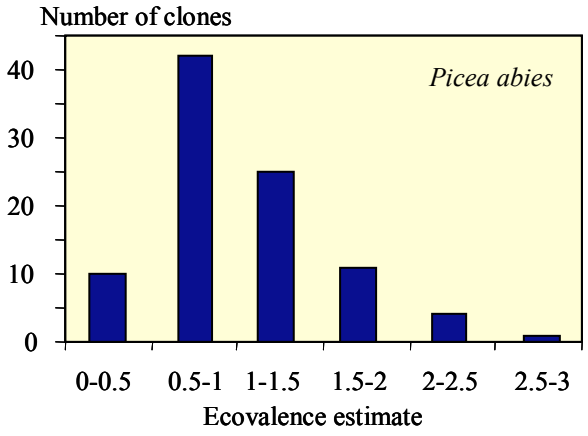


Figure 9-21. The distribution of clones among different classes of ecovalence is shown. Ecovalence is an estimate of the contribution to the clone x trial interaction. The results originate from a series of clonal trials with 96 clones.

Picture 9-8. Cryptomeria japonica *clonal forests in Japan. Photograph Gösta Eriksson.*

The Japanese have propagated *Cryptomeria japonica* for centuries and extensive reforestation with this species occurs (Picture 9-8). Reforestation with *Cryptomeria japonica* cuttings has been successful on the steep slopes of Azorean islands as a means to avoid erosion and to produce wood (Picture 9-9).

Many poplars and willows are easy to propagate vegetatively and are used in the production population. The so-called energy forestry with willows in Sweden relies on vegetative propagation of outstanding clones. Some of the most productive forests in the world consist of *Eucalyptus* clones. At the turn of the century approximately half of all planted *Eucalyptus* forests consisted of clonal plantations. There are thus several examples from all over the world in which vegetatively propagated material is utilised in the production population.

Progeny testing and mating design

Progeny testing plays a major role in forest tree breeding, above all to identify parents with good general combining ability. Selection of parents based on data from progeny

Picture 9-9. Cryptomeria japonica *forest on a mountain slope in Sao Miguel island in the Azorean archipelago. Photograph Gösta Eriksson*

tests is usually designated as selection backward. Estimates of variances is another objective of progeny testing. Such estimates are used for future breeding and for prediction of possible gains from tree breeding. Finally the progeny trials are sources for selection of trees for a new generation of the breeding population. Such a selection is designated selection forward, *i.e.* the best trees in the best families are selected.

There are three main types of mating design:

- Diallel matings
- Factorial matings
- Nested matings

In addition, polycross and open pollination may be used. The meaning of the different types of mating design is given in connection with the presentation of their advantages and disadvantages. All artificial mating (=controlled pollination) work is labour demanding and thus expensive. It is important to clarify the objective of the mating work before it is decided which mating design should be used.

	1	2	3	4	5	6	7	8
1		x	x	x	x	x	x	x
2	x		x	x	x	x	x	x
3	x	x		x	x	x	x	x
4	x	x	x		x	x	x	x
5	x	x	x	x		x	x	x
6	x	x	x	x	x		x	x
7	x	x	x	x	x	x		x
8	x	x	x	x	x	x	x	

Figure 9-22. A complete diallel mating design without selfing. Female vertical and male horisontal in the illustrations of mating design; figures 9-22 - 9-26.

	1	2	3	4	5	6	7	8
1		x	x	x	x	x	x	x
2			x	x	x	x	x	x
3				x	x	x	x	x
4					x	x	x	x
5						x	x	x
6							x	x
7								x
8								

Figure 9-23. Mating design described as half-diallel.

The meaning of diallel mating is that the parents serve both as female and male (Fig. 9-22). If we want to have total information about the genetic quality of a set of trees the best thing to do is to carry out all possible crosses among all parents, ie 1x2, 2x1, 1x3, 3x1 etc. With this

	1	2	3	4	5	6	7	8
1				x	x	x		
2					x	x	x	
3						x	x	x
4							x	x
5								x
6								
7								
8								

Figure 9-24. Partial diallel mating design according to Kempthorne and Curnow.

mating design we shall theoretically obtain the best estimates of additive and non-additive effects as well as selfing and reciprocal effects. The progeny plantation from such a mating design is also the best for selection of the best trees in the best families. This is the only mating design in which all families are present. The major disadvantage with the complete diallel is that it becomes cumbersome when the number of parents is high. If we assume that 50 trees should be progeny tested, a complete diallel mating requires 50 x 49 = 2,450 crosses of the trees. Selfings are not included in this figure. Both mating work and field trials will be too large to make this mating design realistic in applied breeding. Another thing that is frequently overlooked in connection with choice of mating design is that mating designs with large numbers of families require a large homogeneous area of forest land for progeny trials. Mostly it is hard to find forest land larger than 2-3 hectares with a satisfactory homogeneity. A complete diallel mating with 50 parents at a spacing of 2 x 2 meters would only allow 3 plants per family in a field trial of 3 hectares. To avoid the requirement for large progeny trials it is necessary to reduce the number of families. Such reductions of the complete diallel are called partial diallel matings.

Picture 9-10. Isolation of female strobili of Scots pine before they are receptive to prevent fertilization by airborne pollen. A plastic tube is put on top of a twig with strobili and is sealed both in the upper and lower part with foam plastic. Isolation of strobili with paper bags as in Norway spruce occurs only rarely. Photograph Carin Ehrenberg.

	1	2	3	4
5	x	x	x	x
6	x	x	x	x
7	x	x	x	x
8	x	x	x	x
9	x	x	x	x
10	x	x	x	x
11	x	x	x	x
12	x	x	x	x

Figure 9-25. A factorial mating design that is frequently called matings with common testers.

The largest with respect to remaining families after reduction of the complete diallel mating design is the half diallel (Fig. 9-23). As the name says half of all possible matings are carried out. Mostly this is done by excluding the reciprocals. It is assumed that maternal effects can be neglected. A partial diallel that has frequently been used is the one in Figure 9-24, which also excludes selfings. This type of mating is a good compromise among different objectives in progeny testing, such as identification of parents with good general combining ability, estimates of variance components and possibilities for forward selection.

Picture 9-11. Equipment for pollen extraction of individual clones. Owing to the wind pollination of spruces and pines it is of utmost importance to take serious measures to avoid pollen contamination. Shortly before pollen dispersal, twigs are placed in the conical paper bag. When the pollen is ripe the water in the containers is emptied and the paper bag is turned upside down. Pollen drying is started by blowing dry air through the paper bag. Finally, pollen is collected in glass vessels attached at the bottom of the paper bag. Photograph Kjell Lännerholm.

Factorial mating means that a parent either serves as female or as male. When a factorial mating design has a few male clones and numerous females it is designated as common tester mating (Fig. 9-25). The major advantage of common tester design is that the estimates of female GCAs are fairly accurate. The number of unrelated families is low and does not exceed the number of males. This mating is unbalanced with respect to the number of females and males. The estimates of the GCA of each male is very precise. Since the males are few this is a waste of resources. Historically this was the first systematic mating design used world-wide in tree breeding programmes. Earlier, seemingly haphazard matings were carried out. In the early days of tree breeding it was important to compare the performance of plus tree progenies with ordinary seed lots. Since flowering in the young seed orchards was erratic, systematic matings were almost impossible. The early tree breeders had to rely on data from unsystematic matings. The greatest efficiency of factorial matings is obtained at equal numbers of females and males. Also for factorial matings there are possibilities to reduce the number of matings to enable a simplified handling of the progeny testing.

Picture 9-12. Pollination of isolated strobili with a known male. Photograph Carin Ehrenberg.

Disconnected half-diallels (Fig. 9-26) are groups of half-diallels that have no clones in common. This mating design became popular worldwide around 1980 and substituted the common tester mating design in many tree breeding programmes. The major advantage with this mating design is that small half-diallels are easy to complete. The parents are selected according to flowering a certain year. Clones that are not flowering one year may perhaps flower the next year so that another half-diallel can be accomplished that year. Flowering has been a great obstacle in certain species for completion of mating designs using several clones. The greatest disadvantage with all mating designs without connections between groups of progenies is that a comparison of breeding values of parents from different groups is not totally unequivocal.

Single-pair mating means that each parent is mated just to one other parent. This mating design might be good to mate parents with good breeding values for generating families for selection forward. The possibilities to estimate genetic variance components are more or less non-existent.

Polycross and open-pollination are two satisfactory alternatives for estimates of breeding values. Non-additive estimates cannot be obtained in these two cases. Polycross means that each parent is pollinated with a pollen mix, usually with a large number of males. Open pollination means that seeds are harvested from trees without any artificial pollination. At the first selection of plus trees a simultaneous collection of seeds enables an early establishment of progeny trials, which gives a gain in time in the breeding work. Since each parent is represented by one progeny only, the trial area is much less than for other mating designs, even if the number of trees per family should be larger in progeny testing using polycross or open pollination than in other mating designs.

Nested matings

Nested means that the parents are grouped into a series of nests, preferably no less than 20 in each nest. In its most

	1	2	3	4	5	6	7	8
1								
2	x							
3	x	x						
4	x	x	x					
5								
6					x			
7					x	x		
8					x	x	x	

Figure 9-26. Disconnected half-diallel mating design.

complete form each female is mated with pollen mixes from each nest. This may result in some selfing but it is judged as negligible since selfed seedling will be outcompeted by the outcrossed seedlings. The estimates of parental GCA are good if the pollen mix is composed of 20-30 parents. There are possibilities to modify the complete nested design with less labour-demanding designs. Since they do not seem to have been used in forest genetics research or breeding we will not discuss them.

Point of time for selection

For trees with long rotation times amounting to several decades it is impossible to postpone the evaluation of the progeny trials until harvest. Some breeders claim that one third of the rotation time is enough for a ranking of the parents with respect to growth. Even one third of the rotation time means many years for high latitude progeny trials. A ranking of the parents for growth at an age of 15 - 20 years will probably not result in significant mistakes. The long-term growth potential is probably best obtained from the growth increment during the last five or ten years. The breeders are generally careful about selection of the locality for the progeny trial to get as homogeneous ground as possible. However, the phase of establishment is a very sensitive part of the development of a progeny trial. Planting shocks might be random so that some plants are hit severely while others are less affected. The competition with weeds is another matter which might affect juvenile plants in a random way. The effect of such environmental effects will diminish with time and more of the genetic quality will determine later growth.

As trees in a progeny trial grow, they will face increased competition for resources such as water, nutrients, and light from the other trees in the progeny trial. If the competition is allowed to be very strong this will lead to a stronger differentiation among the families. This will facilitate the selection of the best parents. However, if we

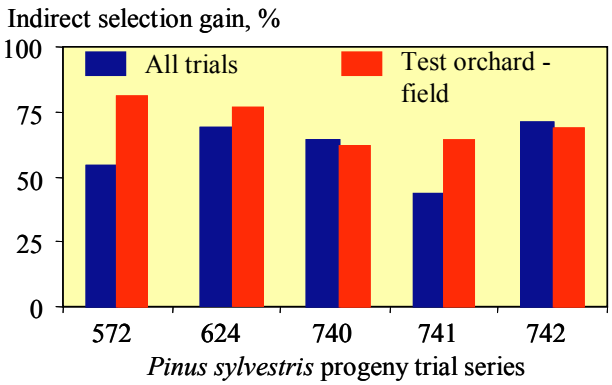


Figure 9-27. Blue columns show indirect selection gain based on data from all trials in a series of Pinus sylvestris *progeny trials. Red columns show indirect selection gain when data from test orchards only, are estimated. Test orchards have denser spacing and are frequently established on farm land.*

are interested in an estimation of future gains via the heritability which is derived from the results in the progeny trial we will probably exaggerate the potential gain from the material under strong competition.

One option to determine the point of time for selection is to estimate age-age correlations, *i.e.* correlations estimated on the same tree individual between the same trait at different ages. For example, in a Swedish progeny trial of *Pinus sylvestris,* high age-age correlations were estimated for tracheid length between ages 11 and 31, and for wood density between ages 8-11, and ages 28-33. The results also showed that the genetic gain per year for these traits was two to three times larger when selection was carried out at age 11 rather at age 31 or 33. This indicates that the optimum selection age might be even lower than 11. Moreover, early tests for these traits should increase the efficiency of the *Pinus sylvestris* tree breeding progam.

In Finland there are two main types of progeny tests in applied tree breeding, conventional progeny trials in the field and so called short term test orchards, mainly on farm land. The aim is to enable an earlier selection than in field trials. A concern with the latter is that they do not reflect the site conditions in the field owing to serious genotype x environment interaction between test orchard and field. Genetic correlations and rank correlations between 40 pairs of progeny trials were estimated for tree heights at age 10. Based on these correlations it was possible to estimate the indirect selection gain. In Fig. 9-27 the average indirect selection gain for series with more than two trials is given. The blue column is an average for all trials in one series of progeny trials, the red column is the average between a test orchard and field progeny trials in the same series. As can be seen the indirect selection gain based on test orchard data is sometimes higher than indirect gains based on all progeny trials within a series. Thus, it seems to be advantageous to focus on test orchards in progeny testing since the loss of information owing to genotype x environment interaction is so small that it is outweighed by the good growth and discrimination between genetic entries in test orchards.

Early tests

Great hopes have been invested in possibilities of predicting future growth performance on seedlings or even seeds. The advantage with early tests is that the circle in Fig. 9-7 can be completed much faster than is possible with long-term field testing. One problem with early testing is to identify the trait or the combination of traits in the juvenile material that gives a strong correlation with the valuable adult traits. Up to the end of the 20th century the early tests for growth have not given any consistent results. Strong juvenile-mature (J-M) correlations were obtained in a few cases while no correlations were found in other cases. It is of interest to analyse the reasons for weak juvenile-mature genetic correlations.

1. Different sets of alleles regulate the trait at the juvenile and mature stages. One probable case might be the presence of free growth in *Picea abies* at the juvenile stage which disappears at a certain age.
2. As discussed in Chapter 5 the same phenotype might be created by several different combinations of alleles. A fast-growing juvenile plant might have a genotype that differs from that of a fast-growing mature tree.
3. The environmental conditions are mostly different in growth chambers, greenhouses, or nurseries and in the field. This may result in a genotype x environment interaction.
4. As discussed above, non-genetic effects may dominate during the phase of establishment. This means that strong J-M genetic correlations cannot be expected until the genetic effects are dominating in the field trials.
5. The results in the field trials may not reflect the genetic capacity fully owing to imperfections in experimental design or to other causes leading to imprecision of the estimates.
6. Since growth is a complex trait, individual components of growth may not give strong J-M correlations. Weighting of the components in an index may be a way to overcome this.
7. The additive variance may be low either at the juvenile or the mature stage.
8. Human failure may have resulted in mislabelling. The scions, the grafts, and the seed lots might have been mixed with wrong identity as a consequence. Pollen contamination may have occurred since it is extremely hard to avoid pollen contamination in wind pollinated species. The experimental plan may not reflect the true identity.

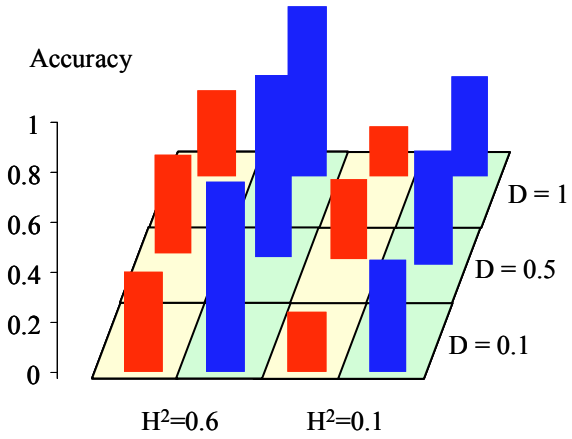


Figure 9-28. The accuracy (= correlation with the true values) based on phenotypic selection (blue columns) or on genomic selection (red columns) over four generations with three levels of dominance; equal to the additive variance, 50%, and 10% of the additive variance. The simulation started with 67 full-sib families and the 50 best parents were selected for the next cycle. The genetic data were considered before generating each cycle.
Genomic selection means that a dense map of markers is required and the predictions are based on the use of all markers.

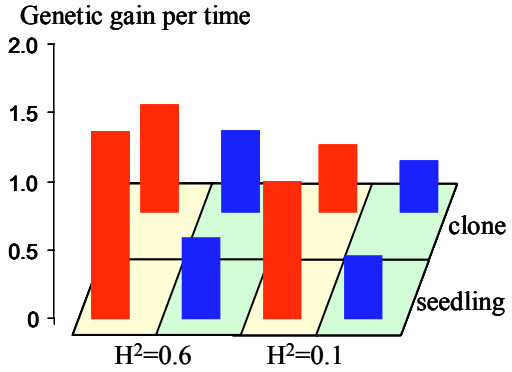


Figure 9-29. The genetic gain per time unit following phenotypic (blue columns) or genomic selection (red columns) after four generations of selection. The gains are mean values for three ratios of dominance variance over additive variance, 1.0, 0.5, and 0.1, respectively. The lengths of the cycles for phenotypic selection was 10, 17 and 17 years. The lengths for genomic selection were 4, 5, and 5 years. Deployment with cuttings or seedlings are shown separately.

The simplest way to develop early tests is to utilise the results from existing field trials. This is possible when the parents of such field trials are present in seed orchards or clonal archives. Crosses can be repeated or seeds might be obtained after open pollination and young siblings to the more mature trees in field trials can be studied at the juvenile stage. Such an early test is called retrospective.

The same Norway spruce material was tested with respect to nutrient efficiency and water availability to test if explanation 6 above was true. However, there was no indication that this was the explanation for the poor juvenile-mature relationships. The most likely reason for poor juvenile-mature genetic correlations is that there are different sets of genes active during the juvenile and adult phase, respectively. Explanation 2 may also be of importance for weak juvenile-mature correlations.

Around 1990 a molecular genetics method for early testing was developed. This method requires the identification of quantitative trait loci, QTL, (see Chapter 5). It is expected that the results from molecular marker techniques will enable early selection of individuals with a desirable phenotype and thus increase genetic gain per time unit. However, detection of QTLs is most efficient for traits that have high heritabilities, which often means that these traits are affected by genes with large effects. But for these traits phenotypic selection is often more efficient. This dilemma can be solved by using marker-assisted selection (MAS) only when a phenotypic selection for a trait with high heritability is more expensive or takes longer time. Traits with low heritabilities can be subdivided into components, each with a relatively higher heritability, for example height growth can be subdivided into time for growth initiation, length of growing period and time for growth cessation.

Around 2010 a new method for use in plant breeding was introduced. The method is coined genomic selection or genome-wide selection. As the latter term suggests, markers from the entire genome are utilized to identify superior trees.

In a simulation study comprising four cycles of selection, three ratios of the dominance variance to the additive variance were included, 1.0, 0.5, and 0.1. Two broad-sense heritabilities were analysed, 0.6 and 0.1. The simulation started with 67 full-sib families and the 50 best parents were selected for the next cycle to generate 25 full-sib families. This selction was repeated until cycle four. The genetic data were considered before generating each cycle. The phenotypic selections took place at 10, 17, and 17 years while the genomic selection took place at 4, 5, and 5 years. Deployment with seedlings or cuttings were also included in the simulations. The accuracy of the various combinations of selection was estimated by correlations with the true genetic values. The simulations carried out were designed to be relevant for *Eucalyptus* breeding.
Fig. 9-28 reveals that the phenotypic selection was superior to the genomic selection for each combination of heritability and variance ratio. As expected the precision was higher at high heritability than at low heritability. However, when the gain per time unit was considered the genomic selection was superior (Fig. 9-29) thanks to the

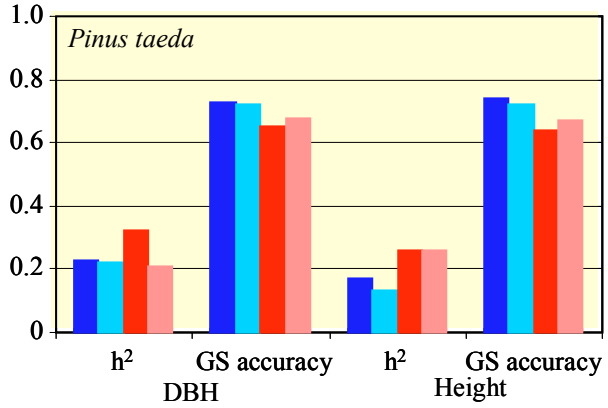


Figure 9-30. Narrow sense heritabilities for breast height diameter and tree height at age 6 in four field trials of Pinus taeda, *two in Georgia (red columns) and two in Florida (blue columns). The accuracy of the genomic selection (GS) for the two traits in each of the four trials is given.*

shorter generation turn-over times. The lower gain per time for clonal deployment depends on the additional time for testing and selection of clones; 7 years.

An investigation comprising four trials with *Pinus taeda* in Georgia and Florida will serve as some early empirical results from genomic selection. Approximately 800 trees from a circular mating design of 32 parents were genotyped. Breast height diameter and tree height at age 6 were assessed. As seen from Fig. 9-30 accuracy of genomic

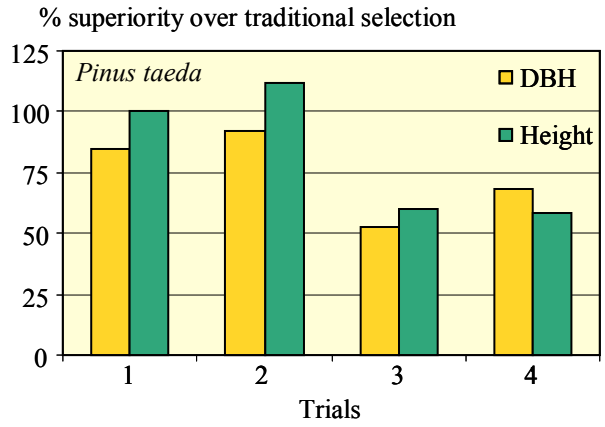


Figure 9-31. Genomic superiority of genomic selection for breast height diameter and tree height at age 6 over phenotypic selection in each of four trials with Pinus taeda. *Trials 1 and 2 are located in Georgia while 3 and 4 are located in Florida.*

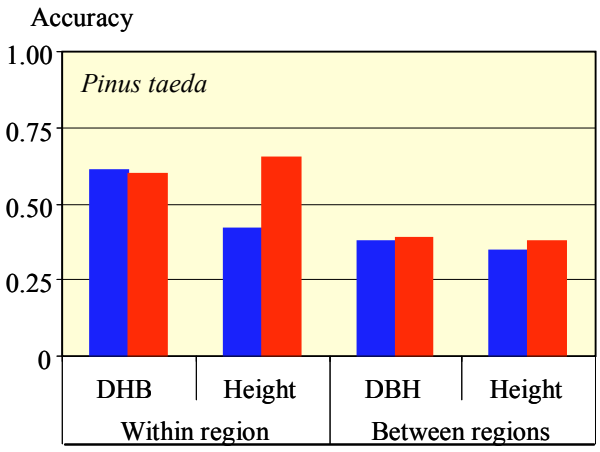
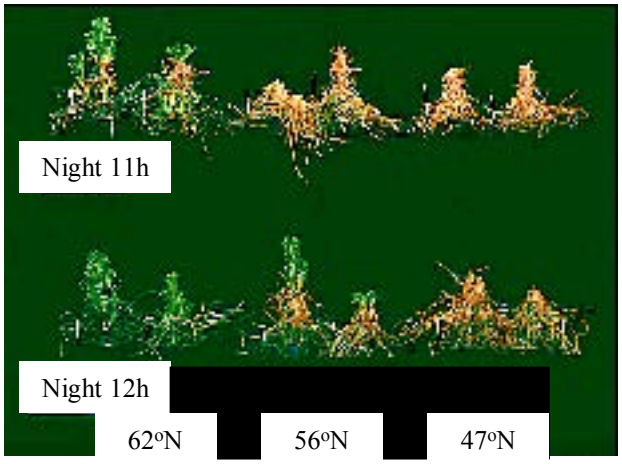


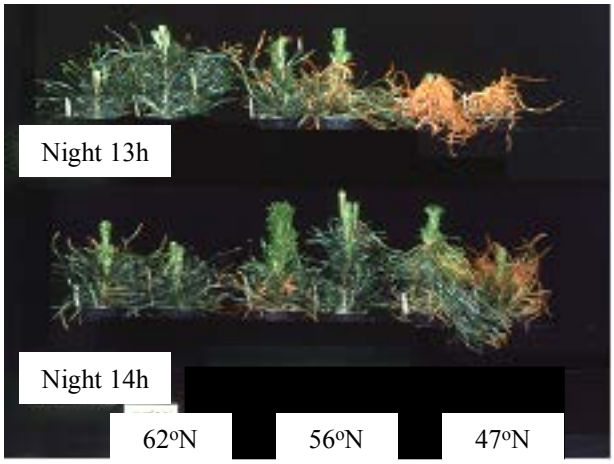
Figure 9-32. The accuracy of genomic selection within and between two regions (Georgia and Florida) for diameter at breast height and tree height at age 6. Each region has two trials of Pinus taeda. *Blue columns refer to genomic selection in the Georgian trials while red columns refer to selection in the Florida trials.*

selection results in accuracies between 0.6 and 0.8 in all trials and for both traits. There is no correlation between heritability and accuracy of genomic selection. When the genomic selection was based on earlier data the accuracy dropped considerably.
In agreement with the results from the simulation study presented above (Fig. 9-29) the genomic selection becomes superior to traditional selection when the gain per time unit is considered (Fig.9-31). There is a conspicuously higher superiority in the Georgian trials than in the Florida trials.

The potential use of genomic selection for prediction of performance at other trials in the same series of trials was also analysed in this study. The results are shown in Fig 9-32. The predictions from one trial to another across regions resulted in accuracies below 0.40 both for DBH and height while the within-region predictions were higher. Differences in climate between the two regions may be responsible for these results.

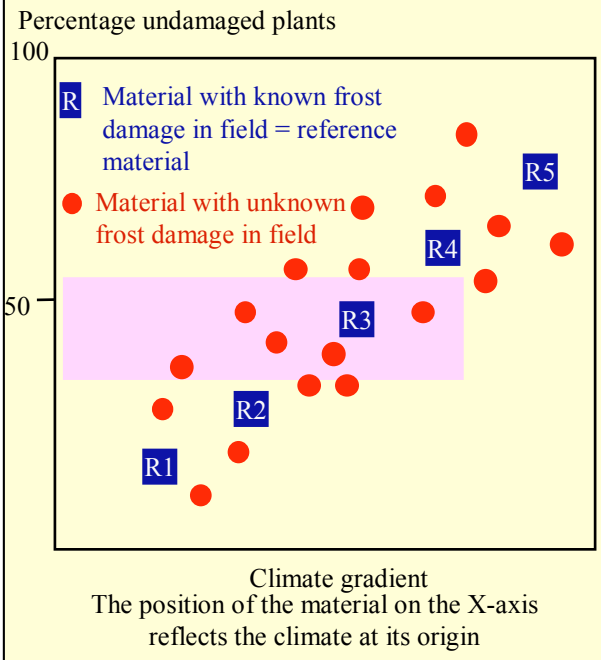
In conclusion, some of the early results from genomic selection are promising but the poor agreement across trials is disappointing. The reason for weak juvenile-mature correlations according to point 2 above (several genotypes might give rise to one specific phenotype; page 151) will not be overcome by genomic selection.





Picture 9-13, left and 9-14, right. Results of early testing for frost hardiness during the autumn for three populations of lodgepole pine from latitudes 47, 56, and 62. Only the above ground parts of the seedlings were exposed to -10ºC during three hours. The freeze testing took place at different times of inwintering, 11 and 12 hours of night (Pict.. 9-13), and 13 and 14 hours (Pict. 9-14). The possibilities to reveal differences between the three populations is larger when freeze testing is carried out at night lengths 12 and 13 hours than at 11 and 14 hours. Photograph Tullikki Lindqvist.

Box 9-5. Early tests for frost tolerance in *Pinus sylvestris*



The freeze testing of a material with unknown frost tolerance takes place after cultivation in a greenhouse at varying night lengths dependent on the origin of the material. Simultaneously a reference material with known frost tolerance is cultivated and freeze tested. As indicated in the figure it is useful to select reference materials with strongly variable frost tolerance. Three weeks after freeze testing the seedlings are examined and the damage classified in a six-degree scale from undamaged to dead plant. A newly tested material with damage intermediate to reference materials 3 and 4 can be used in the climate zone, in which the reference material 3 has satisfactory frost tolerance.

It was relatively easy to develop early tests for frost tolerance (Pictures 9-13 and 9-14). This trait is of greatest significance during the phase of establishment. At this time the plants are close to the ground and the temperatures during clear nights with cool air is much lower than the temperatures recorded by weather stations. Normally the temperature is recorded at 1.3 meter above ground.

In Sweden, freeze testing of individual progenies or bulked seed lots from an orchard is routinely carried out for Scots pine for the interior part of northerly Sweden. The principle of the procedure followed for this kind of freeze testing is illustrated in Box 9-5. With the help of such freeze testings the frost hardiness of a material can be obtained already half a year after seed harvest. Under field conditions the critical period for survival occurs when the young trees are above the snow cover during late winter. However, the critical weather conditions do not appear regularly. It can take some years before the trees are exposed to the critical temperatures. Twenty years is usually the time period required for reliable results as regards frost tolerance of Scots pine in northerly Sweden.

Progress in breeding

With the help of equations such as number 6 in the section about genetic gain in Chapter 5 we can theoretically estimate the genetic gain that can be obtained from different methods of breeding.

If a seed orchard is established and we designate the proportion of selected plus trees as i_p the theoretical gain becomes identical to the gain in equation 7 in Chapter 5, *i.e.* $\Delta G = i_p \sigma_a^2/\sigma_{ph}$. Contrary to this, the seeds from a seedling seed orchard will only have half of that gain, $\frac{1}{2} i_p \sigma_a^2/\sigma_{ph}$, if the seed is collected after open pollination. The reason is that there was no selection among the pollen producing parents.

Box 9-6 Partial gains from different simple breeding methods

Method	Partial gains			
	1	2	3	4
1. Seed from clonal seed orchard without roguing	One gain related to the plus tree selection			
2. Seed from seedling seed orchard without roguing	The gain is A) equal to 1 if the seeds were obtained from crosses among the selected trees or B) is half of that gain if the seeds were obtained from open pollination			
3. Selection backward	One partial gain related to the original plus tree selection	One partial gain related to the selection among the tested plus trees		
4. Selection forward	One partial gain related to the original plus tree selection	One partial gain related to the selection among the tested plus trees	One partial gain related to the selection of the best trees in the best families	
5. Biclonal seed orchard based on progeny testing of full-sib families obtained from controlled crosses in a clonal seed orchard	One partial gain related to the original plus tree selection	One partial gain related to the selection among the tested plus trees		One partial gain from dominance effects

The progeny trials are established in order to guide roguing in existing seed orchards or to guide which crosses should be carried out to obtain a filial generation in which the best trees in the best families should be selected. From Box 9-6 it may be seen that one can obtain different partial gains. It is beyond the scope of this book to give all equations for the different partial gains that might be obtained.

A major objective of seedling seed orchards is to identify the best families and the best trees in those families. Once this information is available, culling of inferior individuals and families can take place (4 in Box 9-6).

Except for biclonal seed orchards, only the additive variance is exploited in the breeding population. In biclonal orchards a major objective is to exploit the dominance variance. There are few cases in conifers in which dominance variance has been shown to be significant. Fig. 9-33 shows significant non-additive effects for several traits in an Australian combined progeny and clone trial with *Tectona grandis*. In most cases the additive effect was larger than the non-additive effect.

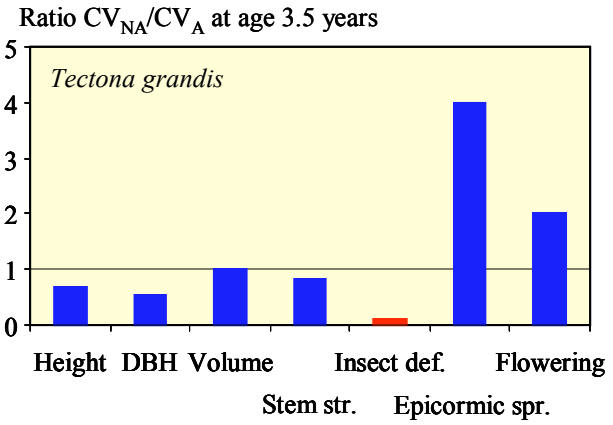


Figure 9-33. The ratio between non-additive variance and additive variance for various traits in an Australian combined progeny and clone trial at latitude 15.33°S and longitude 128.33°E with Tectona grandis. *Blue columns refer to traits that showed significant non-additive effects. str = straightness, def = defoliation, spr = sprouts.*

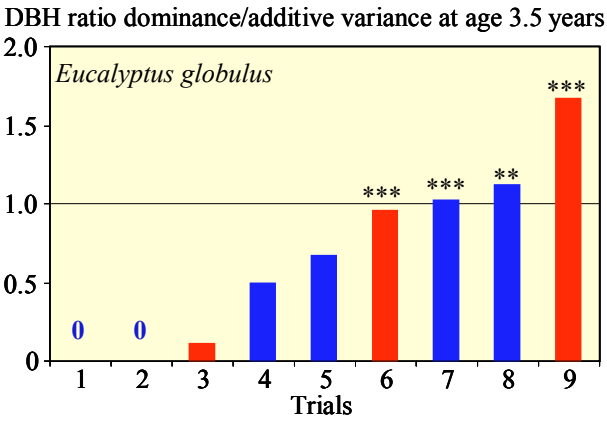


Figure 9-34. The ratio dominance to additiv variance for breast height diameter in nine field trials with Eucalyptus globulus *in western Australia. Red columns refer to a material with 153 full-sibs and the blue columns refer to a material with 94 full-sibs. *** = strongly significant dominance effect.*

Another case is illustrated in Fig. 9-34, in which dominance played a significant role in four of the nine progeny trials.

In certain intensive breeding programmes artificial crosses are carried out among trees with the highest breeding values. Seedlings obtained in this way serve as ortets for vegetative mass propagation in order to obtain the best possible material for the production population.

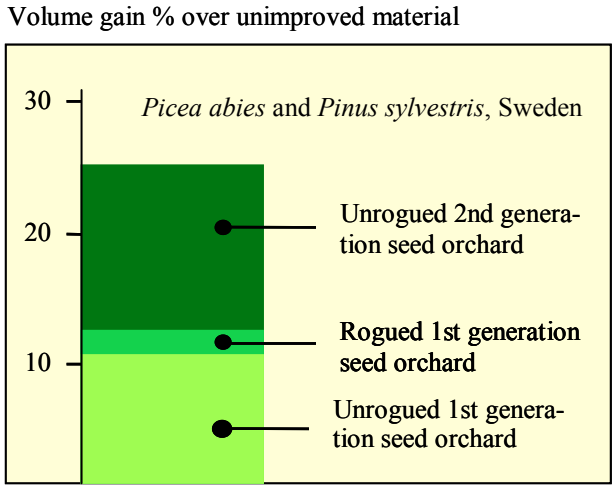


Figure 9-35. First and second generation percentage stem volume gain in Picea abies *and* Pinus sylvestris *averaged over 40 progeny trials.*

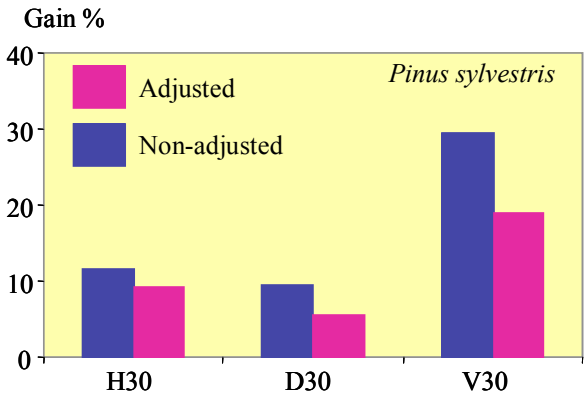


Figure 9-36. The percentage gain at age 30 in selected material for height (H30), breast height diameter (D30), and stem volume (V30). Estimates originate from 36 Pinus sylvestris *progeny trials in northern Sweden. Adjustments were made for patchiness and for competition from adjacent trees.*

In most countries seed orchards were not established until the end of the 1940s. This means that crosses to raise progenies for estimation of realised gains could not be started until 1960. All progeny trials are young and predictions of gains at full rotation cannot be given. However, a large number of progeny trials with fairly uniform data suggest that considerable gains could be obtained.

In 2001 The Forest Research Institute of Sweden summarized the results from approximately 40 progeny trials of *Pinus sylvestris* and *Picea abies*. The average improvement for these seed orchards with untested clones, *i.e.* trees selected in stands, amounted to 10% (Fig. 9-35). An additional gain of 2% can be obtained by roguing in this type of seed orchard. The real improvement, 25%, can be achieved by establishment of a second generation of seed orchards with the best parental clones from the first generation of seed orchards. For the northern harsh parts of Sweden where survival is a serious problem in *Pinus sylvestris* plantations, improved survival increases the gain further.

An estimate of the superiority of bred material was carried out for eleven series of *Pinus sylvestris* progeny trials in northern Sweden. Most trials had single-tree plots. This called for an adjustment for competition from adjacent trees as well as for patchiness because of variation in survival. The results showed that there is a considerable

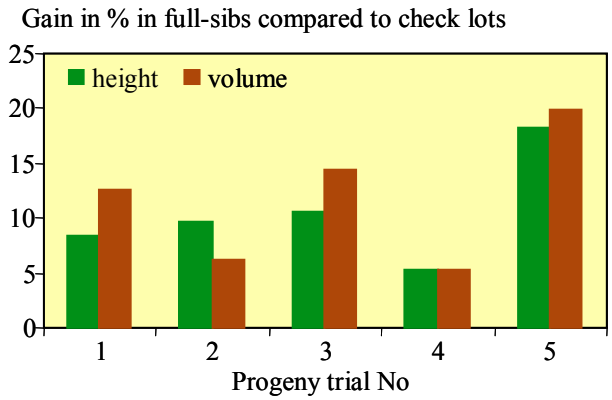


Figure 9-37. The mean percentage gain in Pinus sylvestris *full-sib families compared to check lots in five progeny trials in southern Sweden. Gains in height refer to ages 7-10 while gains in volume in 6 x 6-tree plots refer to ages 26-36.*

reduction of the gain estimates after this adjustment but the gain over check lots is still considerable; for volume at age 30, 19% (Fig. 9-36).

Another example of achievement from *Pinus sylvestris* breeding is shown in Fig. 9-37. In this case the superiority of full-sib families over the check lots varied in the range 5-20%; both for tree height at ages 7-10 and plot volume at ages 26-36. Strong correlations between early tree height and volume at more mature age in the 6 x 6- tree plots were noted. These findings are rewarding for *Pinus sylvestris* breeding.

The reason for locating the Swedish Norway spruce and Scots pine seed orchards on farm land was to have the seed orchard as far away from forests of the same species as possible. This was done to avoid fertilizations with unimproved pollen. Such fertilization is designated as pollen contamination. It was assumed that the contamination decreases with the distance from the unimproved pollen source. Even a total emasculation (taking away of male strobili) within a Scots pine seed orchard situated a few kilometers away from the nearest Scots pine stand did not reduce the seed production in this seed orchard. This clearly illustrates one of the weaknesses with the conventional seed orchards.

For the the northern part of Sweden Scots pine seed orchards were located to warmer climates to stimulate flowering and seed development. This means that any pollen contamination would lead to a reduction in frost

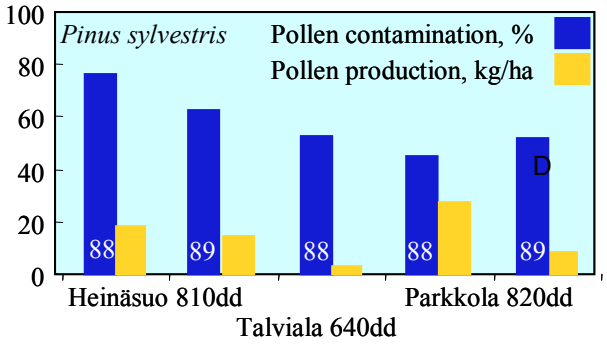


Figure 9-38. The estimated % pollen contamination (blue columns) and pollen production in kg/ha (yellow columns) 1988 and 1989 in three Finnish Pinus sylvestris *seed orchards located in southern Finland with a temperature sum of 1150 degree days. The mean temperature sums of the seed orchard clones at their origins are given.*

tolerance. As long as there is no method that permits us to distinguish hybrids from seedlings obtained from crosses inside seed orchards, the seed crops are hardly of any use at all. This is particularly pronounced when the contamination is 50 %, which will result in a distribution with two peaks. For regions in which the hardiness problem does not exist such as for Scots pine in southern Sweden, contamination will ”only” result in a reduced gain in growth in proportion to the contamination.

With the aid of biochemical markers it was estimated that the contamination amounts to such a high figure as 50 % on average. Cases with more than 50 % contamination are not uncommon. One example of this is given in Fig. 9-38, in which data from three Finnish *Pinus sylvestris* seed orchards located far south of their clonal origins are illustrated. In Finland it was anticipated that a southern location should cause a physiological isolation of southerly located seed orchards. The northern material has a lower temperature demand for reaching receptivity and for pollen dispersal. If this difference in demand is large enough no receptive strobili in the seed orchard clones should remain when pollen dispersal from surrounding stands occur. As seen from the Fig. 9-38 there was no clear relationship between pollen production and the frequency of contamination. The small size of northern grafts in the southern location might be an explanation for the high pollen contamination. It is evident that there was no such physiological isolation during the years of investigation.

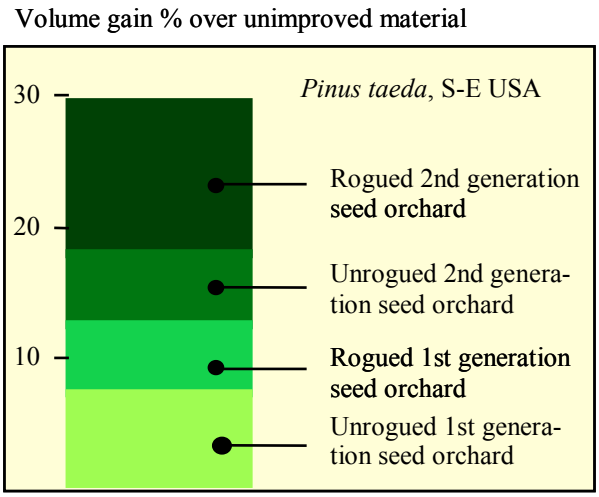


Figure 9-39. The percentage gain in stem volume over unbred material in the Pinus taeda *breeding program of the Tree Breeding Cooperative in south-eastern US.*

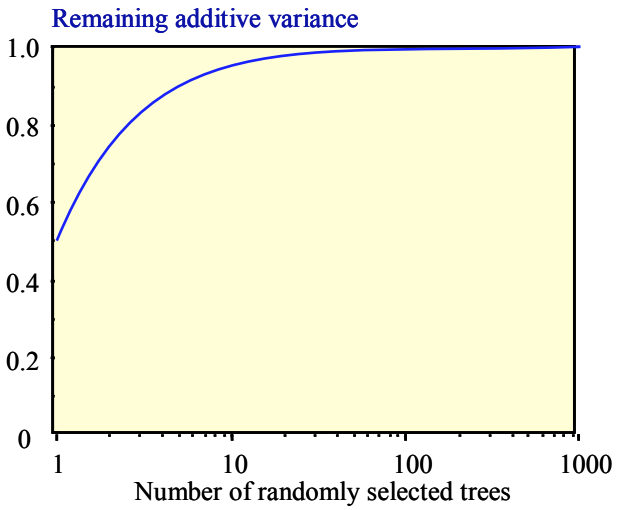


Figure 9-40. The relationship between remaining fraction of the additive variance at random selection of various numbers of individuals. Observe that the scale on the X-axis is logarithmic.

The improvement in the breeding programme of *Pinus taeda* in south-eastern USA is fairly similar to the Swedish Scots pine data (Fig. 9-35). In this case almost 30% gain is expected for the rougued second generation seed orchards (Fig. 9-39). This figure reveals that the gain is increased considerably from the first to second generation of breeding of *Pinus taeda* in south-eastern US. Culling of the poorest clones increases the gain dramatically in the second breeding generation.

Some of the most advanced breeding programmes in the world occur in two cooperatives in south-eastern US. In this region the breeders are facing a large problem with pollen contamination. Owing to the forest ownership there are small land owners who do not utilise any bred material. Therefore, there are fairly large areas with native forests that can spread pollen to the third generation of seed orchards. In the case of 100 % pollen contamination the gain is reduced to half the potential gain. Thus if the gain can be 30 %, a total pollen contamination would reduce the gain to 15 %. In summary it is most urgent to avoid pollen contamination after completion of several cycles in the breeding population. The difference between what is theoretically possible and what is obtained is unfortunately maximised if pollen contamination cannot be avoided.

Considerable gains are also reported for species growing under tropical and subtropical conditions. A progeny trial at latitude 20.33°S with 96 open-pollinated *Pinus caribaea* families in Brazil is an example of this. Selection of the 15% best families and 10% best trees within families suggested an increase in stem volume from 750 dm^3 to 860 dm^3. At age 14 the mean tree height was 23.2 metres.

Besides pollen contamination further problems contribute to deviations from the ideal composition of the seed crop after random mating in a seed orchard. Large differences in the number of female and male strobili per clone occur frequently, especially in young seed orchards. Similarly the point of time of receptivity and pollen dispersal vary among clones in a seed orchard. These factors contribute to a skewed distribution of the contribution to the filial generation.

To overcome the problems with pollen contamination and unequal contribution of the parents to the filial generation, breeders are working actively to develop alternatives to the conventional seed orchards. There is a large potential to improve the gain as indicated in Figs 9-35 and 9-39.

The sustainability of the gain

In model studies in maize and *Drosophila* as well as in some breeding programmes there has been a response to selection for quantitative traits even after 100 generations of directional selection. These results suggest that there is no substantial decrease in the additive variance even if the population sizes were as low as 20-40 individuals. In such small populations exposed to a strong selection, the existing additive variance at the start of the selection would be eroded after 10-20 generations. Since there has been a steady response to selection, new genetic variation must have arisen, or alternatively, previously neutral alleles have contributed to the regulation of the trait in the new genetic environment. Since mutations per locus arise at a rate of one per hundred thousand per generation there must be a large number of loci involved in the regula-

tion of the trait if the hypothesis on mutations is correct. Earlier we have mentioned that the pooled mutation rate for one quantitative trait is considerably higher and might reach one per thousand or even higher. The true explanation for the steady response to selection remains to be determined. Evidently, heterozygosity *per se* is not the explanation.

The knowledge that the variance remains in spite of intensive selection is fundamental, since absence of variance would cause stagnation instead of progress. This is true for traits that the breeders want to improve. What is the situation for traits not included in any breeding programme? Under the assumption that these traits are not linked to traits included in the breeding, the relationship for loss of additive variance at random selection is valid ($1-1/2N_e$). From this formula it is evident that selection of one individual means that 50 % of the additive variance remains (see Fig. 9-40). Even a selection of such a low number as 10 individuals means that 95 % of the additive variance remains. Finally, a randomly selected population with 500 trees has almost the same additive variance as one with 1000 trees. Therefore, we do not gain much by increasing the population size above 500 trees.

Summary

Forest tree breeding is a cyclic process, in which the gains are obtained by selecting the best trees in the breeding population for seed production or for vegetative propagation. Different populations have different functions. The breeding population should safeguard long-term gain in the breeding. Seed orchards serve as propagule population, breeding population, and gene resource population. The concepts of the Multiple Population Breeding System (MPBS) and of nucleus breeding are presented. It is recommended to split the breeding population into 20 subpopulations according to the MPBS concept. This will cause an increased variance among the subpopulations, which facilitates sustainable gains in the breeding.

The advantages and disadvantages of seedling seed orchards, clonal seed orchards, and clonal forestry are discussed. Various types of mating designs are presented. A full diallel cross is the best mating design with respect to estimation of additive and non-additive variance. Owing to the large number of crosses that must be carried out this is not feasible when a large number of clones should be tested. Mating designs have been developed in which a reduced number of crosses are required.

Before the establishment of new seed orchards it is important to find sites with a local climate that stimulates flowering. The consequences of the locality of seed orchards on the characteristics of the progeny, so called after effects, are discussed.

Early selection in progeny trials for hardiness is promising while similar selection for growth traits has so far been unsuccessful. Genomics making use of all molecular markers have recently (2011) been introduced in progeny testing. In early 2013 it is premature to evaluate its potential in tree breeding.

Seeds from existing seed orchards contain a considerable genetic gain. The great weaknesses of the conventional seed orchards all around the world are that the theoretically possible gains are not reached owing to pollen contamination, and that pollen and seed production of the seed orchard clones vary. Differences in the points of time for receptivity and pollen dispersal also contribute to deviations from theoretical expectations. Pollen contamination is very harmful for Scots pine seed orchards for northerly Sweden since they are located far south of the area in which the seed should be used.

Further reading

Jayawickrama, K.J.S. and Carson, M.J. 2000. A breeding strategy for the New Zealand radiata pine breeding cooperative. Silvae Genet. 49: 82-90.

Kvaalen, H. and Johnsen, Ø. 2008. Timing of budset in *Picea abies* is regulated by a memory of temperature during zygotic and somatic embryogenesis. New Phyt. 177:49-59.

Skrøppa, T., Tollefsrud, M.M., Sperisen, C., and Johnsen, Ø. 2010. Rapid change in adaptive performance from one generation to the next in *Picea abies* - Central European trees in a Nordic environment. Tree Gen. Genomes 6:93-99.

Zobel, B. and Talbert, J.T. 1984. Applied forest tree improvement. Wiley, New York.

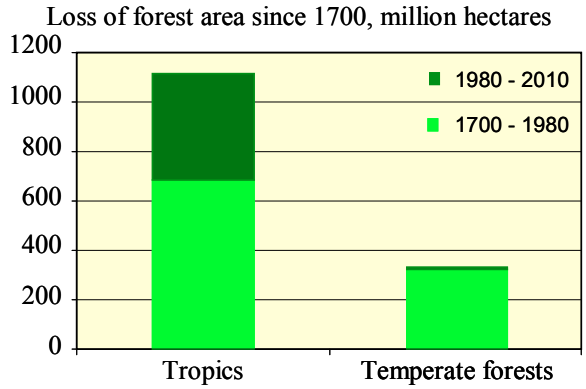


Figure 9-41 The estimated deforestation since year 1700 in The Tropics and in temperate forests. During the period 1980-2010 the deforestation in The Tropics was approximately 325 million hectares. FAO State of the World's Forests 2012.

With the rapid development in science as well as in society as a whole one may ask: What direction will forest tree breeding take in future? Forest genetics, as much as any other forest discipline, depends on attitudes to forestry. Furthermore, forest genetics can benefit from progress in other fields of genetics. We shall briefly present current mega trends in forestry before we turn to forest genetics *sensu stricto*.

Deforestation has been a great concern for a long time. As is evident from Fig. 9-41 the deforestation since year 1,700 in the temperate zone amounts to approximately 400 million hectares. The loss in this part of the world during the last 30 years is less dramatic, around 4 million hectares. This should be compared with the corresponding loss in the Tropics, 325 million hectares. The total loss since 1700 in the tropics amounts to the gigantic area of one billion hectares. Latin America is being strongly affected during recent decades; approximately 10% of the forest area was lost in Latin America from 1980 to 2010 (Fig. 9-42).

The FAO reports from years 2009-2012 project the following main factors as affecting long-term demand for wood products:

- Increase in world population from 6.4 billion in 2005 to 7.5 billion in 2020 and 8.2 billion in 2030.
- Continued increase in global GDP (gross domestic production) from 47 trillion US dollars in 2005 to 100 trillion in 2030, at 2005 prices.
- Regional shifts, owing to the rapid growth of emerging economies especially in Asia.
- Strong impact of environmental organizations, both public and private: more forests will be excluded from wood production.
- Energy policies: increased use of biomass, including wood.

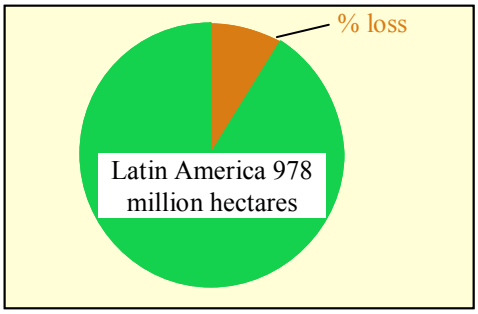


Figure 9-42 The total area of forests in Latin America and the percentage loss of its forest area for the period 1990-2010. FAO State of the World's Forests 2012.

To illustrate: as a result of the rapid economic growth of Southeast Asia, particularly China, the global forestry market is moving from west to east. China now provides 8% of the world's forest products exports, comparable with Sweden, and China is the world's largest exporter of furniture (cf Fig. 9-43). China is at the same time the world's biggest importer of industrial roundwood.

The increased demand for forest products is being met from plantations, which are immensely more productive than natural forests, particularly in the tropics and subtropics (Fig. 9-44). Whereas natural forests are decreasing, the area under plantations is increasing. The total planted forest area is projected to increase from 260 million hectares in 2005 to 310-350 million hectares in 2030, varying with scenario. The increase is most marked in Southeast Asia. China now has the biggest area of planted forest in the world, 61.7 million hectares. Africa probably has great potential, but it is little realized at present.

The rapid expansion of plantations in tropical and subtropical countries means increasing interest in trees

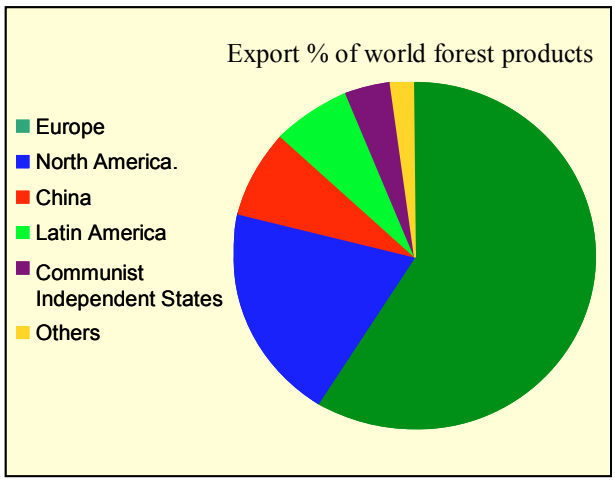


Figure 9-43. The percentage shares of forest products export from some major exporters. FAO State of the World's Forests 2009.

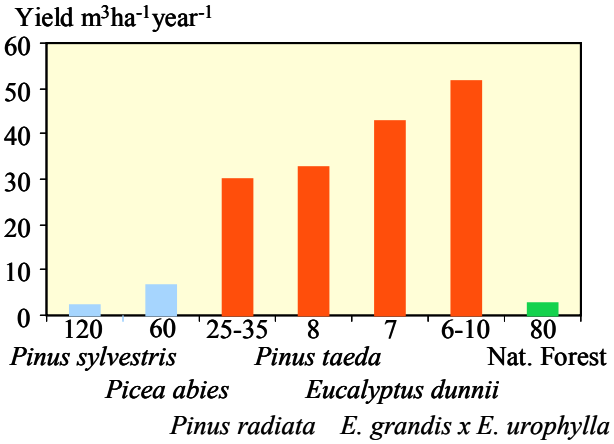


Figure 9-44. The annual yield in cubic meters per hectare for northern (blue) and southern (red) plantations and for natural forests (green). Species (locations): Pinus sylvestris *(N. Sweden)* Picea abies *(S. Sweden),* Pinus radiata *(New Zealand, Chile),* P. taeda *(Brazil, Argentina),* Eucalyptus dunnii *(Brazil),* E. grandis x urophylla *(Brazil, China), natural forests (worldwide). Rotation times in years are indicated below the columns.*

appropriate to these regions. These include particularly eucalypts and other hardwoods. In the words of a North American forestry company, Brazilian *Eucalyptus* is typically harvested after seven years of growth, yielding an average of 17 green tons per acre per year, as compared to a hardwood tree from a naturally regenerated forest in the United States, which is typically harvested after 40 to 50 years of growth and yields an average of two green tons per acre per year.

Apart from buying land in the tropics and subtropics, paper companies in the developed world have responded by developing the technology for high-volume production at low cost, and by attempting to invent complex high value niche products for specific customers. Examples of niche products are various kinds of *smart* paper, including the integration of electronic devices. Nanofibers based on cellulose may be employed here. These developments may require specialized breeding, for example to raise the content or alter the composition of cellulose, or to lower the content or alter the composition of lignin to facilitate isolation of the cellulose. Biofuels may benefit from higher lignin content.

Tree breeders are taking into consideration more specialized wood properties, such as spiral grain angle and shape stability during drying, in addition to straightness and density. With increased genetic knowledge about various quality traits it becomes more important to evaluate the relative importance of such characters. Economic considerations weigh heavily here in creation of optimal selection indices. This is particularly problematic with long rotation times, as it is uncertain what characters will be desirable at the time of felling.

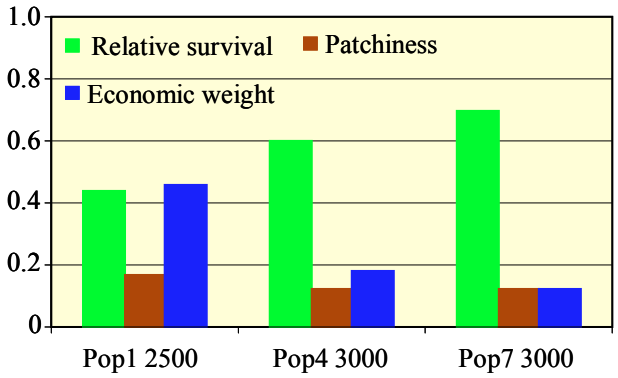


Figure 9-45. The relative survival and patchiness in three Pinus sylvestris *breeding populations with varying number of trees per hectare, 2500-3000, are shown. The economic weights that should be put on survival are shown.*

Even in breeding involving less sophisticated traits such as survival and growth, economic weighting may be useful. In northern Sweden there is high mortality of *Pinus sylvestris* in plantations (see Chapter 7). In Fig. 9-45 the relative survival and patchiness (= empty spots) in progeny trials of three breeding populations are shown. With increasing mortality the yield per hectare becomes more dependent on survival. As a consequence of this, economic weights that should be put on survival in breeding for yield per hectare increase with increasing mortality. Increased patchiness influences the economic weighting in a similar way, but to a somewhat lesser extent.

Earlier there were conflicting interests between forest companies and biofuel producers. The former feared higher wood prices if biofuel would be introduced on large scale. Since the nineties the conflict is rather between environmentalists and wood producers. This competition is expected to remain and even sharpen. This is partly attributed to growing urban populations with limited knowledge about forests.

Global warming is another factor that has raised great concern in most countries. Ecologists have projected that a majority of species will not keep pace with the speed of change. Global warming may strongly influence tree breeding and forest tree gene conservation. In this connection it is important to note that most forest trees have long generation times that cause a slower progress to changed climatic conditions via adaptation than in annual plants.

There are economic incentives in the developed world to exploit advances in molecular genetics, as a means to develop valuable niche products as mentioned above. This has led to an explosion of molecular data from university departments of forest genetics in the developed world, particularly following mass sequencing, see Chapter 2. We have referred to these results rather briefly since to

date they have found limited application in practical forestry. Here we shall outline some of the expectations from recent developments.

The FAO has recently (2010) surveyed the prospects for genetically modified trees in practical forestry. A list of traits that genetic engineers have modified with some success includes:

- resistance to various pathogens, expressed in *Pinus, Picea* and *Populus* species,
- resistance to herbicides, expressed in *Populus, Eucalyptus, Larix, Pinus and Picea*
- increased tolerance to salt, cold and drought stress in species of the above genera
- ability of *Populus* to remove contaminants such as heavy metals from soils, a process called phytoremediation,
- modification of lignin and cell wall biosynthesis in *Eucalyptus, Populus,* and *Picea,* to reduce energy costs in pulp production or to enhance the calorific value of biofuels.

Furthermore the Israeli company FuturaGene has introduced into *Eucalyptus* a gene from *Arabidopsis* that alters the structure of the cell walls to favour rapid growth, enhancing the potential as biofuels. The modified *Eucalyptus* trees, planted on 100 hectare plots in Israel, China and Brazil, can grow 5 metres a year, with 20%-30% more mass than unmodified *Eucalyptus*.

An aspect the FAO 2010 report considers is how to integrate genetically modified traits into tree improvement programmes. It is suggested that GMOs are only used in the production population In the report it is recommended against applying genetic modification to members of the breeding population.

In the important area of flowering control, the authors of the FAO report write: *Despite indications that one or more of the strategies involving flowering control can be successfully employed to engineer transgene confinement, no single method fulfills the basic requirements for long-term commercial use.*

The future of GM trees depends on how far environmentalists are prepared to change their negative attitudes. The demand for forest certification has largely prevented the commercial planting of genetically modified trees, though genetically modified *Populus nigra* is grown on 300-500 ha of land in China. The Forest Stewardship Council, however, now allows forest companies to *look at research* on GM trees. More than 200 field trials with GM forest trees have been documented. A papaya fruit tree, genetically modified to resist a viral disease, has been approved and is in widespread use in Hawaii. The government of Brazil may be prepared to allow commercial scale plantation of GM *Eucalyptus* in a few years' time.

The nearby field of bioengineering is also developing rapidly, *e.g.* in automating somatic embryogenesis, and more generally in high-volume production at low cost.

In conclusion there is little question that traditional tree breeding based on established principles of quantitative genetics, as outlined in the previous chapters of this book, is still highly effective. This is as expected for species that have been consciously selected under domestication for less than a hundred years. Progress is rapid for tropical and subtropical species, which flower in the second or third year and grow sufficiently fast to allow rotation times of five to fifteen years. The pertinence of this is the increasing importance of plantations in Southeast Asia and countries like Brazil. In northern boreal climates, where early selection is usually ineffective before an age of ten years and where rotation times are in the range 30-120 years, traditional breeding for most characters is slow; early evaluation of frost tolerance is a possible exception. At present it is too early to predict the impact of genetic engineering, either in 'south' or 'north'.

Further reading

Berlin, M., Jansson, G., Lönnstedt, L., Danell, Ö., Ericsson, T. 2012. Development of economic forest tree breeding objectives. Scand J For Res 27: 681-691.

Cubbage, F. 2008. Comparative timber investments returns for selected plantations and native forests in the Americas. Timberland Investing World Summit, Sao Paulo, Brazil, 3-5 March 2008.

FAO State of the World's Forests 2009, 2010, 2011, and 2012.

FAO, Rome. 2010. Forests and Genetically Modified Trees. 235 pp.

The Guardian, 15 Nov 2012. The GM tree plantations bred to satisfy the world's energy needs.

UNECE/FAO, 2010. China becomes a global player in forest products market. Geneva Timber and Forest Discussion Paper.

Wintzell, J. 2012. Global demand for wood and fibre in the next 20 years. Kungl. Skogs- och Lantbrukdsakademiens Tidskrift 4:28-30.

Plant production

In this chapter knowledge of the varying requirements for growth cessation and building up of frost hardiness is illustrated from an applied perspective. Genetic aspects of container cultivation as compared to open air cultivation are presented. Finally the possibilities for vegetative propagation are touched upon.

In Scandinavia many companies raise their seedlings indoors in polythene houses. Early on there were some failures since the sowing took place in late February or early March with immediate budset after the development of the cotyledons. During this part of the year the long nights induce immediate budset after germination in northerly materials. Already during the 1960s it was shown that growth cessation is mainly regulated by the night length. The clinal variation in critical night length for budset in Norway spruce was presented in Chapter 7. Once it was realised that the nights were too long, artificial light was used to prolong the day and the plants continued to grow.

The reason for the early sowing was that nurserymen wanted to complete two growth periods during one season. The plants were therefore exposed to 4-6 weeks of 16-hour nights in May and June, after which the plants were moved outdoors where they began their second growth period. This cultivation technique has led to development of robust plants for reforestation.

Since materials of varying origin have different critical night lengths it is important that the nurserymen know approximately the critical night length of each material to avoid too early growth cessation. During the autumn before cold storage or planting it is essential that the plants are frost hardy. In many nurseries artificial night prolongation to 16 hours is carried out to complete the process of attaining stable hardiness. Most materials need a continuous period with such long nights to achieve this. Extremely northern populations have set terminal buds after exposure to one single 16-hour night.

During the 1970s and 1980s many Swedish nurseries stopped their open-air production of seedlings and started to use various types of container for plant production. This means that 1- or 2-year old seedlings instead of 4-year old plants are planted in forests. This in turn means that a material with a much longer growth period is planted in forests since the duration of the growth period declines strongly during the first years (Figure 10-1). This also means that small seedlings are planted in the forests and that the seedlings are closer to the ground, at which the temperature is lowest during clear cool nights. Both these conditions mean that the change of production system leads to reforestation with a plant material at higher risk for frost exposure and frost damage.

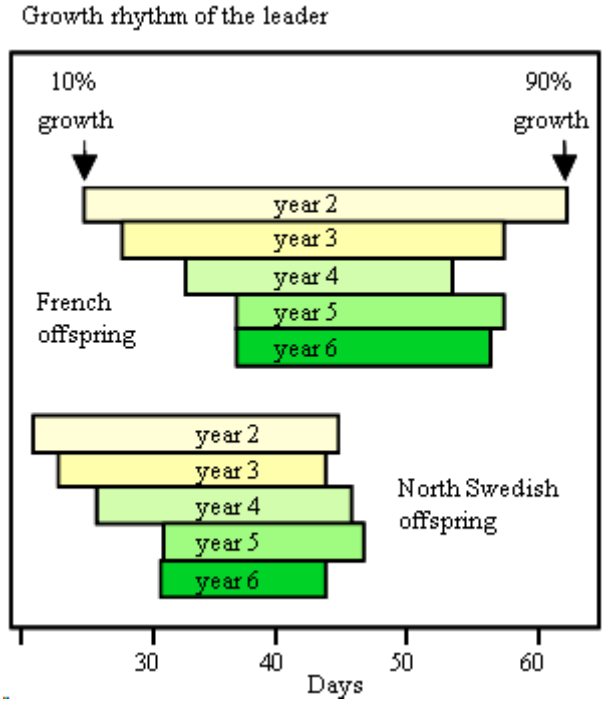


Figure 10-1. The figure illustrates that the duration of the growth period declines with increasing age.

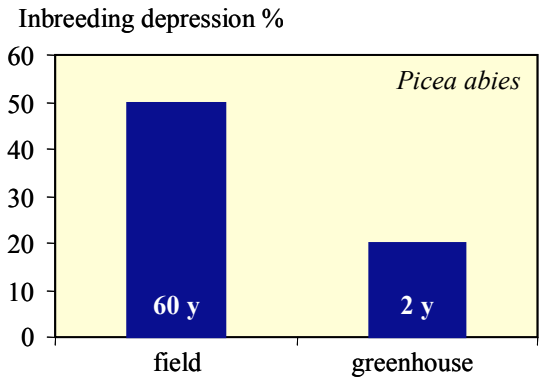


Figure 10-2. The inbreeding depression of genetically identical material in field and in greenhouse. The age in years of assessment is indicated.

Container raising of seedlings also means that each seed should germinate and give rise to one seedling that is planted in the forest. This means that the entire distribution with both poor and good genotypes is represented in the material that is planted in the forest. In open-air cultivation it is assumed that the poor genotypes are outcompeted and never reach the forest. The hypothesis is that good cultivation conditions in the nurseries with adequate water and nutrients lead to minor differences between poor and good genotypes. The performance of selfed Norway spruce in nursery and in field revealed a difference in 30 percentage units (Fig. 10-2) and thus support the hypothesis. If these data are confirmed in other investigations such a cultivation system would lead to higher mortality in the afforestation with a reduced yield in future.

From a theoretical point of view the selection differential is probably not very large between the two types of cultivation. A large proportion of the difference in number of seedlings germinated per kilogram seed must probably be attributed to differences other than genetic. To avoid potential genetic risks connected with container raising of seedlings it is important to have material with high genetic quality.

Some of the problems referred to above can be overcome by regulating the cultivation regimes in nurseries. Thus, there are possibilities to influence the duration of the growth period during one season via the cultivation regime used during the preceding growth period. Cultivation of Norway spruce seedlings for 24 weeks without any night leads to a shortening of the second growth period. Since the probability for exposure to frost decreases with time during spring and increases with time during autumn, the probability for frost exposure is reduced

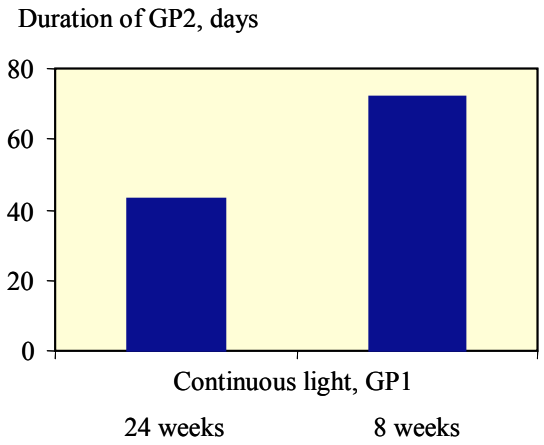


Figure 10-3. Duration of the second growth period, GP2, in Norway spruce seedlings cultivated under continuous light for 8 and 24 weeks during the first growth period, GP1, respectively.

during the second growth period in plants cultivated for 24 weeks without any night during the first growth period. The treatment has caused a more aged performance, *i.e.* shorter growth period, during the second growth period (Fig.10-3), which might last even during the third growth period. Norway spruce seedlings which from their start grow under continuous light also grow continuously with steady cell divisions. Many scientists believe that the number of cell divisions determines whether a plant will be juvenile or adult. This would explain the aged performance of the young Norway spruce seedlings described above.

During the 1970s there was a growing interest in the production of cuttings from valuable genotypes. The main interest was to mass propagate trees which had shown promising results in field trials. It was soon realised that there were great difficulties connected with vegetative propagation. Thus, the percentage rooted Norway spruce cuttings from old trees was low and those which had roots frequently showed the branch type growth rather than the orthotrophic growth characteristic of young seedlings. Frequently the root formation was abnormal with one unbranched root growing perpendicular to the stem. The branchlike growth habit and the abnormal root growth is attributed to ontogenetic aging. Cuttings do not perform like young plants but rather as parts of a mature tree. This type of aging appears already at an age of 5 years in Norway spruce. Cuttings can successfully be produced from 1-4 years old seedlings. Among pines *Pinus radiata* is one example of a species in which vegetative propagation is commercially possible.

If scions are taken from different parts of the crown of an adult Norway spruce tree, those taken at the bottom of the crown give the highest percentage of rooting. The scions taken from the apical part of the crown show most symptoms of ontogenetic aging which might seem remarkable. The scions from the apex are evidently chronologically the youngest but they are formed from a meristem that has passed through hundreds of cell divisions and definitely more than the meristem at the bottom of the tree crown. Once again, this is a sign that the number of cell divisions is critical for the ontogenetic age.

Since the 1970s much research has focused on tissue culture techniques, by which it is possible to obtain plants from ordinary somatic cells. Such techniques are common in horticulture. Great hopes were raised in the 1980s from reports of successful plant regeneration via somatic embryos of Norway spruce. By means of hormone treatments it was possible to obtain cultures in sterile petridishes of hundreds of immature somatic embryos originating from a single somatic cell of an embryo cut from a seed. Treatments with the plant hormone abscisic acid enabled the embryos to mature and subsequently give rise to plants, all with the genotype of the original seed embryo. Commercialization requires development of machines to replace laboratory handwork and reduce the current high costs of plant production, and this is under way. Somatic embryogenesis is also possible with pines such as *Pinus sylvestris, P. taeda* and *P. radiata*, but is more problematic than for the spruces.

The Forest Research Institute of Sweden has developed a computer programme for selection of plant material for reforestation in northern Sweden. Via the Institute's home page anyone has free access to this programme, which gives several options for reforestation material to be used at a particular clear-felled area.

Summary

Genetic knowledge of importance for plant cultivation is mainly related to genetic variation in growth rhythm of different materials. There have been fears that container raising of seedlings where even poor seedlings are planted out in the forests should lead to inclusion of poor genotypes in coming production populations in contrast to the case with open-air cultivation in nurseries. It is assumed that inferior seedlings are outcompeted in the latter type of cultivation, but there is no definite proof for this. There is great interest among breeders in the vegetative propagation of outstanding trees, and this has been done successfully for a small number of tree species.

Further reading

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

11

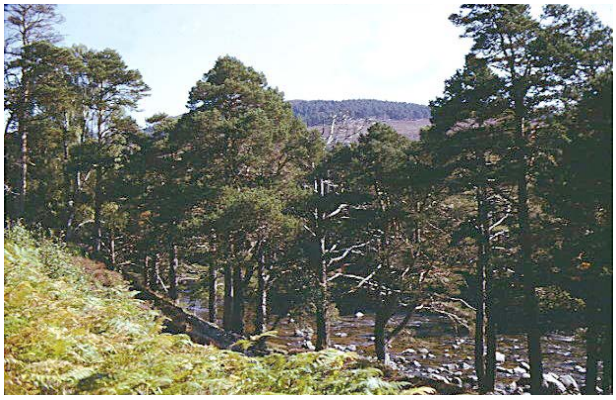
Forest tree gene conservation

The difference between gene and genotype conservation is first explained. After that the three key components of gene conservation - objectives, genetic structure & dynamics, and methods - are presented. Identification of target species is discussed. The discussion of methods occupies the largest part of this chapter.

The prime aim of gene conservation is to save enough genetic variation for the targeted species to enable it to cope with changes in the environment. Expressed in another way, forest tree gene conservation should focus on potential for adaptation. Similarly, broad genetic variation is also required for long-term success in breeding. These matters will be elaborated somewhat more under objectives below. In other cases the reason for conservation may be as is the case for Scots pine, once an important commercial tree in Great Britain, now greatly reduced in number (Picture 11-1).

The future demand for wood or conversion of forestland to crop cultivation is of significance when we plan gene conservation. Without these considerations the most sophisticated gene conservation program may be futile in a long-term perspective. One problem in designing gene conservation programs is that in the overwhelming majority of cases, we do not know the relationship between gene diversity and production of utilities. If this relationship follows curve A in Fig. 11-1 there is no conflict between the two goals whereas curve E describes a situation that is harder to resolve. Curve E suggests that production of utilities and gene conservation should be carried out separately. Research priority should be given to studies of the relationship between diversity and production.

A fundamental question is whether we should conserve genes or genotypes in our gene banks. So far there is no technique available for conservation of genes like books in a library, and for the foreseeable future, gene conservation will take place by conservation of certain genotypes. This means that commonly occurring genes will be conserved, but only in a limited number of genotypes. Is this satisfactory? The question is justified since genes may interact with each other and there can be difficulties in recreating valuable genotypes. In Chapter 3 it was shown that heterozygosity even at a rather limited number of loci gives rise to a large number of genotypes. In cross-fertilizing species, all individuals except for identical twins have a unique genotype. For this reason conservation of each genotype is impossible. Conservation of all genotypes would require a gigantic global museum of all existing individuals. As in many other cases a compromise must be reached, which means that a representative sample of existing genotypes is conserved in such a way



Picture 11-1. A Scots pine population in Scotland. Photograph Gösta Eriksson.

that the largest possible number of genes will be included in the gene conservation programme.

Before we present key components of gene conservation it is useful to repeat some of the knowledge from Chapters 5 and 6, which is of great significance for forest tree gene conservation. Therefore, Figures 11-2 – 11-4 are included once more. The gene frequency constituting the lower limit for conservation has been debated. Some forest geneticists claim that gene conservation should be designed such that rare alleles are included in gene resource populations. Others claim that that they are of limited or no importance for gene conservation.

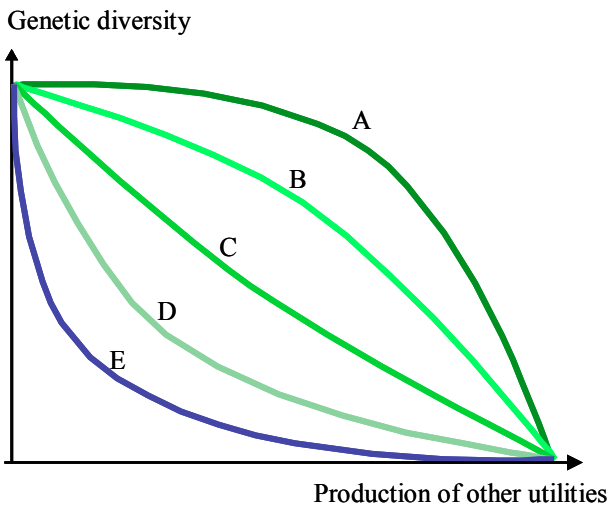


Figure 11-1. Potential relationships between genetic diversity and production of other utilities.

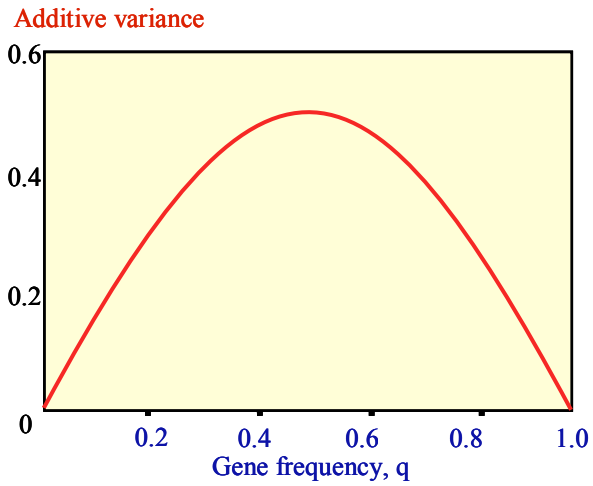


Figure 11-2. The relationship between gene frequency and additive variance with completely additive gene action; a is the value a illustrated in Figure 5-2.

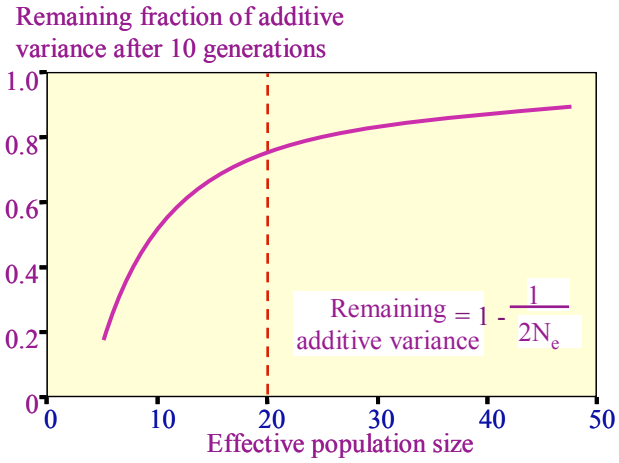


Figure 11-3. Remaining fraction of additive variance after 10 generations as a consequence of genetic drift. It is assumed that the effective population size was constant during these 10 generations. Loss of additive variance is considerable at an effective population size of 20 or lower.

From Fig. 11-2 it is evident that alleles at very low frequencies do not contribute to the additive variance; they do not safeguard the potential for adaptation and are of limited value in gene conservation. Another way to explain this is that rare, recessive alleles mainly occur in heterozygotes and they cannot be increased in frequency by natural selection. Therefore, it is expected that natural populations will have a large number of recessive alleles at low frequencies, which cause a reduction of the vitality of the recessive homozygotes. This was observed in Douglas fir for which a very large experimental material was analysed. The most common reason for a low frequency of rare alleles is that they do not contribute to fitness of the individuals having these alleles. Other rare alleles are probably the result of recent mutations.

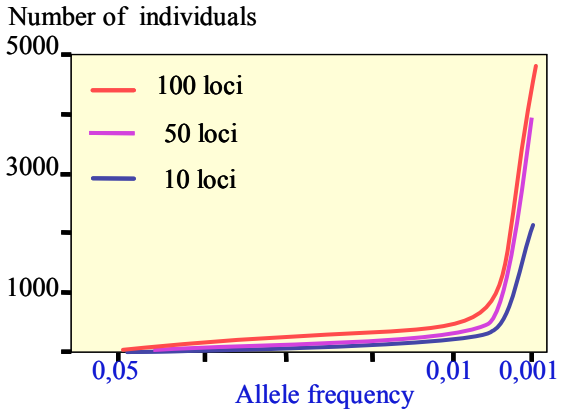


Figure 11-4. The minimum number of individuals required to save one rare allele at each of 10, 50, or 100 loci.

Since loss of additive variance is considerable in populations with an effective population size of 20 or lower (Fig. 11-3) gene resource populations should be large enough to avoid serious losses of additive variance. From Fig 11-4 it is seen that allele frequency is more important than number of low-frequency alleles for the possibility to include this type of allele in gene resource populations.

The three cornerstones of gene conservation

Gene conservation has three cornerstones - the objectives, the genetic structure and dynamics, and the methods. Genetic structure or population structure was discussed in Chapter 7. When the objective is clearly identified and the genetic structure is known, the most adequate method for gene conservation that matches the objective should be developed.

Objectives in gene conservation

Prime objective

In Chapter 6 we stressed that for species in nature, several evolutionary factors may be in operation simultaneously. There is a steady change of gene frequency in a population according to the ambient conditions and/or the genetic qualities (above all the N_e) of the population. Therefore, dynamic forest tree gene conservation has been argued for in most instances. The Swedish parliament took a decision in 1991 according to which, in a word for word translation, *The biological diversity and the genetic variation should be safeguarded...and...naturally occurring plant and animal species should be given conditions for continued existence under natural conditions and in vigorous populations*. This is in agreement with decisions in many other countries that signed the Rio declaration, and agrees with the statement of one of the forefront persons in gene conservation, Michael Soulé: *Conservation genetics exists for one reason only: To promote the fitness of targeted populations*. A related formulation used

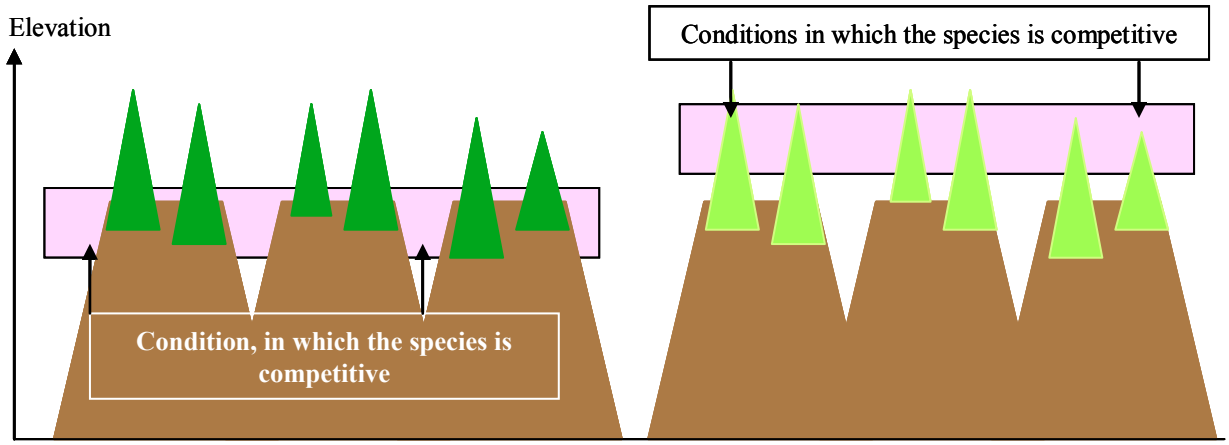


Figure 11-5. Schematic illustration that a species which for climatic reasons at present is competitive on mountain peaks will have these climatic conditions a few hundred meters higher after global warming.

by the EUFORGEN network on noble hardwoods is to safeguard the potential for adaptation of a species. If the potential for adaptation is guaranteed, the species has a greater chance to cope with the changes continuously occurring in the environment.

For the tree species included in breeding programmes it is important to analyse whether it is possible to include breeding within dynamic gene conservation. Simultaneous gene conservation and breeding might be one objective.

Other objectives

Preservation of the present genetic constitution is another objective in gene conservation. Preservation means that the conservation is static. Behind this objective we may distinguish four different reasons:

1. The need to have a reference material for comparisons in future research
2. The belief that natural selection has chiselled out individuals that are perfectly adapted to the site condition where they live.
3. The material has highly desired characteristics that may be lost if the present constitution is not preserved
4. Wild tree species may hybridise with highly bred cultivars

In breeding of agricultural crops point 1 has proved useful and may be useful in forestry as well.

In Chapter 6 it was clarified that point 2 is incorrect. The present genetic structure is one of many possible and it is transient. However, it is a good starting material for dynamic gene conservation.

Point 3 is frequently referred to as gene conservation although it might be better to classify it as a breeding or production objective since it is the human utility of a tree species that is the motive for preservation.

Wild fruit trees such as apples and pears are rarely occurring and as such exposed to great threats of hybridization with cultivated varieties. Preservation of the wild status is one objective.

Some scientists argue for conservation of unknown genetic variation. The reason for this is the expectation that useful substances for mankind might be detected.

Another objective is to save populations that are endangered directly or indirectly by human activities. Tree species that today have their distribution restricted to a low number of mountain peaks, face a serious threat if there is a climatic change with higher temperatures as a result (Fig. 11-5). At such a change there is no possibility for the species to migrate to higher elevation. *Abies fraserii* in the Appalachian mountains in south-eastern USA and *Abies pinsapo* in southern Spain and North-Africa are examples of species that may suffer if there is considerable global warming. The cost for saving the gene resources of such species will be considerable. Especially riparian species in the industrialised world have been exposed to urbanisation, since the banks of many rivers have been stabilised, preventing the natural dynamics of rivers and thereby the natural regeneration sites of such species. Fragmentation of populations is one result of such activities. Biological threats will be discussed below under Grouping of Species.

The objectives discussed so far have concerned a single species, *i.e.* the species targeted in gene conservation. For most forest tree species, many other species are dependent on them for their existence. In this case the tree species is designated as an ecological keystone species. By associated species is meant a species dependent on other species for its existence. A totally objective separation between keystone species and non-keystone species does not exist. There are many transition cases since species are always dependent on other species to some extent.

It is obvious that forest trees owing to their age and size are of great significance for many other species. A final objective for gene conservation is to include the conservation of species associated with a target species.

Genetic structure

In Chapter 7, several possible population structures were presented. The strategy for gene conservation depends on the genetic structure of the species in question. Knowledge of the genetic structure is indispensable for genetically satisfactory gene conservation. There will never be enough funding for studies of the genetic structure of all species, so such studies will be restricted to a few commercially important species. Measures to be taken in absence of knowledge about genetic structure will be discussed under "Gene conservation methods".

In situ and *ex situ* gene conservation

Traditionally two main methods have been distinguished in gene conservation, *in situ* and *ex situ*. *In situ* means "on the spot" and is understood by most forest geneticists as conservation of naturally regenerated forests. *Ex situ* means that gene conservation is carried out by seed or pollen banks or that the gene resource population occurs in some kind of plantation. A certain confusion of concepts exists since many gene conservationists of agricultural crops interpret the *in situ* concept in another way. They regard a growing crop as *in situ* while *ex situ* for them is limited to banks of seed, pollen, or tissue culture.

Owing to this confusion of concepts it would be better to classify methods according to the function of the different gene resource populations. However, the *in situ* and *ex situ* terms are so commonly used that a new but unequivocal terminology would not be able to outcompete these terms.

Before an analysis of the different methods is carried out it is important to emphasize that gene conservation will always have too limited funding. Therefore, it is important to develop methods that unite as many objectives as possible. It is also important to give priority to certain species. Species given priority are referred to as target species. Once target species are identified a grouping of species with respect to gene conservation methods has to be carried out.

Target species

There are several options to select a species as a target species:

- scientific reason
- threat
- charisma
- economic reasons

For scientific reasons there is a desire to choose species in order to combine different ecological characteristics such as distribution, pollen vector, seed dispersal, and stage in ecosystem. We shall return to these characteristics in the next section.

If species are selected according to their ecological characteristics and designated as ecological keystone species, they will probably be maintained as target species. Whether a species has any close relatives is another scientific reason, on which to base the selection of target species. Some scientists suggest that species without any close relative should not be selected as target species, since they appear to belong to an evolutionary dead end. If target species are selected among species which have shown recent speciation the funding is spent on vigorous species with high potential for continued evolution. Other scientists claim the opposite, that species without any close relatives are probably genetically unique and deserve to be target species. This type of species is frequently endemic, *i.e.* occurring within one restricted area only. Such species are sometimes endangered, and have therefore often been conserved. For practical reasons it is natural that this has been the case. However, an analysis of the potential for adaptation of such a species ought to be carried out before decisions on large investments are taken.

The selection of charismatic species might seem to be an incorrect way to utilize limited funding. The spotted owl in North Western USA is an example of a charismatic species that has taken large resources. Such an investment might be justified, since it draws public attention to conservation issues and might make it easier to raise funding for gene conservation.

We are facing an explosion of the human population never experienced before. In this perspective the demand for fibres for various purposes will probably increase dramatically. The human population increase also contributes to an aggravation of the effects of pollutants and climatic change. Improvement in the living conditions in developing countries is one way to come closer to economic equality among countries. One means to achieve this goal is to utilize the renewable forest resources. The demand for wood will probably increase dramatically up to the middle of the 21st century. For the period 2010-2030 an estimate of the increase of wood demand for industry was 700 million m^3.

In countries with hundreds of tree species a scoring system has been used to identify target species. Scientists, farmers, local peasants, and business people scored the species with respect to utility, ecological importance, and threat.

Probably a balancing of the economic and the ecological reasons is a good starting point for selection of target species. The scoring mentioned above is very close to such a balancing. The various reasons have to be judged for each

individual case. It is also of great significance that the methods for gene conservation that we suggest will stand future pressure of demand for wood. If not, the gene resource populations may be cut, like most of the park trees in Sarajevo during the civil war in former Yugoslavia.

Grouping of species in gene conservation

Ecological characteristics

Tree species differ in their characteristics and may require different methods for their gene conservation. Of interest is to identify whether species can be grouped with respect to gene conservation using species characteristics such as:

Distribution, wide range - limited; continuous - scattered; large - small populations
Mating system, wind pollinated - animal pollinated
Stage in ecosystem , climax - intermediate - pioneer species.

Population size covers everything from the extreme situation of large random mating populations to widely scattered single trees. The effective population size is of great significance for random mating. A species consisting of many small and scattered populations without any gene flow among the populations will give rise to large but non-adaptive genetic variation among populations in contrast to the adaptive variation that may exist in random mating populations. The species are therefore first grouped according to rarity, *i.e*. whether they are rarely or commonly occurring.

Some of the rare tree species are intermediate species and may therefore be cut during thinning to promote the growth of climax species. Owing to limited taxonomic knowledge foresters may unintentionally remove rare species during thinning.

Wind pollination and pollination by insects, birds, or bats are two major types of mating system. Studies of the mating system by means of isozymes have revealed that mating system influences the genetic structure of a species. Wind pollinated species have generally a higher within-population/among-population variation ratio than insect pollinated species (Chapter 6). This is attributed to gene flow over larger distances of wind pollinated species than in insect pollinated species. This does not mean that every wind-pollinated species has wider pollen dispersal than every insect pollinated species. Generally, an insect pollinated species will need a larger number of gene resource populations than a wind-pollinated species since the differentiation in a given area is assumed to be larger for the insect pollinated species than for the wind-pollinated species. However, the difference between the two types of mating system is probably of another magnitude than differences between rarely and commonly occurring species.

By stage in ecosystem we mean whether a species is a pioneer species, a climax species or takes a position between these two extremes. Typically, pioneers invade open areas with fairly homogeneous growth conditions that do not call for a large genetic variation. Rather, once a genotype with good adaptedness to the prevailing conditions at the open area arises, it would, teleologically speaking, be an advantage for the species in a short-term perspective to rely on that genotype. Therefore, asexual propagation of highly adapted genotypes as in *Taraxacum vulgare* would be advantageous. Contrary to this, climax species experience heterogeneity both in space and time during their lifetime, and genetic variation within populations must be assumed to be advantageous. The climax – pioneer difference in genetic variation is analogous to the contrast wind pollination – insect pollination and is probably of a lower magnitude than the classification: rarely occurring – commonly occurring.

Involvement in breeding activities

Long-term breeding efforts require breeding populations with satisfactory additive variance, which is also a major prerequisite for gene conservation. If this requirement for a large additive variance is fulfilled, gene conservation is well taken care of in breeding. One prerequisite is that managed forests do not differ too much from natural forests. Whether or not a species is included in breeding might therefore be used in grouping of species. Moreover, the intensity of breeding might vary and as a corollary the amount of additive variance in the breeding population might vary. The objectives in breeding might include one or several traits. When breeding objectives in a species comprise such disparate traits as high-quality timber and nuts it is not self-evident that improvement can be achieved in one common breeding population. This is especially pronounced for chestnut and walnut, in which selection for nut quality and yield has gone on for millennia while timber improvement has not taken place to any large extent. So gene conservation in multipurpose species needs special treatment.

Biological threats

Besides the threats caused by human activities, biological threats in the form of diseases or pests play a prominent role. Well-known cases are the serious fungal diseases in elms in Europe and America and the American chestnut (*Castanea dentata*). Only the most northerly populations of wych elm (*Ulmus glabra*) in Europe are not affected by the Dutch elm disease since the *Scolytus* insects, which transmit the disease from tree to tree, do not survive under the harsh northern conditions. A serious threat to *Fraxinus excelsior* as recently emerged.

Forest tree gene conservation methods

We shall first once more return to the multiple population breeding system, MPBS, in its role for combined breeding and gene conservation. The advantage of splitting the combined gene resource and breeding population into subpopulations is visualised with the help of Figure 11-6. Each point in the cube is assumed to be one subpopulation, which gives a total number of 64 subpopulations. It needs to be emphasized that the growth conditions are not as simple as indicated in this graph. It may very well be that certain of these 64 combinations do not exist in reality. The merit of this subdivision is that each subpopulation might be enriched with alleles promoting fitness under this particular combination of environmental conditions. This means that rare alleles that are valuable under extreme environmental conditions might increase in frequency. Such alleles could be lost at random if there was just one large gene resource population. To strengthen this still more, some of the gene resource subpopulations might be planted outside the present range of the species. This will lead to a broader genetic variation than if we have one large gene resource population. The MPBS method of gene conservation and breeding is gaining terrain which means that tree species included in intensive breeding programs do not need a separate gene conservation activity.

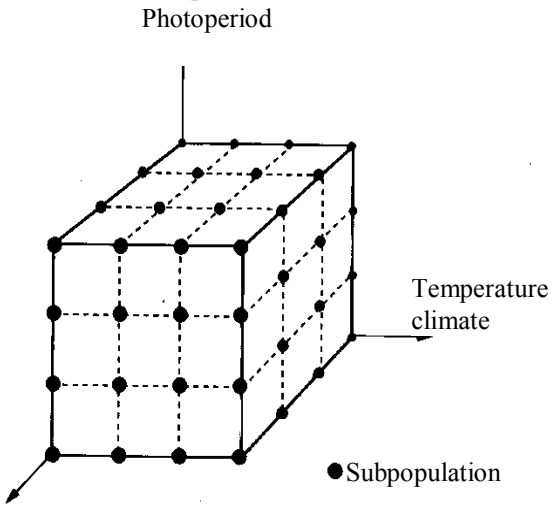


Figure 11-6. The principle for gene conservation by splitting the gene resource population into subpopulations, which will adapt to different combinations of photoperiod, temperature climate, and nutrient availability. Via natural selection an increased adaptedness for the specific combination of environmental conditions will be obtained in the different subpopulations. This would not be achieved in one single, large gene resource population growing at one site.

To reiterate the summary of the merits of MPBS: The main advantage of the MPBS is that it combines the capture of the total existing genetic variation with a satisfactory variation within each subpopulation and that it allows the target populations to adapt to the prevailing environmental conditions. Another advantage is that the speed of evolution might be faster in a population of 50 trees than in a large population containing thousands of trees.

Besides MPBS another system, coined HOPE, Hierarchical Open Ended, was developed for simultaneous gene conservation and sustainable breeding of agricultural crops for one environmental condition (see Box 11-1) and is of limited relevance for forest trees that will grow under variable site conditions. Another disadvantage is long time to flowering, which means that backcrosses would take several decades to complete. This is in sharp contrast to crop species such as wheat, maize or rice, in which one backcross per year may be accomplished.

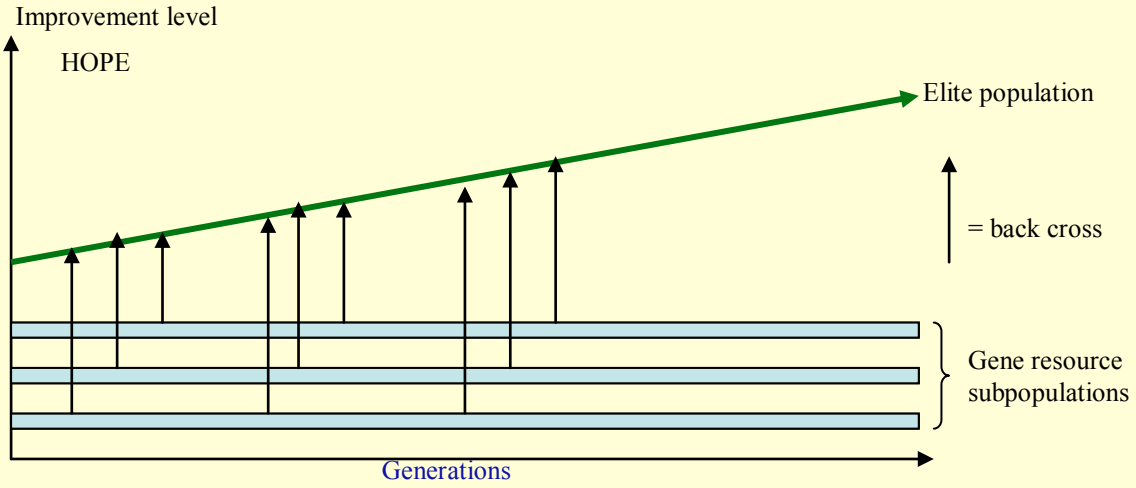
In Tables 11-3 and 11-4 we have summarised the methods that should be used to match different objectives in forest tree gene conservation. The greatest emphasis is given to the prime objective of gene conservation, to safeguard the potential for adaptation of the target species. Before coming to specifics about methods it is of importance to discuss how to select gene resource populations in the absence of genetic knowledge. In this case we have to select the subpopulations based on knowledge about the life history traits and the genetic structure these traits might have given rise to. This means that we may benefit from what we have learned from Chapter 6. For species with random mating populations, disruptive natural selection and gene flow are the two dominating evolutionary factors.

Box 11-1 Comparison of the two systems aiming at a combined gene conservation and tree breeding.

HOPE stands for Hierarchial OPen Ended system, which implies that new material may continuously be incorporated into the system. MPBS stands for Multiple Population Breeding System, implying that the system has many equivalent subpopulations.

The level of improvement is shown on the Y-axis. On the X-axis there are many generations of breeding over time. In HOPE there is a transfer of genetic material from the unimproved level and less improved gene resource subpopulations to the green elite population through backcrossing. The level of improvement remains more or less constant in all subpopulations except for the elite population. The elite population has a narrow genetic base. The cultivars for commercial production are generated from the elite population. For each breeding generation the gaps between the elite population and the subpopulations are broadened. HOPE is of limited importance for long-generation species such as is the case for most forest trees.

Over the time the gaps between all the 19 subpopulations in the MPBS system are broadened while keeping satisfactory genetic variation (= additive variance) within each subpopulation. This means that the total additive variance in this case increases over time. Selection of material for seed orchard establishment or clonal propagation takes place in some of the subpopulations according to the demand for reforestation material. Thus, if there is a demand for reforestation for fibre farming, clones are selected from the uppermost subpopulation. When breeding of a species is carried out according to the MPBS system it conserves the genetic variation of that species in a good way.



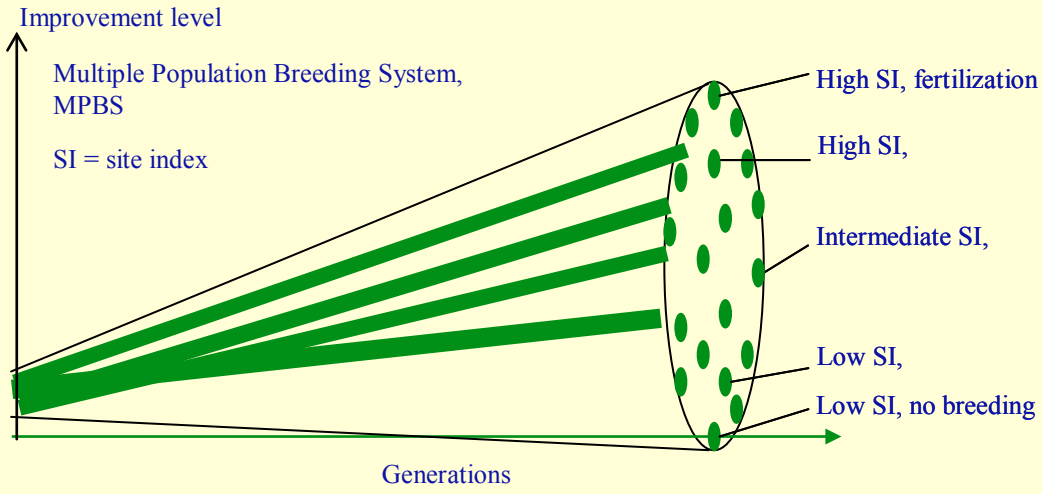


Table 11-1. Educated guesses about population differentiation in species with random mating populations

		Disruptive natural selection between populations	
		weak	strong
Gene flow	limited	Some among-population variation for random reasons	Important among-population differentiation
	strong	Very limited among-population differentiation + a very large variation within populations	Some among-population differentiation + a large within-population variation

Table 11-2. Educated guesses about variation among and within populations in species with non-random mating populations.

	weak disruptive selection		strong disruptive selection	
	Limited gene flow	Considerable gene flow	Limited gene flow	considerable gene flow
Genetic drift	Variation between populations			
	++	+	+++	++
	Variation within populations			
	+	+++	+	++

In Table 11-1 educated guesses about within – and among-population variation are given for contrasting combinations of disruptive selection and gene flow. Studies have shown that species with pollen vectors flying over short distances will probably be more differentiated than species that are wind pollinated. This is probably also the case for species with scattered distributions with no or limited gene flow among the scattered populations. In the case of non-random mating populations we also have to consider genetic drift and its impact on within- and among-population variation (Table 11-2). The strongest population differentiation is projected for the combination: genetic drift + strong disruptive selection + limited gene flow. The contrasting combination, weak disruptive selection and large gene flow will have the lowest differentiation of populations. This combination is also expected to have the largest within-population variation. In cases with weak disruptive selection and limited gene flow, genetic drift will be the dominating evolutionary factor. Since genetic drift leads to fixation of alleles, the variation within populations is expected to be low for this combination. It ought to be stressed that these projections are theoretical and should be used with care, and only when information on adaptive differentiation is lacking.

Safeguarding the potential for adaptation

As stated above, species included in serious long-term breeding programmes may not need separate gene conservation programmes. The main method for this type of species will be the *ex situ* MPBS (Table 11-3, page 178). This means that there will be a series of progeny trials in which the best phenotypes are crossed to obtain a new generation in the combined breeding and gene resource population. This process is repeated in a recurrent way. Possibly the combined breeding and gene resource subpopulations should be complemented with additional populations when the MPBS subpopulations do not well cover the entire genetic variation of the species.

Within the EU funded CASCADE project, "Securing gene conservation, adaptive and breeding potential of a multipurpose tree species (*Castanea sativa*) in a changing environment", conservation values were developed by Gabriele Bucci for adaptive traits (ATCV), pathogen tolerance (PTCV), and marker traits (MTCV). The number of populations in this study varied from 6 for adaptive traits to 78 for markers. The full use of the ATCVs and the PTCVs cannot be take place here since the number of studied populations was too limited. However, the calculations used in this project can be applied in future for sweet chestnut or any other tree species studied in detail.

The additive trait conservation value (ATCV) can be based on the evolutionary potential or population divergence. The latter is based on how much a specific population differs from the other populations studied. As seen from Fig. 11-7, the Greek population Paiko was the

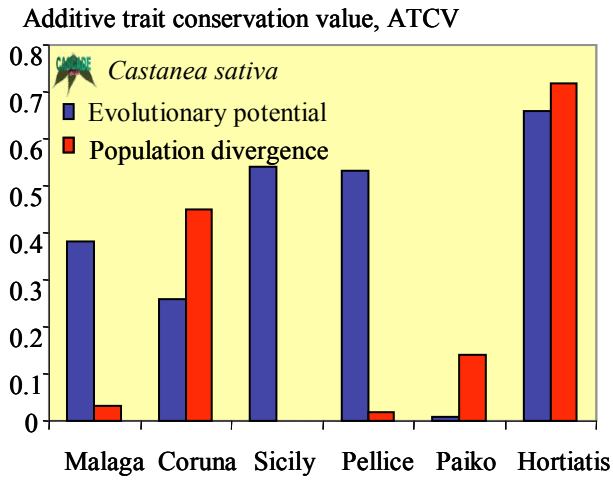


Figure 11-7. Adaptive trait conservation value, ATCV, of six Castanea sativa *populations. ATCV considers the potential of the population to evolve and its unique genetic constitution.*

only population that showed low evolutionary potential. The Spanish population Coruna and the Greek population Hortiatis showed the largest population divergence. The former showed good juvenile growth and the latter showed poor growth.

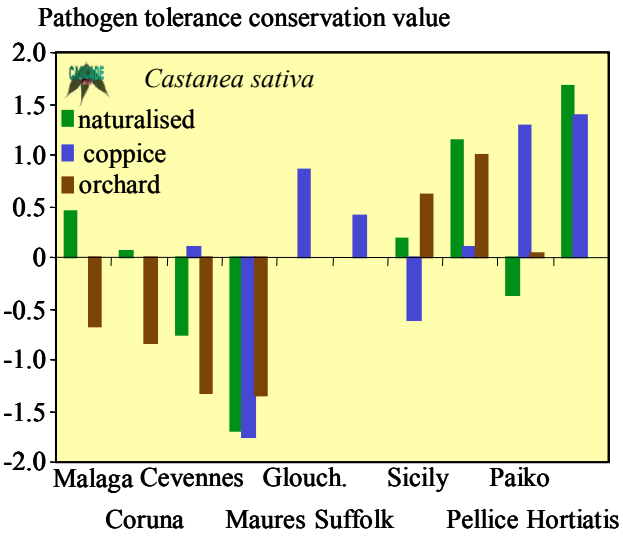


Figure 11-8. Pathogen trait conservation value, PTCV, of Castanea sativa *populations. The PTCV combines high tolerance to* Phytophthora cambivora *and high potential for improvement of tolerance against this pathogen. The value is given for naturalised, coppice and orchard populations separately.*

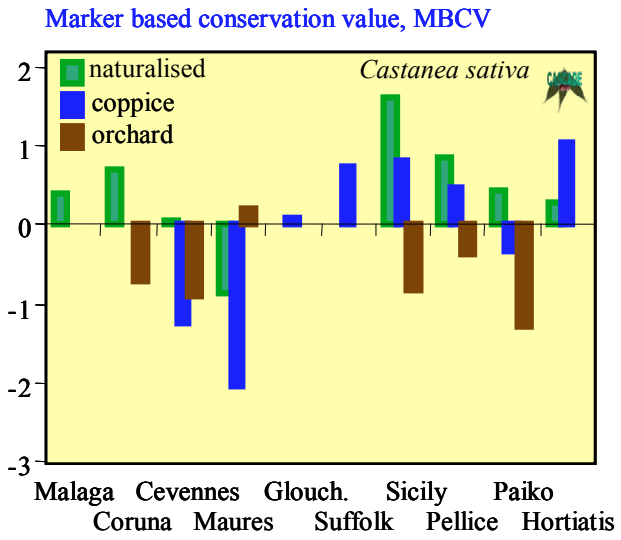


Figure 11-9. Marker-based conservation value, MBCV, of Castanea sativa *populations from 9 regions in Europe. The MBCV is mainly attributed to richness of genetic variability in individual populations. The value is given for naturalised, coppice and orchard populations separately.*

The pathogen tolerance conservation value, PTCV, was based on the inoculations of the material with one strain of *Phytophthora cambivora* and it was calculated separately for three domestication levels, naturalised, coppice, and orchard populations. The PTCV was calculated in such a way that a high PTCV value means good tolerance against *P. cambivora* as well as large evolutionary potential for improvement of tolerance. The two coppice populations from Greece as well as the naturalised Greek population from Hortiatis showed high PTCVs (Fig. 11-8). The French populations and the Spanish orchard populations had low PTCVs.

Three estimates were used for derivation of the marker-based conservation value MBCV, expected heterozygosity H_e, F_{ST} and N_e. For the markers it turned out that H_e had the greatest influence on MBCV. Generally the orchard populations showed the lowest H_e as expected for grafted material. The southern Greek populations had a genetic constitution differing from most other populations and for that reason the Greek populations have a special value for the network of gene resource populations (Fig. 11-9). Noteworthy is the high H_e in both English populations, (Glouchestershire and Suffolk), which are both coppice forests.

Table 11-3. Gene conservation methods to meet the objective of safeguarding the potential for adaptation in various groups of species.

Commonly occurring: Species included in intensive breeding	*Ex situ* MPBS + complementation with populations in the wild when needed
Commonly occurring: No breeding or low-intensity breeding	*In situ* MPBS selected according to genetic knowledge
Commonly occurring: No breeding or low-intensity breeding without any genetic knowledge	*In situ* MPBS selected on ecogeographic principles
Commonly occurring: Multipurpose breeding: wood and nuts	MPBS for wood in naturalised forests + clone archives for nuts
Commonly occurring: Endangered by Dutch elm dicease	Low clone hedges + *in situ* MPBS whenever possible
Rarely occurring: With possibilities for investment	Clone archives + progeny plantations for each ecogeographic zone
Rarely occurring: low-cost alternative	promotion of the growth conditions + delivery of seedlings free of charge to forest land owners

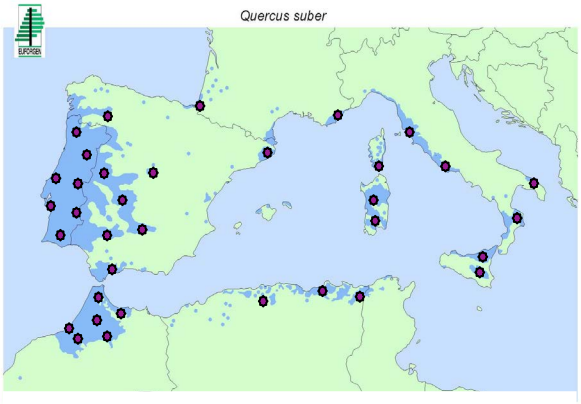


Figure 11-10. Distribution (blue areas) of and suggested gene resource populations for cork oak, Quercus suber.

In cases where there is low-intensity breeding or no breeding but we know the genetic differentiation of the species, the less intensive *in situ* MPBS method is recommended (Table 11-3). This method is also recommended for those cases for which we lack the desired genetic knowledge. Cork oak (Picture 11-2), *Quercus suber*, is one example of a species for which knowledge about

Picture 11-2. Harvesting of cork from a cork oak tree. Photograph Gösta Eriksson.

genetic differentiation of adaptive traits is just emerging (year 2006). Based on existing knowledge from juvenile experiments, the subpopulations suggested in Fig. 11-10 may be one solution for gene conservation of cork oak. During the nineties a similar approach was suggested for conservation of *Tectona grandis* in Thailand.

The planned and partly implemented strategy for forest tree gene conservation in Sweden (2012) will be given as an example how forest tree gene conservation in the absence of genetic knowledge can be accomplished. In Sweden five types of protected areas may be utilised for conservation of forest trees and their associated species:

National parks. The total number is 29 with an area of 700,000 hectares.

Nature reserves. The total number is 3,200 with an area of 4 million hectares.

Habitat protection areas, small land or water areas that are important environments for threatened plants or animals, or especially important to protect for other reasons. The total number is 6,250 with an area of 19,400 hectares

Ecoparks, large (≈ 5,000 hectares), connected landscapes throughout Sweden with high biodiversity conservation and environmental protection ambitions. The total number is 36 with an area of 175,000 hectares.

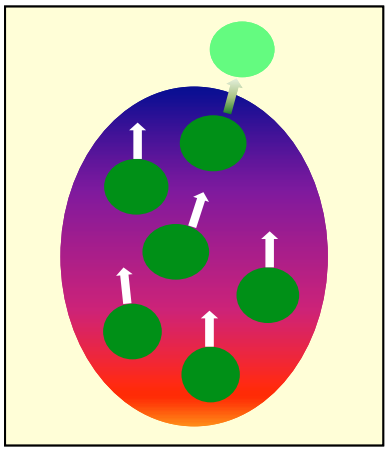


Figure 11-12. A principle for mitigation of global change in gene conservation. The current distribution area is inside the oval with decreasing temperatures upwards. The darker green circles represent existing populations. The arrows indicate transfers of material to a cooler climate and even planting outside the present range of the species dsitribution.

Nature conservation agreements are voluntary agreements between the Swedish Forest Agency and forest owners valid up to 50 years. The total number is 4,000 with an area of 27,000 hectares.

With such a large size and number of protected areas it would be possible to identify some of them as *in situ* gene conservation areas of species for which we lack genetic knowledge. The condition is that they contain a satisfactory number of flowering trees. A minimum number of 15 for the most rarely occurring species is required. The conservation areas will be selected according to ecogeographic principles.

In the case of global warming it might be neccessary to transfer populations to temperature climates that are cooler today (Fig. 11-12) but which may have the present temperature conditions after warming. Even transplanting outside the present range of distribution might be advisable.

Picture 11-3 (above) and 11-4 (below). 11-3 Fruit orchard of Castanea sativa. *11-4.* Castanea sativa *high forest being either naturally regenerated or naturalised,* i.e. *conversion of coppice or fruit orchard to high forest. Photograph Fiorella Villani.*

In the Mediterranean region, breeding for nut yield and quality in chestnut (*Castanea sativa*, Picture 11-3) and walnut (*Juglans regia*) has gone on for millennia. Grafting of superior nut producing genotypes has taken place, which means that fruit orchards usually have few cultivars/genotypes. Some of them are even male sterile. In high forests (Picture 11-4), adaptation to the prevailing conditions has probably taken place, whereas the Darwinian fitness in most cultivars for nut production may be low.

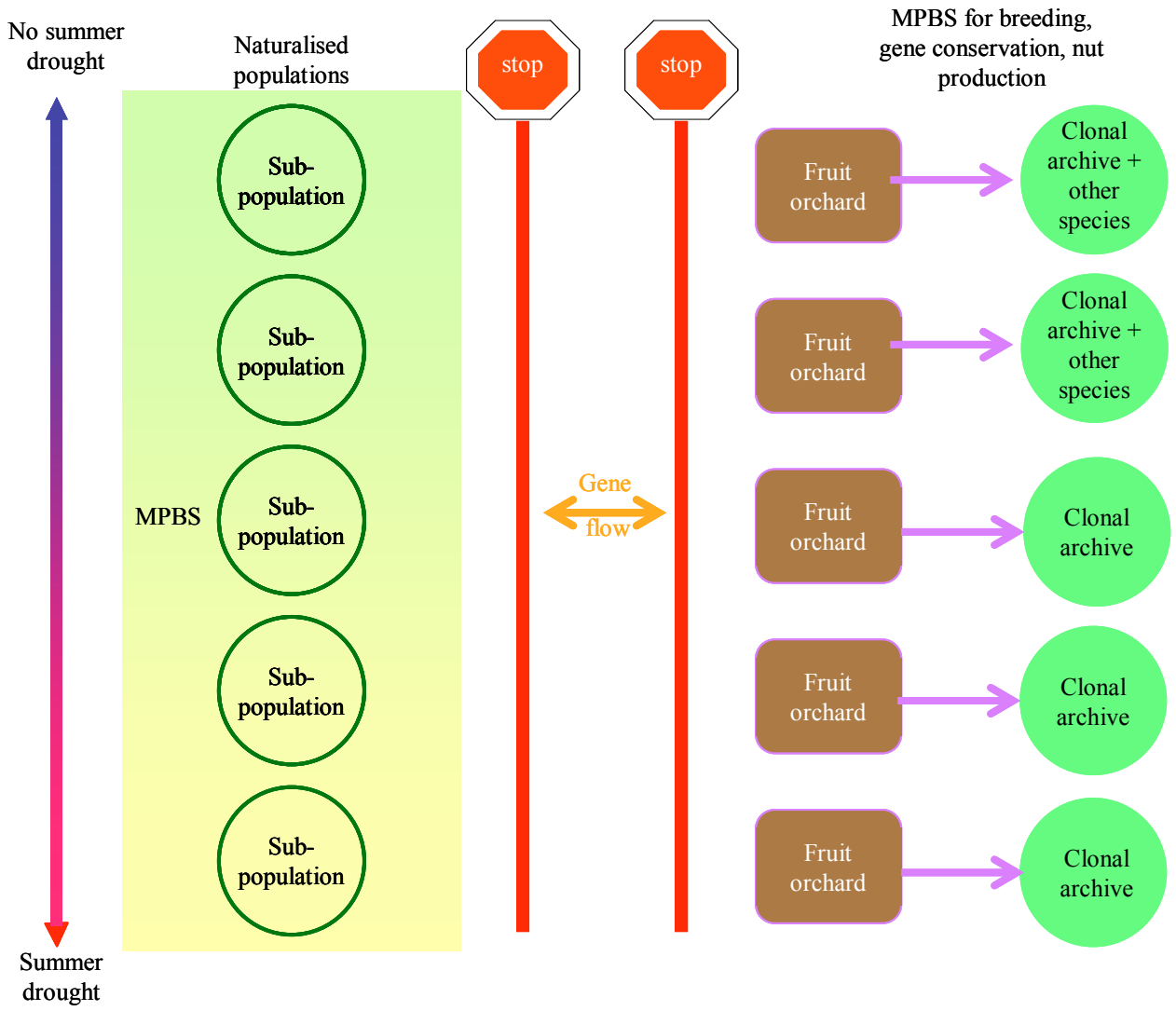


Figure 11-12. Suggested principle for gene conservation of Castanea sativa. *Separate conservation for wood production and fruit production are suggested. For both purposes the multiple population breeding system concept will be applied.*

According to the concepts introduced in Chapter 7, the nut cultivars might have a high degree of domestic fitness while populations in the wild have some degree of Darwinian fitness. This might cause a problem for gene conservation if there is a large gene flow from cultivars to gene resource populations, since there is a fear that such a gene flow would drastically reduce the Darwinian fitness of the populations in the wild. A similar problem is due to the introduction of the Asian chestnut species *Castanea crenata* and *C. mollisima* for hybridisation with *C. sativa* to obtain hybrids tolerant to diseases caused by *Cryphonectria* and *Phytophtora* species. The species hybrids are less drought tolerant and have another growth rhythm. The latter means that they cannot be used in areas in which late spring frosts are a constraint for chestnut growth. To solve the problems mentioned, the *ex situ* MPBS method is suggested for breeding to improve wood yield and quality. If funding for that is not available the *in situ* MPBS is recommended for the naturalised forest populations. The *in situ* subpopulations should be selected such that gene flow from cultivars is minimised (Fig. 11-12). Probably the nut breeding has suffered from low effective population sizes. Therefore, a series of clonal archives in different ecogeographic regions is suggested. This will permit more efficient breeding when there are more genotypes represented. For areas with no summer drought and limited spring frost problems, clones of the two Asian species might be included in the clonal archives.

Dutch elm disease affects wych elm most seriously but in cycles. The long-term gene conservation of this species must rely on low hedges (Picture 11-5). Such hedges do not constitute a breeding ground for the insect vectors (*Scolytus* insects). Whenever possible the *in situ* MPBS method is suggested. To overcome the problem with Dutch elm disease and other serious diseases in a long term perspective, breeding for disease tolerance should be carried out. A restoration programme of the severely affected American chestnut is proceeding by hybridisation of this species with disease tolerant Asian species.

Picture 11-5. Vegetatively propagated wych elm, Ulmus glabra, *grown as hedge. This prevents the attacks of the* Scolytus *insects, which spread the Dutch elm disease. Photograph Eric Collin.*

One focal point for gene conservation of rarely occurring tree species is to increase the effective population size to avoid random genetic drift. The most costly way of doing this is to collect scions of trees and produce grafts for clone archives or seed orchards. This approach is used for *Sorbus torminalis* in Germany and *Ulmus laevis* in Finland. The seeds obtained from the seed orchards are used to raise seedlings which will be planted in forests. The seedlings and trees will be exposed to natural selection. Clones from different ecogeographic regions should not be mixed. Therefore, this intensive gene conservation will be carried out according to the MPBS method. In most cases such a high-cost method will not be practicable, instead it is recommended that seedlings are raised and offered to forest landowners free of charge in order to raise the population size. To overcome the problem of unintentional cutting of rare trees during thinnings, taxonomic training of all kinds of foresters might be a remedy. Many of the rare tree species in temperate forests are intermediate species that might be outcompeted by climax species. Once foresters are aware of the existence of rare species they might even be promoted in silvicultural operations by thinning of competing tree species.

For all types of *in situ* gene resource populations it is important that their regeneration is guaranteed. Natural regeneration of some gene resource populations may in some cases fail owing to severe competition from other species such that the regeneration takes place from the wrong species. It is evident that this is a dead end for that gene resource population. In this case, active measures must be taken to support the regeneration of the target species. This has to be done even if the gene resource happens to grow in a protected area with "hands off" management regime.

Table 11-4. Gene conservation methods to meet some gene conservation objectives other than safeguarding the potential for adaptation.

Objective	Method
Preservation: as reference for future experimentation; avoidance of contamination from cultivars	Clone archives, seed orchards, seed banks (pollen banks)
Unknown variation	Encompass as much variability as possible; is mostly obtained if the MPBS method is applied
Threat: from pollution or other anthropogenic causes, urbanization	Clone archives, seed orchards, seed banks, complementary plantations
Gene conservation of associated species:	Large MPBS, 200 – 300 ha including management of some subpopulations while a few others are nature reserves

Methods for other objectives in gene conservation (Table 11-4)

Preservation of the existing genetic constitution means freezing the current genetic structure. The need for reference material (Reason No 1 for preservation presented under Objectives above) is most simply satisfied for many tree species by storage of seeds or other propagules. Seeds, acorn or nuts of several tree species cannot be kept in long-term storage. For these species clone archives are the remedy and such archives are used for conservation of wild relatives to fruit trees such as apple, pear and cherry. Pollen storage is also an alternative for static gene conservation.

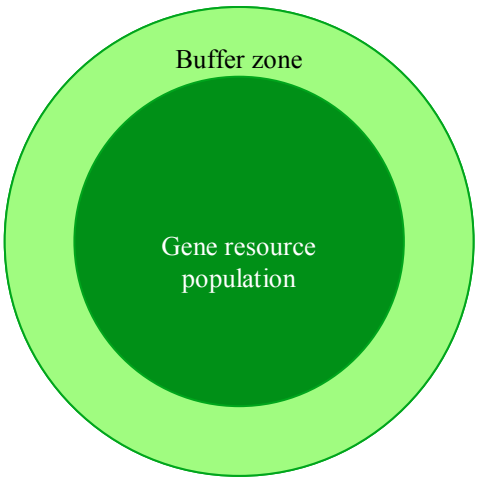


Figure 11-13. The principle design for preservation of the core gene resource population from undesired gene flow by having a buffer zone.

To match reason No 3 for preservation the material has highly desired characteristics that may be lost if the present constitution is not preserved. Therefore, a large buffer zone (Fig. 11-13) around the population/forest that should be preserved, is suggested. However, it should be noted that total preservation is not possible unless the site conditions remain constant, which is highly unlikely.

To safeguard unknown genetic variation the only solution is to try to include as much of the existing genetic variability as possible. This is probably most efficiently done by the MPBS method..

Threat. In Germany great efforts have been devoted to saving *Picea abies* populations exposed to air pollution. This was done by collecting scions for grafting and establishment of clone archives outside the polluted area. The unique Guadeloup population of *Pinus radiata* has no natural regeneration owing to the goat population of this island. During the 1990s several seed collections were carried out. Seedlings raised from these seed collections were established in California, Australia, and New Zealand.

For riparian species that have lost part of their habitats, the *in situ* MPBS is suggested if breeding is not under consideration. If the fragmentation has gone far, with a strong reduction of the effective population size, complementary plantations with seedlings or cuttings (black poplars, *Populus nigra*) are recommended. Clearings along natural riverbanks may be needed to obtain a satisfactory regeneration.

Conservation of associated species. Many laymen believe that nature reserves with old forests (Picture 11-6) are the most important for conservation of associated species. Snags and old trees under decomposition are certainly of great importance for a variety of organisms dependent on mulm. In Fig. 11-14 we present an attempted projection of the impact on biodiversity during the process of decomposition. To begin with there is a steady increase along with the decomposition. At a certain level of decomposition the richness of biodiversity reaches a maximum level. After that there is a decline in richness and finally a minimum level of biodiversity is noted. *Salix caprea* is a keystone species that harbours more biodiversity than any other tree species in Sweden. A large number of butterflies, beetles, and fungi are dependent on *S. caprea* for their existence. During the juvenile phase several butterfly larvae and beetles feed from the young leaves. As the tree matures, other wood boring insects attack the wood. During flowering certain bees and other nectar searching insects benefit from the rich flowering. Thus, parallel to the aging process of the *S. caprea* trees there is gradual replacement within each of these three categories of organisms. This is not unique for *S. caprea*; rather in most ecosystems, species appear during different stages of the succession (cf Fig. 11-15).

Picture 11-6. A nature reserve at latitude 59°N in eastern Sweden. Photograph Gösta Eriksson.

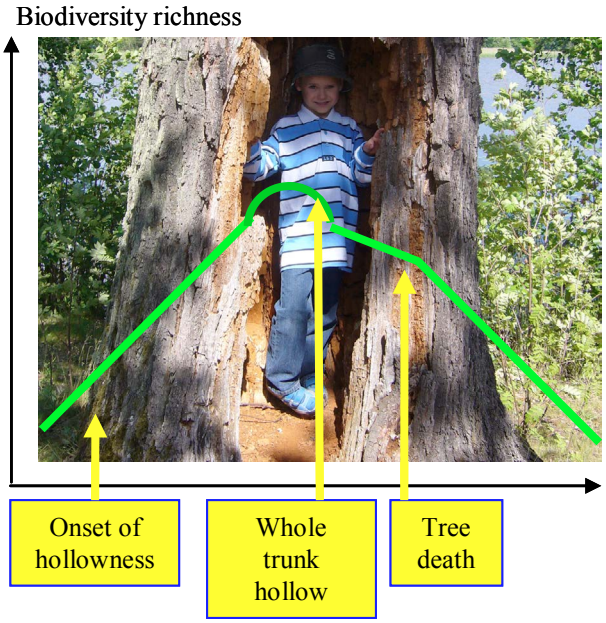


Figure 11-14. This Quercus robur *tree is used to illustrate the expected importance of the decomposition process for richness of biodiversity during the breakdown. The decomposition of parental material for production of non-living organic material is crucial for the existence of certain associated species. The curve was developed together with Ola Bengtsson. Photograph Gösta Eriksson.*

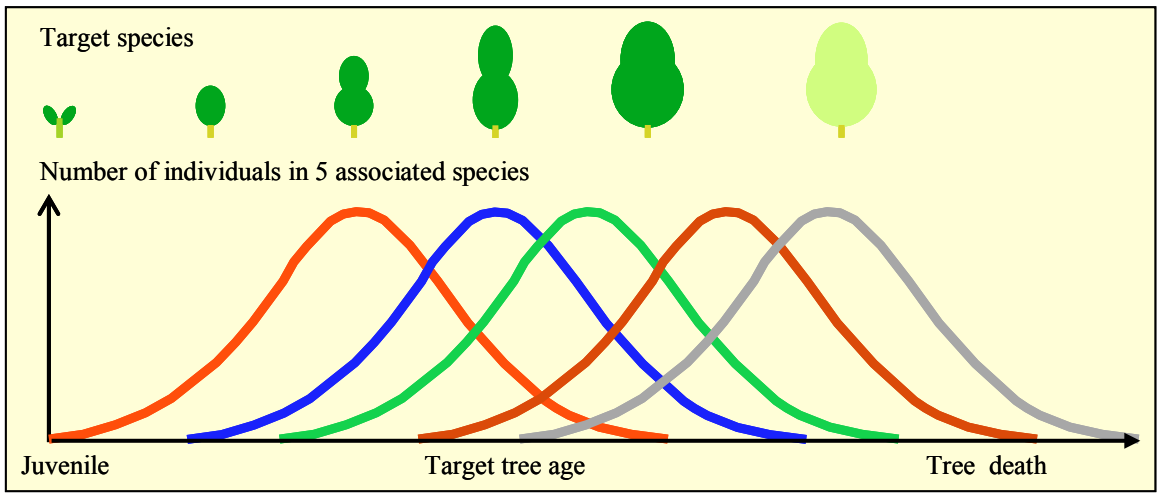


Figure 11-15. Schematic illustration of change in species composition during the course of development during aging of a target tree species.

Picture 11-7 and 11-8. The two pictures were taken of the same Quercus robur *population growing approximately 200 metres apart. The forest in the above picture does not have much human intervention while the lower is managed to acquire a park landscape character. Photograph Gösta Eriksson.*

The management regime may also influence the associated species. Pictures 11-7 and 11-8 were taken a few hundred metres from each other. They reveal that the flora is strongly dependent on the management regime. In the upper picture limited management takes place while the lower population is managed as in a park landscape. It ought to be stressed that several species are dependent on human activities; many species would become extinct if no human activities are allowed in gene resource populations. Other species depend on virgin forests without any human intervention. It is important that both managed and untouched forests are included in the gene conservation of associated species. This is particularly well demonstrated in the *Fagus sylvatica – Abies alba* climax forest in Slovenia (Picture 11-9). In this nature reserve no management is permitted, which has resulted in regene-

Picture 11-9. A mixed Abies alba *and* Fagus sylvatica *climax forest in Slovenia. Note that regeneration consists of* Fagus sylvatica *seedlings only. Photograph Gösta Eriksson.*

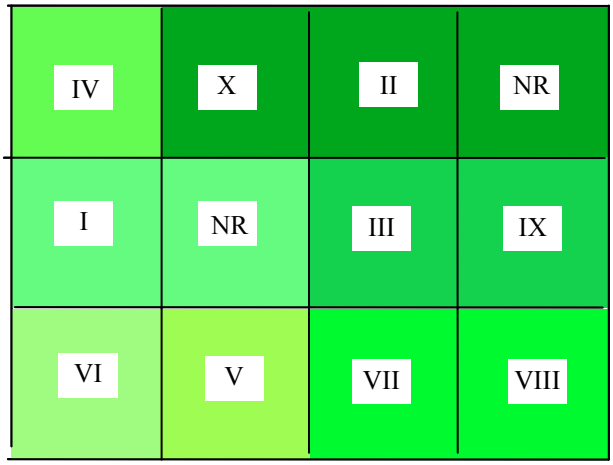


Figure 11-16. Schematic illustration of how a large gene resource population, 200-300 hectares, might be subdivided into 12 plots, 10 of which have different age classes, I- X, and two plots are nature reserves, NR. Different site indices are indicated with different colours. Plots I-X may be managed for production of utilities.

ration of *Fagus sylvatica* only, since all *Abies alba* seedlings were eaten by deer. If no management is allowed this mixed climax forest will turn into one-species climax forest over time. It should be noted that this Slovenian nature reserve was not classified as a gene resource population of *Abies alba*.

Many of the rare or endangered species depend on specific habitats for their survival.
For a satisfactory gene conservation of the associated species it is important to include:
Different age classes of the target species
Different management regimes
Different site conditions
Therefore, to accomplish these prerequisites we suggest that some of the subpopulations in the MPBS are extended to 200-300 hectares. It is important to design the gene conservation so that all stages from the juvenile to the over-mature phase with dead trees are represented (Fig. 11-16). Different management regimes are recommended and even controlled forest fire might be necessary. The subpopulations should be selected so that they cover the site conditions occupied by the target species. Within each of these large subpopulations, as broad cover of site conditions as possible should be aimed at. If these suggestions are followed, the gene conservation of both target and associated species will be taken care of.

Miscellaneous

Clone archives, provenance and progeny trials are a form of gene resource population. Their major merit is that they can be utilized for crosses in gene conservation and

Picture 11-10. A Castanea sativa *coppice population. Photograph Fiorella Villani.*

breeding. The genetic structure of the seed in a provenance trial is hard to predict. In most cases there will be a mixture of within-provenance crosses, crosses among the provenances in the trial, and hybrids between the provenances in the trial and the surrounding stands. Especially for wind-pollinated species with wide pollen dispersal, the latter will be significant. In many progeny trials the parents originate from one population and the seed crop will be more homogeneous than seeds from a provenance trial. Depending on the composition of the clone archives, the seed crop from them will either have a similar structure as in a provenance trial or in a progeny trial.

Botanical gardens mostly contain one or a few trees of each species and it might be questioned whether they should be regarded as gene resources. Some botanical gardens carry out an active gene conservation on annual and perennial herbs.

Coppice populations occur in some species such as *Castanea sativa* (Picture 11-10). Mostly, this type of silviculture prevents a natural regeneration since any seedlings occurring are outcompeted by the vigorously growing stems in the coppice population. This means that no adaptation takes place in coppice populations and they are incompatible with the safeguarding of the potential for adaptation. Picture 11-11 was taken in a *Taxus baccata* forest in Sardinia. In this population there were no seedlings on the ground, and thus no regeneration. The shoots on the trunks were the only juvenile material. This presents an analogous situation to coppice forests of *Castanea sativa*.
Protected areas play a significant role in gene conserva-

Picture 11-11. Picture from a Taxus baccata *forest in Sardinia. No regeneration was found on the ground, the stem shoots shown constitute the only juvenile material in this forest. Photograph Gösta Eriksson.*

tion in developing countries. There is an increased understanding that the previous philosophy of "hands off", which means that any human intervention is banned from protected areas, has to be revised to reach goals in gene conservation. There is also an increased understanding that sustainable use of the natural resources in protected areas by local people ought to be integrated with conservation. Gene conservation in forests that are included in protected areas in future will more easily reach its conservation goal if a management plan is developed for the protected area. There is a need that governmental organisations include non-governmental groups, indigenous people, community groups, and the private sector in establishment and management of protected areas. It is fundamental that an appropriate management of the protected area is carried out, such that forest gene resources are not lost or degraded unintentionally. If local people are involved in establishment of protected areas the risk for degradation or loss of such gene resources is probably much reduced. It is worth restating that silvicultural treatments to guarantee regeneration of gene resource populations are of greatest significance in management of protected areas.

Especially for tropical forests the regeneration issue is most relevant. Logging of very valuable tree species that occur at low densities, 1-2 mature trees per hectare, poses a risk for the continued survival of such species. In these cases measures to promote regeneration should be taken. Common sense gives ideas about how to promote regeneration:

- Logging of trees is permitted only if the tree has reached a minimum breast height diameter. This will allow younger trees to reach flowering age.
- Logging at optimum time, *e.g.* after maturation of seeds or fruits and not during main flowering.
- Opening of forests; the size of the openings is dependent on the stage in the ecosystem of the tree species. Pioneer tree species are light demanding while climax species usually are shade tolerant.
- Liberation cutting of competing species and inhibition of grass growth.
- Planting is a costly operation but may be needed in some cases.

Finally logging for commercial reasons means that there may be some revenue from forests. This in turn may convey a message to peasants that forests have an economic value. For that reason slash and burn for shifting agriculture hopefully will become less attractive.

Species hybridisation and gene conservation

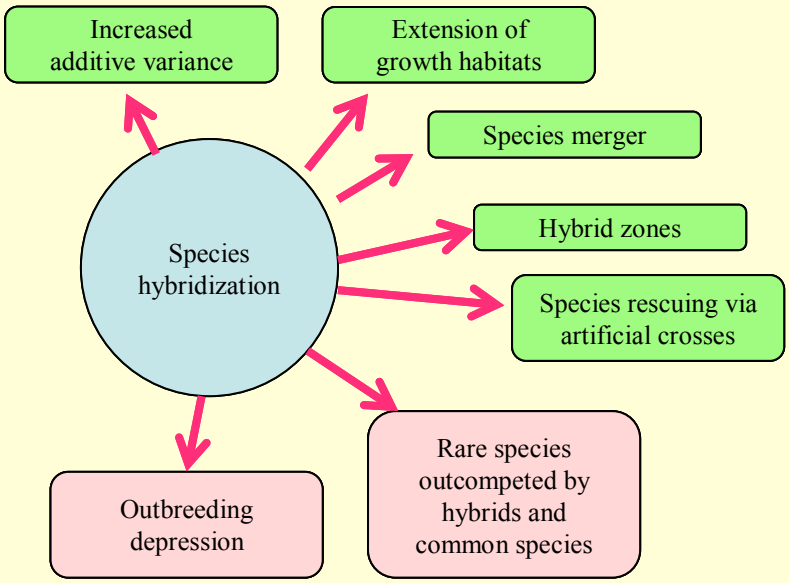
Hybridization is common in some genera and must be regarded as a part of evolution in nature. The role of hybridization is somewhat controversial. Some regard hybridization as something that must be totally avoided while others regard hybridization as a means to increase the additive variance and thereby save genetic material that would be lost without hybridization. In Box 11-2 some possible outcomes of species hybridization are given.

In nature *Trochetiopsis erythroxylon* became extinct during the 1950s. Seeds were collected from the tree before extinction but they were affected by large inbreeding depression. During the 1980s two trees of the related species *Trochetiopsis ebenus* were detected. Crosses were carried out between these two species, which resulted in vigorous offspring. This project did not lead to saving the two species but genes from these two extremely rare species were preserved.

Efforts are being made to save the American chestnut, *Castanea dentata*, which just survives as root suckers owing to the devastating chestnut blight disease caused by *Cryphonectria parasitica*. Crosses are carried out with East Asian *Castanea* species to get resistant hybrids. Via back crossing it is hoped that the American chestnut with resistance against chestnut blight will be restored. Efforts are also made to identify and transfer resistance genes by modern molecular genetics methods.

A possible loss of Darwinian fitness in *Castanea sativa* populations in nature owing to hybridisation with East Asian *Castanea* species was discussed in connection with conservation of *Castanea sativa*.

Box 11-2 Possible outcomes of species hybridization



Species hybridization may result in increase of the additive variance. It is easy to imagine that combination of two different genomes will increase the additive variance. The increased variance may in turn give the hybrids opportunities to be competitive in new environments.

A final outcome of large-scale hybridization may be that the two species merge to a new species or that certain areas will continue to harbour hybrids. Thus, stable areas with hybrids are formed.

For species close to extinction artificial hybridization can in some cases be the only remedy to rescue a species. Two examples, *Castanea dentata* and two *Trochetiopsis* species, are presented in the text.

The two boxes with reddish background constitute more critical cases for rarely occurring species and are treated in the text and illustrated in Figures 11-17 and 11-18.

Species hybridization may be a threat to rarely occurring species. If the hybrids between the rare species (red circle in Fig 11-17) and the common species have higher fitness than the rare species, the rare species may eventually be eliminated. Since the census number is much larger in the common species the gene flow will mainly be unilateral from the common to the rare species. If the hybrid between the two species has a higher fitness than the rare species, hybrids will increase in number at the cost of the rare species. Over time the hybrids will become more and more similar to the common species.

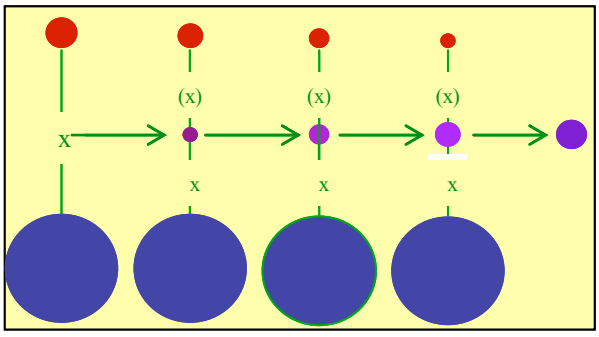


Figure 11-17. Schematic illustration of the risk for loss of a rarely occurring species (red) which hybridizes with a commonly occurring species (blue) giving rise to hybrids (lilac) with higher fitness than the rare species.

Another situation is illustrated in Fig.11-18. In this case the hybrid has an inferior fitness compared to both parental species. This is usually referred to as outbreeding depression. The rare species (red circle) may in such a situation waste its gametes in crosses with the commonly occurring species or the hybrid, leading to reduction in number of the rare species.

Sustainable forestry

Sustainable forestry, when it is being most environmentally conscious, can be regarded as a form of gene conser-

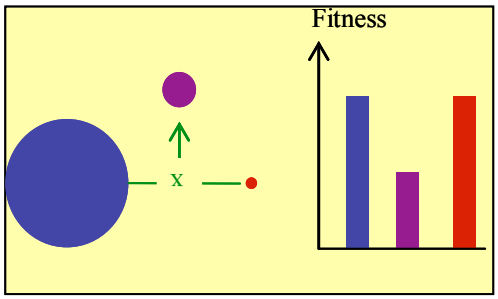


Figure 11-18. Schematic illustration of the risk for loss of a rarely occurring species (red), which hybridizes with a commonly occurring species (blue) giving rise to hybrids (lilac) with lower fitness than the rare species.

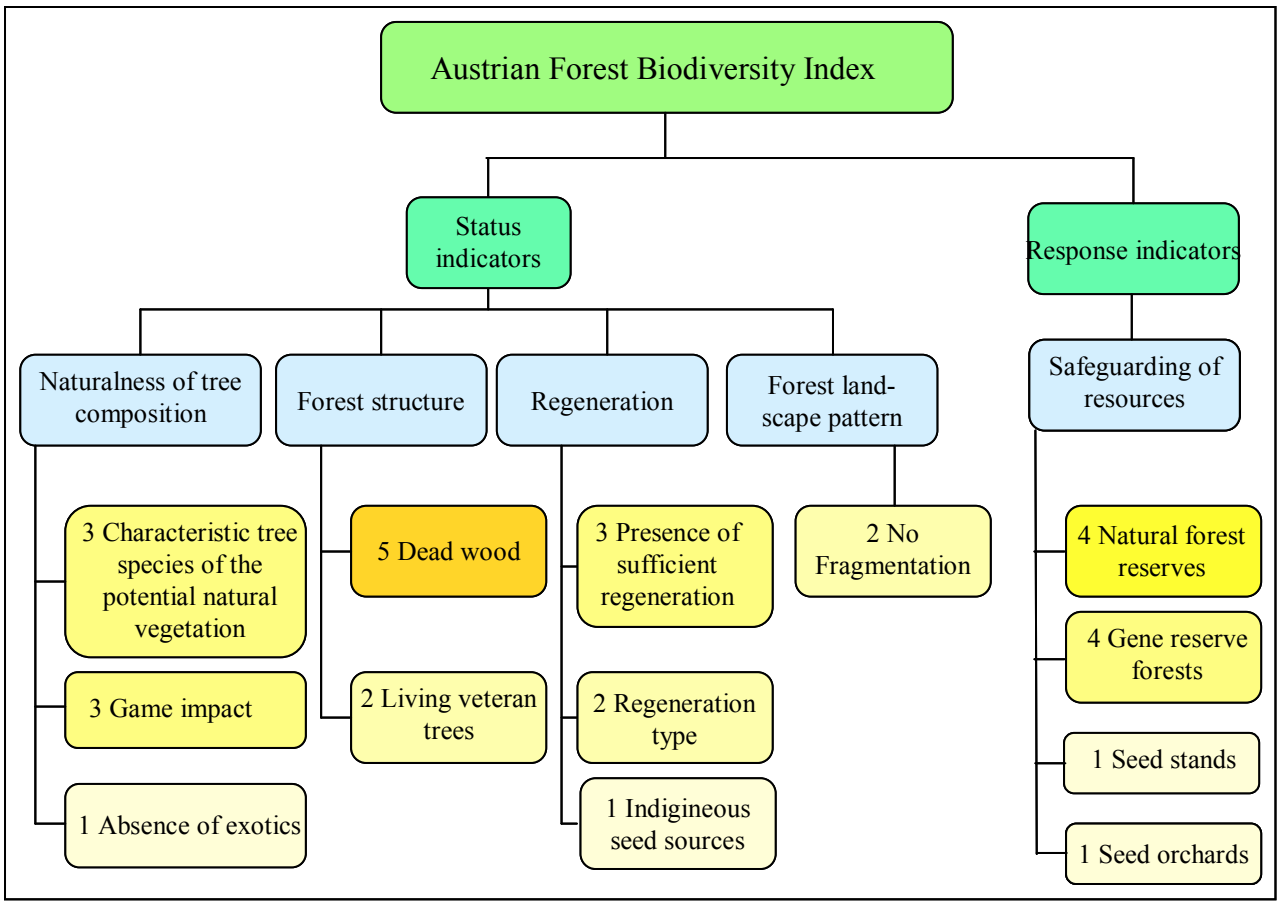


Figure 11-19. Components in the Austrian classification system for biodiversity; slightly modified from the Austrian original. Key factors are shown as light blue, indicators and their weights have different nuances of yellow

vation; both for target species and associated species. One problem in sustainable forestry is how to quantify the sustainability. One attempt at quantification was done in Austria (Fig. 11-19). The effort was to have as simple as possible a system that still gives pertinent information on sustainability. In this classification five key factors were identified; four of them describing the status and one the response to activities. Nine indicators were identified under the four status indicators and four indicators under the response factor. Each indicator was given a weight on a scale 1-5 according to its importance for sustainability. At each forest stand the species that constitute the ”natural” vegetation are identified. If the stand exclusively contains the tree species that is expected to grow there it is given a value of 100. This value is then multiplied by 3 since the weight of this indicator is three. If the stand contains a certain percentage of a tree species that is not considered to be a component of the potential natural vegetation, the value is reduced in proportion to this percentage. If there is no exotic species the ”exotic indicator” is given a value of 100; the indicator weight of exotics is 1. The value of this indicator is reduced dependent on the fraction of exotics in the stand. With only exotic tree species the value is 0.

It might be noted that dead wood has the highest indicator value, 5. The reason for this is that many associated species are dependent on dead wood for well-being and survival. Similarly, the presence of veteran trees (large and old trees) are assumed to be of significance for associated species but not to the same extent as dead wood. Presence of sufficient regeneration is relevant during the phase of stand establishment and it is given a weight of 3. For species that never form stands, seed orchards are of positive value. For stand forming species, natural forest reserves and gene reserve forests (= gene resource populations) bring positive value to the index. It is anticipated that there is no human intervention in the Forest reserves. However, hunting is allowed to avoid too great impact from the game stock.

The maximum index value that may be obtained = 13 indicators x 31 weight values x 100 value points for each indicator = 40,300.

The Austrian index focuses on Darwinian fitness. It also assumes that the existence of associated species are dependent on the ”potential natural vegetation” and that any transferred population of the target species will be less efficient for conservation of the associated species. This assumption remains to be proven.

If all forest land has a *sustainable forestry* silvicultural regime, the gene conservation will be dispersed to all forests. As is evident from earlier chapters of this book, there are no population genetics needs for such a gene conservation of a target species. Associated species might

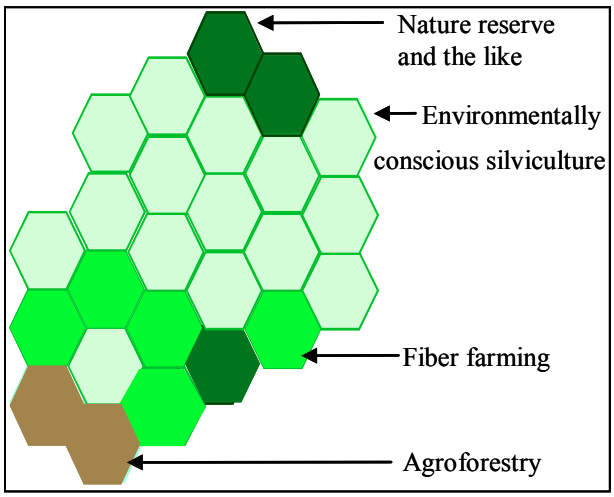


Figure 11-20. Schematic illustration how gene conservation and forest management can be done at landscape level.

be dependent on larger number than required for the target species, especially if it is an ecological keystone species. The requirement is rarely so large that all forest land is needed for gene conservation.

All projections of future demand for wood suggest a steady increase of the demand, partly owing to the dramatic increase in the human population. In this perspective many scientists advocate gene conservation on a landscape basis rather than on a stand basis. This means that certain forests constitute nature reserves, others have a total focus on production of wood, still others take an intermediate position to these two extremes. Such a landscape approach is anticipated to satisfy different objectives in a better way than one silvicultural regime over the entire area. A schematic sketch of how this can be achieved is given in Fig. 11-20. This illustration applies to the tropics in the first place but might in its purely forestry parts be applied outside the tropics as well. This is in line with a statement in FAO's State of the World's Forests 2012. *The world is large enough to allow different forests to be managed for different values and outputs:some forests can be protected; others can be intensively managed for wood; and others can be managed for multiple uses.*

In New Zealand most of the wood is supplied from plantations of *Pinus radiata*. This is an example of a landscape approach and it has probably been of great importance for keeping the unique domestic forests untouched (Picture 11-11).

Figure 11-21 illustrates the decline in self-sufficiency of forest products in California from the Second World War until 1990s. The main reason for this is that forest land, to an increasing extent, has been set aside as various kinds of nature reserves. In the early years, the demand for wood in California was satisfied by "imports" from the neighbouring states, Oregon and Washington. When larger areas in the latter states also were converted to na-

Picture 11-11. Native forest on the northern island of New Zealand. Photograph Gösta Eriksson.

ture reserves and the demand for wood increased, imports came also from British Columbia. During the nineties the demand had to be satisfied by wood from tropical Asia. Thus, the strong protection and gene conservation of plants and animals in California have increased the pressure on forests in other countries. This pressure is most serious for the endangered forests in South-East Asia. The lesson to learn from this example is that we ought to have a global and landscape perspective on forest tree gene conservation.

We have tried to summarise this global perspective in Fig. 11-22. As stated several times the human population will increase dramatically. Human aspirations for a better life will also increase. Expansion of the human po-

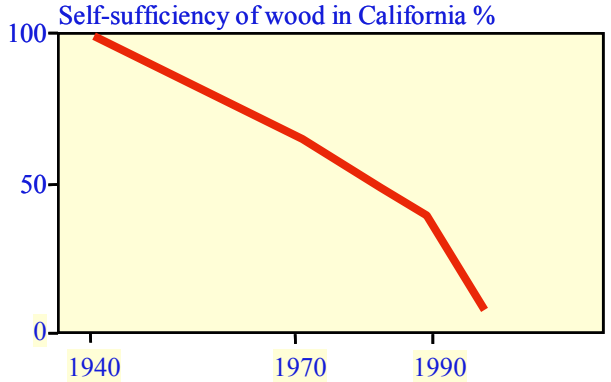


Figure 11-21. The self-sufficiency of wood in California for years 1940-1995.

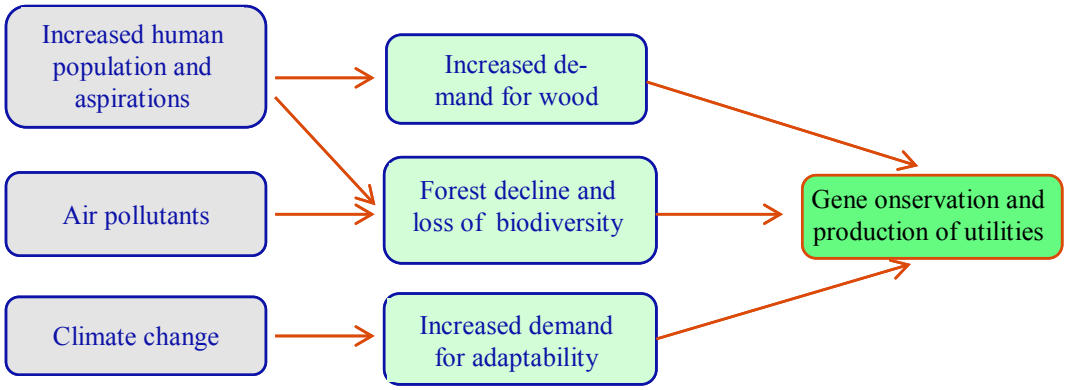


Figure 11-22. Reasons for linking production and gene conservation. See also text.

pulation will also result in increased air pollution. These conditions will lead to increased demand for wood and as a corollary of this, forest decline and loss of biodiversity. Rapid global change means that large adaptability is required. All these conditions mean that we should not treat gene conservation isolated from production of human utilities. Such a production is much dependent on tree breeding. Thus, tree breeding, production of utilities, and gene conservation ought to be done in conjunction.

Genetic pollution

Some scientists have expressed a great fear that the adaptation that has caused an increased adaptedness of a population in nature will be destroyed if there is gene flow from surrounding, introduced populations. Such a gene flow is designated as introgression. Some have even called such gene flow pollution, which is an emotionally strong word. The use of the word pollution probably emanates from

the belief that the adaptedness is perfect and
that any gene flow will reduce the adaptedness
of the recipient population
and
the belief that any gene flow will break a fine-tuned genetic set-up of the recipient population
leading to drastic reduction in fitness.

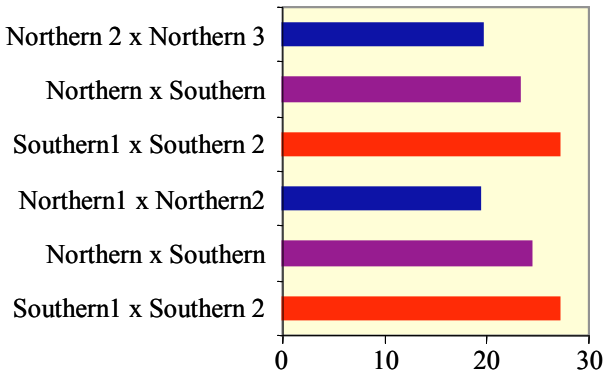


Figure 11-23. Number of days to budburst in three types of matings in Norway spruce, southern x southern, southern x northern, and northern x northern. For the hybrids northern x southern the mean values of the four possible crosses between two northern and two southern parents are given.

The latter belief requires that a specific adaptation to particular site conditions has taken place such that the activity of many genes depends on the presence of many other genes. This implies the evolution of what is often referred to as coadapted gene complexes. Once this specific combination of genes is broken up by crosses with alien pollen the adaptedness would be drastically reduced. Such situations probably exist but it is unlikely for the majority of wind pollinated tree species such as Norway spruce, Scots pine, loblolly pine, slash pine, Douglas fir, sessile oak, cork oak, sweet chestnut and many other wind-pollinated species with a wide and continuous distribution.

In southern Sweden many of the stands originate from eastern and south-eastern Europe. To evaluate if introgression from these introduced provenances to the Swedish Norway spruce populations is of any significance we need to know whether such an introgression causes:

1. a change but with a possibility to recreate the genetic constitution of the domestic population
2. a change that is irreversible.

To get an apprehension of which alternative might be true for Norway spruce we benefit from the knowledge that there is an additive gene action for important traits. As an example of additive gene action, data for budburst in different crosses are illustrated in Fig. 11-23. We have selected budburst since this trait is of great significance for survival of Norway spruce plants; mainly it is a question of avoidance of low temperature exposure of the frost sensitive stages just after budburst. In each part of the graph the mean values for the four hybrids *northern x southern* and the intra-provenance crosses are shown. As is seen from the graph the mean values of the hybrids are close to the means between the *northern x northern* and *the southern x southern crosses*. In one case the mean is somewhat closer to the northern cross, in the other the opposite situation prevails. From Norway spruce and Scots pine we have several such examples suggesting an additive gene action. This means that gene flow to an autochthonous south Swedish Norway spruce population will lead to a progeny that will be intermediate to the two origins. Especially if such hybridisations occur between widely differing populations there will be an increased additive variance. Via backcrosses it is possible

to recreate the domestic population, certainly a very cumbersome task, but possible. It should be noted that we do not know how the situation is for the trees pollinated by insects, which fly over short distances and thus may give rise to specific adaptation (cf Fig. 6-16). For such tree species gene flow might be more serious.

For traits of adaptive significance, gene flow causes a change in allele frequency in the recipient population. Difference in gene frequencies among populations is one result of natural selection. Therefore, there is principally no difference between gene flow and natural selection; in both cases a change in gene frequency takes place. Pollution is too strong a word to use for gene flow, though it must be admitted that gene flow will in most cases reduce the Darwinian fitness of the recipient population.

Different levels of a conservation programme

For practical reasons one has frequently distinguished between conservation at the ecosystem, the species, and the gene levels. Even if there are practical reasons for doing so there is no biological reason (see Chapter 6). It was stressed that genetic differentiation between populations or species is the same type of process in a dynamic evolution. To limit the conservation to the species level only, is a neglect of the fact that speciation is merely a part of the continuous evolution that takes place. Moreover, ecosystems are not stable or static since ecosystems are composed of species, which in turn are composed of populations, which are participants in a dynamic evolutionary process. Fossil data give support to this since they show that climatic change caused different migrations of the different components in an ecosystem. Thus, ecosystems did not migrate as ecosystems but rather each constituent species migrated independently of each other.

Summary

A gene conservation programme consists of three main components; objectives, genetic knowledge, and methods.

Many objectives might be identified, of which the most important is to safeguard the future potential for adaptation of the species. Other objectives are to preserve the present genetic constitution to have as a means for comparisons in the future. Preservation of the unique qualities of some populations used for production of highly valued human utilities is another objective in gene conservation. Simultaneous gene conservation and breeding, and conservation of associated species, are other objectives. Certain populations might be threatened and they deserve to be conserved for this reason.
The methods in gene conservation should ensure that objectives in gene conservation are fulfilled while taking the structure and dynamics of the gene resource into account. For the majority of target species we lack knowledge about the genetic structure. From the ecological characteristics of the species educated guesses about the genetic structure have to be made in these cases. Gene conservation according to the Multiple Population Breeding System is the best way to meet the prime objective of gene conservation. This method cannot always be applied in its most sophisticated form, *i.e.* as *ex situ* plantations of the subpopulations. Less intensive variants of this method can be used by simply selecting the subpopulations in existing forests with the intention of safeguarding as much as possible of existing adaptedness. For the gene conservation of associated species an enlargement of some of the subpopulations to a few hundred hectares is suggested. Preferably, all stages of succession in the ecosystem should be represented.

Since genotypes, populations, and species are all components of an ecosystem it is biologically artificial to separate conservation methods for these three levels.

The essence of dynamic and static gene conservation is illustrated in Figure 11-24. It needs to be emphasized that regeneration of gene resource populations is crucial for long-term success of gene conservation. Since the demand for forestland is expected to increase in future it is important to minimise the risk that the gene resource population will be exploited for other purposes. Especially, for the developing countries it is important that conservation is tightly linked to local communities to guarantee their support of the conservation measures.

Finally, we recommend a landscape perspective in forest tree gene conservation.

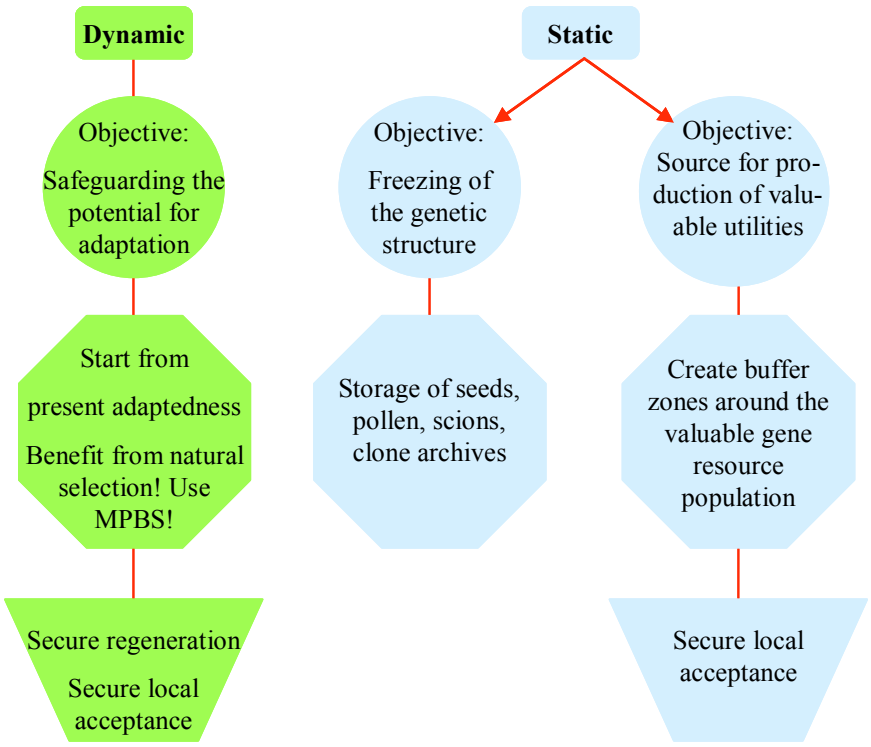


Figure 11-24. A synthesised summary of dynamic and static gene conservation.

Further reading

Geburek, Th., Milasowszky, N., Frank, G., Konrad, H., and Schadauer, K. 2009. The Austrian forest biodiversity index: All in one. Ecol. Indicators 10:753-761.

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ. FAO 2012. State of the World's Forests, 48pp.

Measuring and monitoring biodiversity in tropical and temperate forests 1995. Eds. T.J.B. Boyle and B Boontawee. Center for International Forest Research (CIFOR)

Publications from EUFORGEN.

Forest genetic resources conservation and management, 3 volumes. 2001 - 2004. FAO, Danida Forest Seed Centre, and IPGRI.

The global need for food, fibre, and fuel. 2012. Kungl. Skogs och Lantbruksakademiens Tidskrift 151 (4). 91pp.

Consequences of different breeding activities and silvicultural methods for the new generation of trees

First the impact of breeding and silviculture on the progeny generation is presented, then the impact of fragmentation on genetic variability. Finally the demands for genetic variation in the breeding and the production populations are outlined.

The first question to raise is: To what extent do various breeding or silvicultural activities lead to drastic genetic changes in the filial generation? The knowledge about the genetic consequences of different breeding or silvicultural activities is limited even if there was an increasing number of studies related to these issues from the late 1990s and onwards. Most of these studies were carried out with isozyme markers and they might therefore not well reflect what the consequences have been for traits of adaptive significance. However, if they do not reveal a loss of variability compared to the situation in natural stands it is unlikely that there have been losses in adaptive trait variability.

There are several occasions when there is a potential for genetic change during the course: breeding - raising of seedlings - silvicultural practice. In Fig 12-1 we have visualised a chronological step of events, which may have genetic consequences.

Seed orchards are almost always composed of trees from different stands. This means that relatedness, which might have existed in individual stands, will be broken when clones are brought together in a seed orchard. Therefore, the genetic variability in seed orchards is frequently higher than in natural stands. This has been confirmed by isozyme analysis.

As regards the progenies from seed orchards we know that changes have occurred both with respect to growth and stem quality, even if we do not know the alleles that have caused this effect. Moreover, genetic change is what is aimed at in breeding. Breeding may influence other traits by being correlated with the selected trait either through pleitropy or close linkage. The knowledge of this matter is limited. Since the heritability is mostly low and the selection differential is not strong, we do not expect any great changes in traits not involved in breeding. The results available do not suggest any major changes in other traits.

Genetic drift and increased inbreeding might occur as a consequence of differences in female and male flowering frequencies among clones as well as asynchrony in receptivity and pollen dispersal among clones (cf Chapter 9). For tree species that are wind-pollinated and widely spread there is low probability that genetic drift or inbreeding will be of any significance in the progeny generation if the number of clones in the seed orchard is not extremely low. As pointed out in chapter 6 the loss of additive variance per generation is equal to $1/2N_e$. Most available data, which mainly originate from conifers, indicate that there is no genetic drift or inbreeding. The situation may be different in species that are not wind-pollinated and where the distribution is scattered.

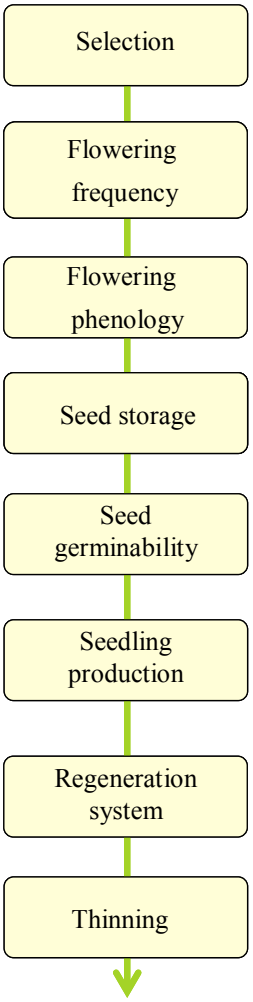


Figure 12-1. A chronological illustration of factors in breeding and silviculture, which may influence the genetic composition.

Some studies showed that seed storage, seed germinability, and raising of seedlings had changed the genetic composition from that at harvest. The probability for deviations from the ideal composition increases with decreasing number of clones.

As regards clonal forestry the genetic variation might be narrowed down considerably. If the characteristics of the clones are well known we may also design clonal mixtures to have a specific genetic variation. Since self regeneration is not an objective of clonal forestry the occurrence of genetic drift or inbreeding is not relevant for clonal forestry. On the other hand this will be of interest if the clonal forest is used as a seed tree stand in the future. The amount of inbreeding will depend on the number of clones in the clonal plantation and gene flow from surrounding forest stands. In many countries the regulations for the number of clones required for clonal forestry do not give rise to any concern for considerable negative consequences of clonal forestry. Only for the cases when large areas have few clones and self regeneration is permitted there are potential risks that the following generation will suffer from inbreeding depression.

Studies on the impact of different regeneration methods on the genetic variability in the progeny populations have not revealed any large differences whether the studies were carried out in North-America, Australia, or tropical Asia. There was a tendency for the lowest genetic variability to occur in the offspring from unmanaged stands. In the tropical forests with low occurrence of many species it is useful to set limits with respect to the size of the trees that may be logged.

In one Malaysian lowland mixed dipterocarp forest, the effect of logging was studied approximately 40 years after logging. An adjacent unlogged stand was used as reference. Of the six species studied only one had a lower frequency of observed isozyme heterozygotes in the logged stands than in the unlogged control. The average increase of observed heterozygosity in the six species in the logged stand was slightly above 15 %.

A Canadian study of the mating pattern in forests with four different regeneration regimes, including self regeneration, showed high levels of inbreeding in all regimes. If this holds for other forests as well it means that self regeneration can lead to some inbreeding depression in the regenerated material.

The number of alleles present after different types of silvicultural activites is frequently reported. In some instances there are losses of alleles but it mostly concerns rare alleles, which probably are of no or very limited significance for additive variance. The effect of thinning on the expected heterozygosity was reported in a Canadian study of two stands. Thinning was carried out to promote the growth of Douglas fir. In Fig. 12-2 the change in species composition in the two stands is illustrated. This study is of significance since we will see the impact of a large change in species composition on genetic constitution. In the first stand the Douglas fir percentage increased from 72.4% to 84.6% while the corresponding figures for the

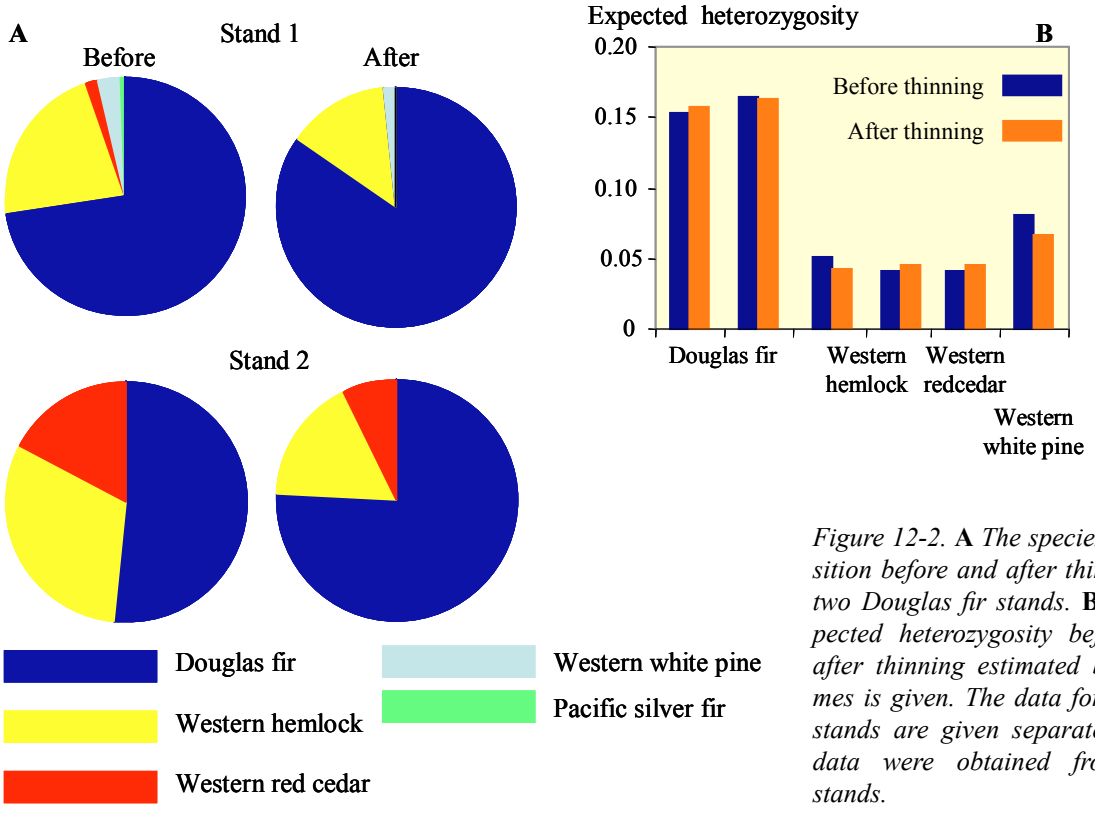


Figure 12-2. **A** *The species composition before and after thinning of two Douglas fir stands.* **B** *The expected heterozygosity before and after thinning estimated by isozymes is given. The data for the two stands are given separately when data were obtained from both stands.*

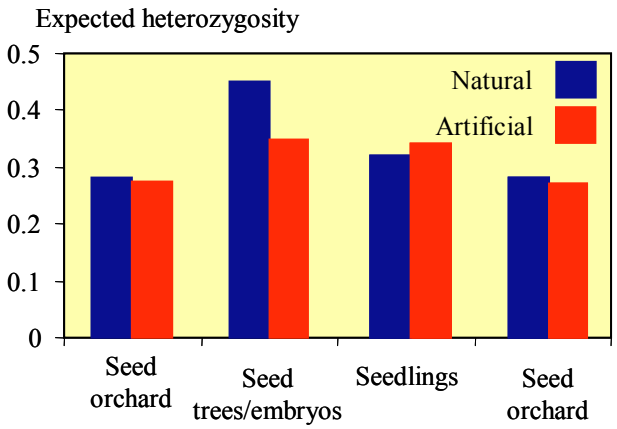


Fig 12-3. Expected heterozygosity in natural and artificially regenerated populations. The types of artificial regeneration are indicated. Details are given in the text.

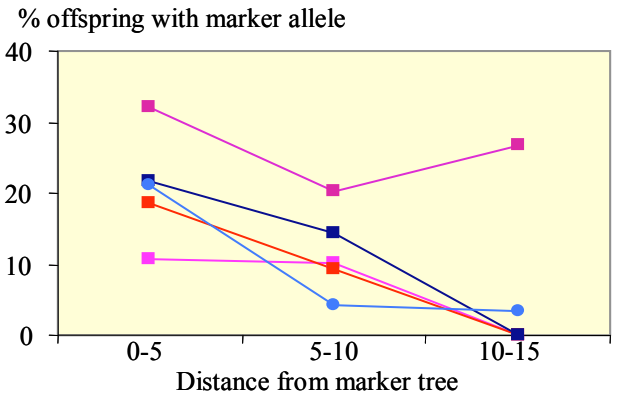


Fig 12-4. The percentage of seedlings with isozyme marker allele GOT-B1 in concentric circles around five marker trees.

other stand were 51.4 and 75.9%. Two species, western redcedar and pacific silver fir, were lost in the thinning, which was not unexpected since they did not pass two percent in the stand before thinning. In spite of the large change in species frequency the expected heterozygosity did not change much in any of the remaining species. Even in western hemlock, which lost 7 rare alleles, the expected heterozygosity did not change.

Data from comparisons between natural stands and their artificially regenerated progenies of *Pinus sylvestris* were compared by aid of isozymes (Fig. 12-3). The first pair comprised three natural stands and seed orchards from northern Sweden. Only in pair number 2 there was a difference between artificial and natural regeneration. In that case embryos were compared with adult trees. When the adult trees were compared with 10-20 years old trees in the same stand there was no difference. Pair No 3 consisted of seedlings grown in a nursery or after natural regeneration in the same stand. Finally, pair No 4 consisted of seed crops from three natural stands and two seed orchards. The absence of a difference between artificially and naturally regenerated populations is expected since the isozyme markers used are assumed to be neutral.

By aid of isozyme marker alleles, *i.e.* alleles in very low frequencies, allele dispersal in a *Pinus sylvestris* seed tree stand with low density in northern Sweden, 18 trees per hectare, was studied. The stand was located at 65.48°N and 400 masl, which means a harsh locality in northern Sweden. It may be hypothesised that the regeneration in such a stand would be problematic. Five trees contained one rare allele. All seedlings in concentric circles with a radius of 5, 10 or 15 meters were screened. Out of the total number of 431 seedlings only six carried a marker allele. Such a low number made it impossible to estimate dispersal with some certainty. An additional study was carried out with another marker, which occurred in higher frequency than the three other rare alleles. This allele occurred in five trees and the detected percentages in the three concentric circles are illustrated in Fig. 12-4. In all trees except one there is a drop in the percentage by increasing distance from the female marker tree. Based on certain assumptions the estimated contribution to the offspring within the five meter radius was 25%. Within the radius of 15 meters it was suggested that no more than 10% of the alleles originated from the marker tree. It should be noted that estimations based on non-rare alleles is rather problematic since long-distance pollen carrying such an allele is hard to separate from the same allele in the female marker tree. It was concluded that a large fraction of the genes in the regeneration does not originate from the seed trees in the stand. There may be a seed bank in the ground containing seeds from trees that were cut to form the seed tree stand.

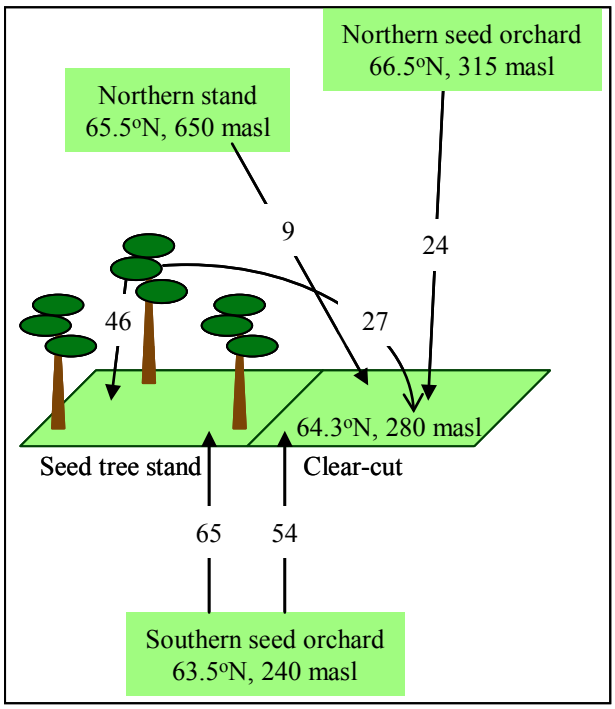


Fig. 12-5. The percentage mortality from start of the experiment to age 11 in four genetic entries of Pinus sylvestris *planted in a seed tree stand and in the adjacent clear cut area. Geographic data are given, means for the clones in the two seed orchards are shown.*

With the purpose to study the role of genetics for reforestation of *Pinus sylvestris* in a harsh area in Northern Sweden survival and growth of different seed sources following sowing or planting in a seed tree stand and in the adjacent clear-cut area was carried out. Geographic data are given in Fig. 12-5. The loss of seedlings from start of the experiment up to age 11 by percentage of survival as well as percentage of 2 x 2 meter squares without surviving seedlings was assessed. As seen from Figs. 12-5 and 12-6 the following conclusions can be drawn.

1. Planting leads to lower mortality than sowing
2. Mortality is higher under seed trees than in the clear-cut area
3. The local seed source is not the best for survival
4. The ranking of the genetic entries is the same for the two ways to study survival

The mortality percentage after sowing was around 90 % in all materials and thus much higher than after planting, which varied in the range 9-65% (cf Fig. 12-5). The southern seed orchard progenies had the poorest survival, which is expected from its northern transfer. The northern seed tree stand progenies had the lowest mortality thanks to its northern and high elevation origin. It should be

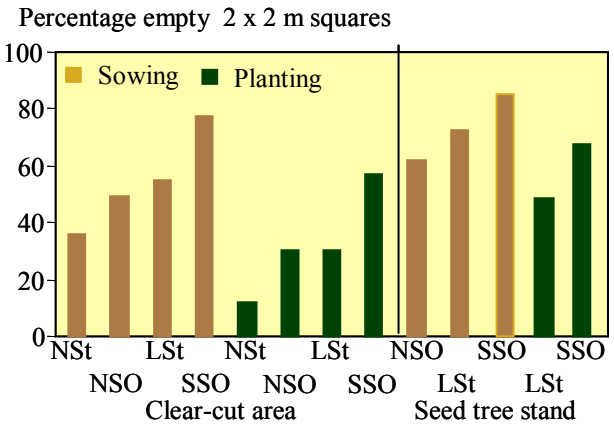


Fig 12-6. The percentage of 2 x 2 meter squares without living plants in a clear-cut area and a seed tree stand of Pinus sylvestris *at a test locality, latitude 64.3°N and 280 masl. NSt = northern stand, LSt = local stand, NSO = northern seed orchard, SSO = southern seed orchard.*

mentioned that mortality may continue for another ten years at such a test locality as used in this study.

From Fig. 12-7 it is clear that planting leads to superior tree growth compared to sowing. Furthermore, the growth is better in the clear-cut area than in the seed tree stand. It should be noted that there was not enough material from the northern seed orchard for planting in the seed tree stand. Similarly, growth data from the northern stand was only reported for the clear cut area.

Individual collection of open pollinated seed from 20 trees in the seed tree stand was carried out. These seeds were used in two replications and the coefficient for additive variance for height was estimated at 1.4% and the family effect was non-significant.

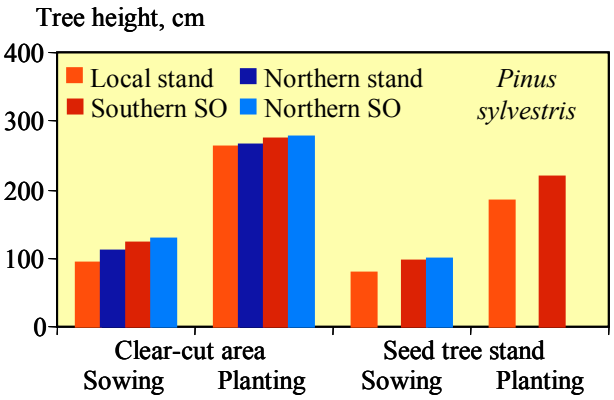


Fig 12-7 The tree heights at age 11 in the four treatments; planting or sowing in a clear-cut area or in an adjacent seed tree stand with three different types of genetic entries.

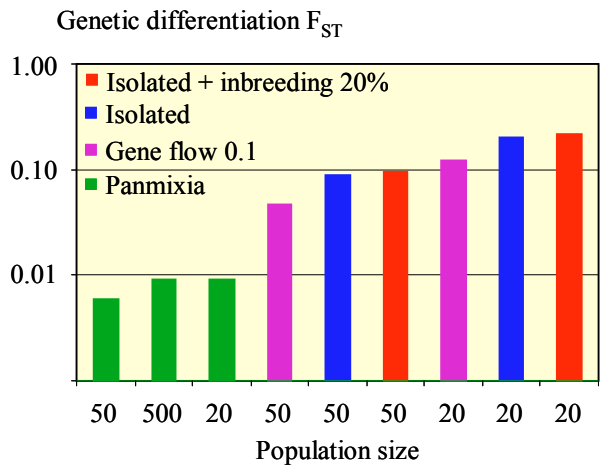


Figure 12-8. The genetic differentiation after 10 generations at different population sizes, 20,50, or 500 individuals. Differentiation was estimated at a gene flow of 0.1 and isolated populations with or without 20 % inbreeding. Note the logarithmic y axis.

Fragmentation

The genetic impact of fragmentation has attracted interest among forest geneticists. The results of a simulation carried out over 10 generations are shown in Fig. 12-8 At panmixia there was virtually no differentiation at any of the three population sizes included, 20, 50, or 500 individuals. At a gene flow of 0.1 the differentiation was larger in the population with 20 individuals than in the 50-population. Similarly, the differentiation was larger in the small population at 20% inbreeding. It is evident from this simulation that limited gene flow has a strong effect on differentiation.

In another study the impact of three types of pollinators, insects, birds, and wind on genetic diversity was analysed on data from 97 publications. Alleles per locus, % polymorphic loci, expected heterozygosity, and inbreeding were studied. There was no difference between the three types of pollinator as regards inbreeding and no difference between fragmented and non-fragmented populations. In Fig. 12-9 rank correlation coefficients are used to test differences between fragmented an non-fragmented populations. One striking result was noted, the absence of differences in these three genetic parameters in bird pollinated woody species. It was speculated that birds have to fly over longer distances in case of fragmentation. Differences were noted for the two other pollinators for all three genetic parameters. However, only in one case there was a significant difference between fragmented versus non-fragmented, number of alleles per locus for insect pollinated species. It should also be remarked that the degree of explanation was fairly low in all cases.
The genetic diversity in the adult populations was generally larger than in the juvenile progeny populations.

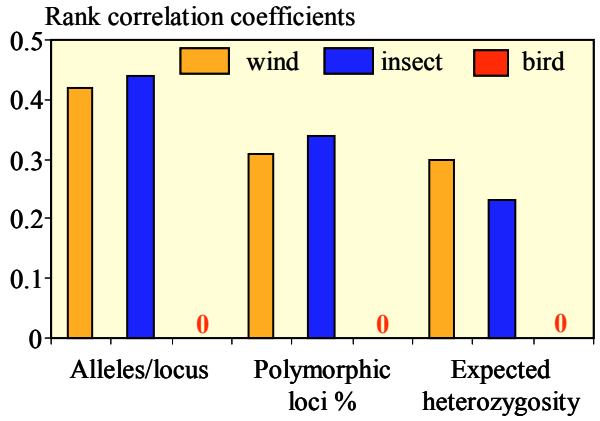


Figure 12-9. The impact of fragmentation on alleles per locus, percentage of polymorphic loci, and expected heterozygosity in species with three types of pollinators, wind, insects, or birds. The study was based on 97 woody species. The larger the correlation coefficient the larger the effect of fragmentation.

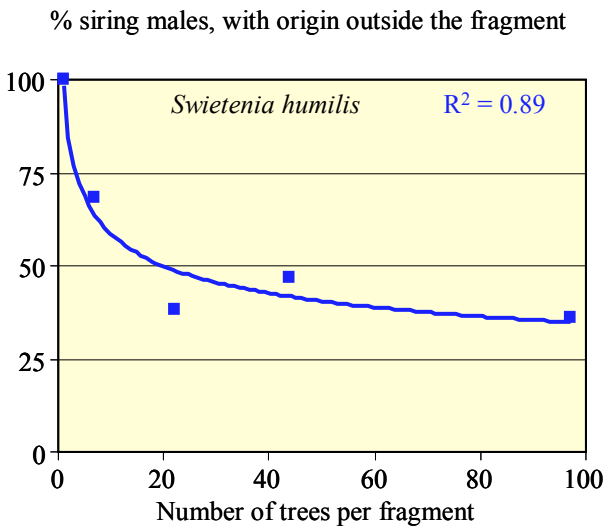


Figure 12-10. The relationship between percentage of siring males with origin outside fragments and number of trees in the fragments.

It is *a priori* expected that the fragment size will influence the impact of pollen coming from outside sources. The smaller the size the larger number of sirings with pollen from sources outside the fragment. One example of this is shown in Fig. 12-10, in which data from fragmented stands of *Swietenia humilis* in Honduras are shown.

Some geneticists have stressed that repeated cutting of the best trees over many generations may lead to genetic erosion, which sometimes is called dysgenic selection. The probability for this must be regarded as low. In spite of this, the stem form of the two oak species *Quercus petrea* and *Q. robur* suggests that such an erosion may have taken place in Denmark owing to repeated selective cuttings of the trees with the best stem form. When decisions are taken about which trees should be left in a seed tree stand it is not possible to select the phenotypically superior trees only, since the spacing after thinning must

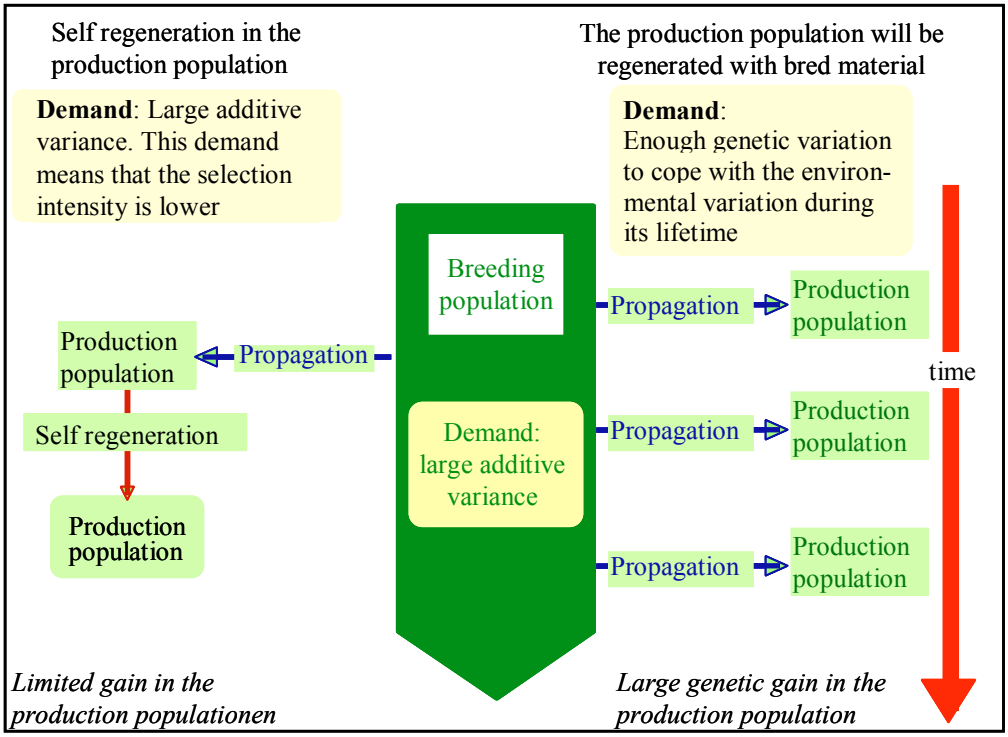


Figure 12-11. Schematic illustration of the requirements for additive variance and genetic variation in the production population at self regeneration of this population (left) or at artificial regeneration with the best material obtained from the breeding population at each occasion (right).

be fairly even in a seed tree stand. This means that the selection differential is smaller than if we consider only the proportion of trees remaining in the seed tree stand. As a corollary of this we do not expect any large genetic changes from thinnings.

Some caution as regards the relevance of these studies for adaptive traits is justified since many studies were carried out with different types of markers.

The demand for genetic variation in the production population

Laymen frequently claim that the genetic variation in the production population must be as large as the variation in the breeding population. In the chapter about tree breeding we have learnt that a large additive variance is crucial for success in breeding. We should therefore focus on the additive variance when we are discussing the demand for genetic variation in a parental population. When the purpose of a population is not to produce a new generation we may simply talk about demand for genetic variation in that population instead of demand for additive variance. In Figure 12-11 two situations are illustrated schematically. To the left we assume that the production population will be used in self regeneration. To avoid genetic drift and inbreeding in this case, the demand for additive variance is as large as in the breeding population. If we need to keep the additive variance it means that we cannot use the same selection intensity as when we obtain the production population from seed orchards or vegetatively from clonal hedges (to the right in Fig. 12-11). In the latter case we might figuratively skim the cream off the milk. This causes a narrowing of the additive variance. This does not matter as long as we have genetic variation enough in the production population to cope with the environmental conditions during its life time. At each occasion when reforestation is to be carried out the plant material is obtained from the genetically most advanced seed orchards or clonal hedges. Genetic progress may be much larger in this case than when the production population is to be used for regeneration.

Even if we never intend to use the production population for self regeneration the demand for genetic variation in the production population might vary. This has mainly been discussed in connection with clonal forestry. Figure 12-12 is based on a scientific paper from a group of scientists who have discussed the demand for genetic variation in relation to clonal forestry. The figure must not be perceived as exact differences in demand for genetic variation in any of the contrasting pairs. Above all, the distances between the two areas do not constitute any precise estimate of the demand for genetic variation.

The demand for genetic variation is least when the rotation time is short and when the cultivation takes place under uniform conditions. Heterogeneous environment

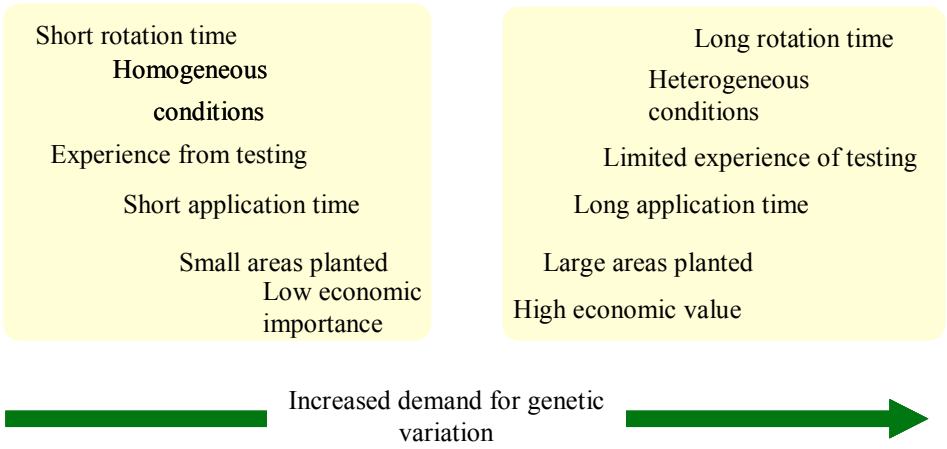


Figure 12-12. Schematic illustration of factors influencing the requirement for genetic variation in the production population.

and long generation times require broad genetic variation to mitigate the variations in time and space that might occur. To reach an understanding of what conditions require the largest genetic variation in the production population we might argue in an analogous way concerning the other contrasting pairs in this figure. In this context it is important to remember once again that both growth under heterogeneous environments and long rotation times probably promote evolution of large phenotypic plasticity in a species. If this is the case the demand for genetic variation is slightly reduced.

Summary

There is limited information about the consequences of different silvicultural methods and breeding activities for genetic changes in future production populations. All kinds of breeding aim at genetic change of the target traits. There are no signs that breeding has caused any dramatic changes in non-targeted traits. A large additive variance is required if self regeneration of the production population is envisaged. When the production population is replaced by material from the breeding population, the genetic variation can be less as long as the production population has enough variation to cope with the environmental conditions during its rotation time. The lower the demand for genetic variation the stronger the selection and the higher the gain.

Further reading

Conservation and management of Forest Genetic Resources in Europe. Eds. T. Geburek & J Turok. 2005. Arbora Publ.

Forest Genetics and Sustainability, Section 5 Sustained management of genetic resources - the impact of forestry 1999. Kluwer Acad. Publ., Editor Csaba Mátyás.

Glossary

Reference is given to the page in the text where the term is introduced.

Adaptability The ability to respond genetically or phenotypically to changed environmental conditions. 71

Adaptation The process of genetic change of a population, owing to natural selection, resulting in a better adaptedness in a specific environment. 71

Adaptedness The degree to which an organism is able to live and reproduce in a given set of environments. 71

Additive gene action When alleles at two or more loci combine additively, the gene action is described as additive; this means that the value of a genotype with respect to alleles at several loci is the sum of the values attributable to the alleles at the separate loci. If alleles at the same or different loci interact the gene action is non-additive. 63 *See* also **Dominance** 47 and **Epistasis** 50

Additive genetic variance The part of the total genetic variance due to additive gene effects; the variance of breeding values. Additive variance can be exploited in mass selection. 61

Adenine A purin basis, one of the four nucleotide basis of DNA and RNA. Adenine is paired with thymine in the double helix. 22 Fig. 2-1

After-effects If the performance of a progeny depends on the conditions during seed maturation or conditions during preceding growth period(s), there are said to be after-effects. This phenomenon has also been called **preconditioning**. 144

Allele One of two or several alternative forms of a gene that can exist at a single locus; if the number of alleles is larger than 2 the alleles form a system of multiple alleles; if the number of alleles in the same population is two or more and relatively common, the alleles are said to be polymorphic. Each individual chromosome has just one allele at each locus. 11

Allele fixation Allele fixation at a locus has taken place when there is only one type of allele at that locus in a population. 77

Allopolyploidy *see* **Polyploidy** 90

Allozyme *see* **Isozyme** 94

Alternative splicing different protein molecules are generated from the primary DNA transcript by changing the number and order of exons in the final mRNA after splicing out the introns. 27

Amplified Fragment Length Polymorphism (**AFLP**) A DNA marker that is based on the polymerase chain reaction (PCR) technique for amplification of restriction fragments and is exploited in the construction of genetic maps of chromosomes; the technique allows a high number of polymorphic loci to be detected. AFLP markers usually show dominance (the heterozygote cannot be distinguished from the homozygote). 94

Antipods The three nuclei located in the pole opposite to the egg apparatus in the embryo sac. 15

Archegonium Female, multicellular, sex organ of most gymnosperms in which a single egg cell is produced. 14

Artificial selection Usually selection of superior phenotypes by man. *See* also **Natural selection** 141

Autopolyploidy see **Polyploidy** 90

Backcrosses Repeated crosses with one of the original parents in each generation. Backcrosses are usually made to incorporate a single desirable trait from a species or a variety. 134

Base pair and **base pairing** The pairing between the nucleotide base adenine-thymine (adenine-uracil in RNA) or cytosine-guanine leads to formation of base pairs; commonly abbreviated bp. 21 Fig. 2-1

Biclonal seed orchard *see* **Seed orchard** 143

Biochemical markers Qualitatively inherited genetic traits that are revealed by biochemical methods. 55

Bivalent A pair of homologous chromosomes, each consisting of two chromatids, appear during the first meiotic division; the number of bivalents is equal to half the chromosome number. 12

Bottle neck The occurrence of reduced effective population size during one or more generations. 35

Breeding population *see* **Population** 136

Breeding value The genotypic value of an individual judged by the mean value of its progeny. If an individual is crossed with a large number of randomly selected individuals in a population, its breeding value = the double deviation of its mean from the grand mean of this population. The breeding value is 2 x the general combining ability. 62

cDNA *see* **Complementary DNA** 33

Central dogma The flow of genetic information is from nucleic acid to protein, never in the reverse direction. More popularly, DNA makes RNA, and RNA makes protein. 28

Centromere The region of a chromosome that is essential for chromosome movements during cell divisions. 10

Chi-square test (χ^2 **test**) A statistical test to assess the goodness of fit between an observed and an expected segregation. 49

Chloroplast The cell organelle in plants in which photosynthesis takes place; chloroplasts have several circular DNA molecules containing approximately 120 genes. Chloroplasts are strikingly similar to cyanobacteria (previously called blue-green algae). 32

Chromatid One of the two subunits of a duplicated chromosome. 11

Chromosomal optimum The degree of polyploidy that gives rise to the most vigorous growth. 133

Chromosome In eucaryotes, a DNA molecule that contains genes in linear order to which numerous proteins are bound and that has a telomere at each end and a centromere. Chromosomes are dark-staining with basic dyes and microscopically observable in the cell during mitosis. 9

Cline A continuous change of population means along an ecological gradient attributed to changes in allele frequency, *see* also **Ecocline** 82

Clonal seed orchard *see* **Seed orchard** 143

Clone genetically identical individuals propagated vegetatively: grafts, cuttings, root suckers, somatic embryos. 147

Codominance Both alleles at a heterozygous locus can be identified phenotypically. 11

Codon A triplet of nucleotides in an mRNA molecule that codes for a particular amino acid or a stop signal in protein synthesis. 29

Coefficient of additive genetic variation. (**CV_A**) The ratio between additive genetic standard deviation and the mean value of a trait expressed as percentage; CVA = $100\sigma_A/x$. 62

Coevolution Mutual evolutionary changes in two interacting species as a response to changes in these species, *e.g.* host-parasite interactions. 87

Combining ability Two types exist:

- **General Combining Ability** (**GCA**) the value of an individual judged by the mean value of its progeny. If an individual is mated to a large number of randomly selected individuals in a population, GCA = the deviation of the mean of its progeny from the overall mean of the entire population. High GCA usually implies the presence of genes with additive effects. 64
- **Specific Combining Ability** (**SCA**) specific pairs of parents give a progeny that strongly deviates from what is expected based on their general combining ability. High SCA usually implies the presence of dominance or epistasis. 64

Common tester *see* **Mating design** 151

Complementary DNA, (cDNA) A DNA molecule that is synthesised from an mRNA molecule by the enzyme reverse transcriptase; cDNA has no introns. 33

Controlled pollination Female flowers are isolated before they are receptive to prevent pollination with unknown male pollen. At maximum receptivity female flowers are dusted with pollen from one specific male or from a mixture of males. 149

Critical night length for budset The night length at which 50% of the plants belonging to a genetic entry are induced to form an apical bud. 103

Crossing-over Reciprocal exchange of non-sisterchromatid segments in a pair of homologous chromosomes resulting in the recombination of genes within a linkage group. 12

Cytology Chromosome cytology deals with the microscopic studies of chromosome number, size, morphology, and behaviour during nuclear divisions. 9

Cytosine A pyrimidine base, one of the four nucleotide bases of DNA and RNA. Cytosine is paired with guanine in the DNA double helix. 22 Fig. 2-1

Cytosol The remaining compartment of the cytoplasm in which the organelles have been excluded.

Darwinian fitness 115 *see* **Fitness** 71

Degenerate code means that more than one codon encodes one particular amino acid. 29

Deletion Loss of a chromosomal segment. 14

Diallel crosses *see* **Mating design** 143

Dihybrid cross A cross between two individuals heterozygous at two different loci. 48

Dihybrid segregation *see* **Segregation** 48

Diploid An individual with two sets of homologous chromosomes (denoted 2x). 10

Disconnected half-diallel *see* **Mating design** 152

DNA, (deoxyribonucleic acid) The carrier of the hereditary material in most organisms; DNA is a double helix consisting of 4 nucleotide bases, (adenine, cytosine, guanine, thymin), one deoxyribose residue, and one phosphate group. 21

DNA fingerprinting A method to generate a pattern of DNA restriction fragments that is unique to an individual. 26

DNA library A collection of transformed cells each of which contains DNA fragments that represent the total genome of a species (genomic library) or contains cDNA fragments (cDNA library). 33

DNA replication Synthesis of DNA leading to the duplication of chromosomes. 21

DNA sequencing The technique for determining the base (nucleotide) sequence of a DNA molecule. 35

DNA vector A DNA molecule, that can replicate in a cell, into which a gene or a DNA segment has been inserted by recombinant DNA techniques; can serve as a vehicle to transfer a gene or a DNA segment to a host cell; bacterial plasmids are frequently used as vectors. 34

Dominance The interaction of alleles at homologous loci; the degree of deviation of the heterozygote from the mean value of the two homozygotes at the locus. 47

Dominant allele The allele (**A**) that is phenotypically expressed in a heterozygous (**Aa**) individual as well as in a homozygous individual (**AA**); at complete dominance both **Aa** and **AA** have the same phenotype. 11

Duplication A chromosome aberration in which more than one copy of a chromosome segment is present in the haploid genome. In a tandem duplication the two segments are adjacent. 14

Dysgenic selection repeated cutting of the best trees over several generations causing a deterioration of the remaining population. 197

Early test Such a test aims at identification of good predictors for adult performance in juvenile material. 153

Ecocline Sometimes used to distinguish clinal variation from ecotypic variation in a species, *see* **Cline.** 82

Ecosystem is a complex set of relationships among the living resources, habitats, and residents of an area. 84

Ecotype Group of individuals in a species with a certain adaptedness to the conditions at a specific site. 82

Effective population size, (N_e) In a simplified version it is the number of individuals contributing to the filial generation. 54

Egg apparatus The egg cell and the two synergids in the embryo sac in angiosperms. 15

Emasculation Removal of male organs - anthers, male strobili - prior to pollination. 159

Embryo sac The female haplophase in higher plants usually developed from one of the macrospores. 14

Embryonic lethal The pooled effect of several vitality-reducing genes corresponding to the effect of one gene showing monohybrid inheritance. 132

Endemic A species is endemic if it occurs naturally only within one area. 172

Endosperm The triploid tissue in seeds of angiosperms. This term is sometimes erroneously used for the haploid female gametophyte in conifers. 14

Endosymbiont An organism that lives in symbiosis in cells or tissues of another organism. 32

Endosymbiotic hypothesis The proposal that microchondria and chloroplasts were originally free living organisms that entered into a symbiotic relationship with nucleated cells. 32

Epigenetics An epigenetic change is an alteration in genetic information where the sequence of bases in the DNA remains the same. The epigenetic change often results from methylation of DNA bases or changes in histone structure. 36

Epistasis Interaction between alleles at different loci, *i.e.* denotes the non-additive effects between loci. 50

EST *see* **Expressed Sequence Tags** 94

Eukaryote An organism in which the cells have a nucleus and other membrane-bound organelles, in contrast to a prokaryote like bacteria which lack these features. Fungi, algae, protozoa, higher plants, and animals are all eukaryotes. 27

Evolution Cumulative change in the genetic composition of a population through time. 71

Expressed Sequence Tags, EST A partial cDNA sequence, *i.e.* a sequence within the coding region of a gene. 94

Exon *see* **Gene** 27

F_1 generation Offspring from a cross between parents, progenies from crosses between F_1 individuals are called F_2, and so on. 47

F statistics F statistics are useful means to get information on population differentiation and amount of inbreeding. 55

Factorial mating *see* **Mating design** 151

Family The progeny from a controlled cross or from open pollination of one individual.

- **full-sibs** a progeny with both parents in common. 42
- **half-sibs** a progeny with one parent, usually the female, in common. 42

Fitness An expression for the average contribution of one allele or one genotype to the progeny of an individual in relation to the contribution of other individuals in the same population.

- **Darwinian fitness** The adaptedness in nature, which means the ability of an individual within a population to transfer its genes to the next generation, usually relative to that of other individuals within the same population, in contrast to domestic fitness. 71 115
- **Domestic fitness** The ability of a genetic entry to produce utilities for man. 71 115

F_{ST} an estimate of population differentiation for marker genes. 55

Gamete A mature reproductive cell that is haploid. 13

Gene A unit that transfers information from one generation to the next; is a segment of DNA of a chromosome (or RNA in certain viruses) with similar biochemical function; most genes in eukaryotes have (1) coding sequences (exons) that are transcribed to mRNA that in turn are transformed to proteins, (2) inserted non-coding sequences (introns), (3) a promoter, a regulating part that enables transcription, and (4) terminal (stop) sequences. 24

Gene bank Collection of genotypes; seed bank, tissue culture bank, clone archives, genetic tests, the main objective of which is preservation of genetic material. 169

Gene cloning Insertion of a DNA fragment, carrying a gene, into a vector molecule, such as a plasmid, capable of replication in the same or a different organism. 33

Gene conservation

- ***ex situ*** in forestry it generally stands for storage or cultivation of a gene resource population. 172
- ***in situ*** in forestry it generally stands for a naturally regenerated gene resource population. 172

Gene fixation *see* **Allele fixation** 77

Gene flow Migration to a recipient population from another population with a different allele frequency. For wind-pollinated species gene flow is mainly the result of pollen dispersal. 72 77

Gene frequency The frequency of a gene in a population; gene frequencies are usually expressed as fractions of 1. 53

Gene map The genes or small chromosomal segments that have been located to their respective chromosomes are arranged linearly in the map and the distances between the genes on the chromosome are usually known.

Gene resource population *see* **Population** 136

Genecology The study of adaptation to varying environmental conditions. 71

General combining ability (GCA) *see* **Combining ability** 64

Generative cell In many gymnosperms, the cell of the male gametophyte that divides to form the stalk and spermatogenous cells; in angiosperms, the generative nucleus of the male gametophyte that divides to form two sperm nuclei. 15

Genetic code The series of 64 triplets of bases, mRNA codons, each of which specifies one of the 20 amino acids in proteins or the signals for initiation and termination of polypeptide synthesis. 28

Genetic correlation Correlation of breeding values, an estimate of the degree to which certain genes influence two different quantitative traits. 69

Genetic drift Random fixation of alleles in small populations. 72

Genetic engineering, or **recombinant DNA technology** The use of molecular genetics techniques to produce DNA molecules containing new genes or new combination of genes for the purpose of generating organisms with new desired characteristics. 33

Genetic entry Stands for clone, clonal mixture, half-sib family, full-sib family, population or provenance. 115

Genetic gain The mean progress of the progeny compared to the original population. 68

Genetic roguing Culling of gentically inferior individuals (in seed orchards). 158

Genetic structure The distribution of the genetic variation within and among populations. 93

Genetically modified organism (GMO) Microorganism, plant, or animal in which genetic engineering techniques have been used to modify specific parts of its genome. Transgenic plants are GMOs, but genetic engineering can modify a plant's genome without stably introducing a gene from another species. 33 164

Genome One set of chromosomes; the gametes of diploid organisms have one genome, the gametes of polyploid species have two genomes or more. 35

Genomics Study of the sequences and properties of entire genomes. 35

Genotype (1) the sum of genes, the genetic constitution; (2) the alleles at one or more loci. 11

Genotype x environment interaction In a somewhat simplyfied way, a rank change of genetic entries from one environment to another. 65

Genetically modified organism (GMO) Microorganism, plant, or animal in which genetic engineering techniques have been used to modify specific parts of its genome. Transgenic plants are GMOs, but genetic engineering can modify a plant's genome without stably introducing a gene from another species.

GMO see **Genetically modified organism** 33 164

G_{ST} an estimate of population differentiation for marker genes. 55

Guanine A purin base, one of the four necleotides of DNA and RNA; guanine pairs with cytosine in the DNA double helix. 22 Fig. 2-1

Haploid chromosome number (**n**) The number of chromosomes in a haploid cell; gametes are haploid; the megagametophyte in conifers is haploid. 14

Hardy-Weinberg law The allele frequencies and genotype frequencies are constant from generation to generation in a random mating population with no selection, mutation or migration. 53

Heredity tendency for like to procreate like. 21

Heritability (**h^2**) The ratio of additive variance to phenotypic variance. The heritability of a certain trait is an estimate of the resemblance between individuals for that trait and it takes values between 0 and 1. 61

Heterosis Occurrence of increased size or vitality in hybrids compared with their parents or the parental generation. 68

Heterozygote An individual that forms more than one kind of gamete since it carries dissimilar alleles of one or more genes or dissimilar gene arrangement such as inversion and translocation heterozygotes. 11

Histones a group of small nuclear proteins rich in basic aminoacids that form the core of a nucleosome. 24

Homologous chromosomes Chromosomes that are identical with respect to size, form, and type of genes but the alleles at a locus may differ. Diploid organisms have two homologous chromosomes that pair during meiosis. 10

Homozygote An individual that carries the same alleles of one or more genes. 11

HOPE, Hierarchial OPen Ended A breeding system in which genes continuously and stepwise can be transferred via crosses to an elite population; the degree of improvement increases with each step. 175

Housekeeping genes code for essential functions common to all or most cells in an organism. 30

Hybrid Progeny produced by mating of genetically different parents. 47

Inbreeding Selfing or mating between related individuals. 66

Inbreeding coefficient, F An estimate of identity by descent of alleles; identity by descent means that copies of one and the same allele in an ancestor have been brought together in an offspring. 66

Inbreeding depression Reduction of vitality after inbreeding. 66

Incompatibility Prevention of selfing or of mating between different individuals, usually caused by genes for self-incompatibility. The term is also used for the hindrance of good union of graft and root stock. 142

Intron *see* **Gene** 27

Inversion The reversal of the linear sequence of the genes in a segment of a chromosome owing to erroneous reunion of two breaks in the same chromosome. 14

Isozyme or **allozyme** Enzymes existing in different molecular forms but with function similar in character. 94

Jumping genes see **transposones** 27

Junk DNA DNA not encoding proteins or RNA, but may have other functions not yet identified. 27

Juvenile - mature correlation Correlation between the expression of a trait in the juvenile stage and in the mature stage. 153

Karyotype Description of the chromosomes of a species including chromosome number, size, and morphology *e.g.* position of the centromere; in some instances, the karyotype can provide information on the relationship between species. 9

Linkage The genes are not inherited independently of each other but rather as if they were linked to each other since they are located on the same chromosome. The larger the distance between two genes the weaker the linkage. Genes located far apart on the same chromosome usually appear unlinked, because at least one crossing-over will take place in the region between the two genes. 11

Linkage disequilibrium means that the alleles $\mathbf{a}_1$ and $\mathbf{b}_1$ always occur in the gametes of one parent and that $\mathbf{a}_2$ and $\mathbf{b}_2$ always occur together in the gametes of the other parent. 58

Linkage group All genes present in the same chromosome. 11

Locus (plural **loci**) Fixed position of a gene on a chromosome. 11

Macrospore mother cell A cell that gives rise to the female gamete. 12

Marginal population A population close to the limit of distribution of a species. 89

Maternal effect Influence of the mother on the progeny that is not of genetic nature. 151

Mating design Systematic crosses of varying character 149:

- **Common tester** A special case of factorial crosses, usually a lower number of males than females are used. 151
- **Disconnected half-diallel** In a half-diallel only half of the possible crosses are carried out, disconnected means that the parents are split into groups, in each group a half-diallel cross is carried out. 152
- **Factorial** A mating design in which one group of parents are used as females and another group is used as males. 151
- **Full-diallel mating** A mating in which all parents are crossed with all other parents including reciprocal crosses. 150
- **Nested** One female may be mated to one series of males while another female is mated to another series of males. 152
- **Partial diallel** A limited number of the theoretically possible matings according to a full-diallel are carried out. 150
- **Polycross** Artificial pollination with a mixture of pollen from several individuals. 152

Mating pattern The matings that are realized, *i.e.* the zygotes formed in a population. 73

Mating system There are two major types: wind pollination and animal pollination; the latter type can be pollination by insects, birds, and bats. 173

Megagametophyte The result of the free nuclei formation in the embryo sac. It is haploid. 14

Meiosis The process of nuclear division that leads to the formation of haploid gametes; the nucleus of the pollen mother cell or megaspore mother cell divides twice: in the first division the homologous chromosomes separate, in the second division the chromosomes divide. 12

Mendelian inheritance The rules of hereditary transmission from one generation to the next; two alleles of a gene segregate from each other in meiosis and pass to different gametes; alleles belonging to different loci segregate independently, and combine randomly in the progeny, except for loci near each other on the same chromosomes, so-called linked genes; this is valid for qualitative and quantitative traits but are usually impossible to detect for quantitative traits. 47

Messenger RNA (**mRNA**) The information stored in DNA is transcribed to mRNA, which in turn translates it into proteins. 29

Metabolomics Study of all the metabolites produced in a cell, tissue, or organism. 35

Microarray DNA segments printed on a slide and used to reveal the genes that are active in a particular tissue at a particular moment. 39

Microsatellite Highly repetitive, polymorphic short tandemly repeated sequences of DNA; 2-6/8 base pair repeat units; also called tandem repeat (STR) or simple sequence repeat, (SSR). Microsatellites can be used for DNA fingerprinting. 26

Minisatellites Tandemly repeated DNA sequences of 2-8 base pairs. 26

Mitochondrion An organell, 1-3 μm x 1 μm, occurring in each eucaryotic cell. Mitochondria are the most important energy sources in cells and contain enzymes involved in the final steps of oxidation of organic material to carbon dioxide and water. 32

Mitosis The division of the nucleus in somatic cells leading to the formation of two daughter nuclei, which are enclosed in two separate cells after the division is completed. 11

Molecular clock Based upon the hypothesis that mutations in a gene occur at equal rate during the course of evolution as long as the function of the gene is unchanged.31

Monohybrid segregation *see* **Segregation** 48

MPBS *see* **Multiple Population Breeding System** 138

Mulm dead materia from decomposition of living organisms and their excrements in hollow trees. 182

Multiple alleles *see* **Alleles** 49

Multiple Population Breeding System (**MPBS**) Split of the breeding or gene resource population into approximately 20 subpopulations that are cultivated under different environmental conditions or are exposed to different selection criteria. 138

Mutation A heritable change not caused by segregation or genetic recombination. It can arise by chemical change in DNA or a structural change in the DNA or chromosome; see also inversion and translocation. Mutations are neutral if they do not change the fitness of the organism. 22

Natural selection Improvement of adaptedness via differential transfer of alleles to the next generation. It requires that there is a genetically conditioned phenotypic variation causing variable fitness. 72

- **Directional natural selection** individuals with extreme phenotypes in one tail of the distribution contribute more to the progeny generation than others. 75
- **Disruptive natural selection** individuals with extreme phenotypes in both tails of the distribution contribute more to the progeny generation than others. 75
- **Stabilizing selection** individuals close to the mean of the distribution contribute more to the progeny generation than others. *See* also **Artificial selection.** 74

Nested mating design *see* **Mating design** 152

Night length The duration of the dark period. 17

Nonsense DNA *see* **Junk DNA** 27

Norm of reaction The phenotypic expression of a genetic entry along an environmental gradient. 73

Nucleolar organizer A region of the chromosome containing the genes for ribosomal RNA, also called secondary constriction. 10

Nucleosome Structural component in eukaryotic chromosomes. It consists of 8 histones (2 of each of H2A, H2B, H3, H4) which are proteins binding to DNA. This structure is called octamer. The DNA-molecule is wound twice around the nucleosome. 24

Nucleotide The building block of nucleic acids; it is composed of a sugar molecule (deoxyribose in DNA, ribose in RNA), a phosphate group, and an organic nitrogen base (purine or pyrimidine). 21

Nucleus breeding The breeding population is split into one small nucleus population (usually 50-70 trees) and one large subpopulation (= 250 trees); the selection intensity is largest in the nucleus subpopulation; over the generations the gap in progress between the two subpopulations will be broadened. 140

Nutrient efficiency a plant's ability to produce biomass in relation to available nutrients, whether it can be attributed to uptake of nutrients from a substrate or to utilization of nutrients. 126

Nutrient utilization The dry matter produced per amount of nutrient in the whole plant or parts of it. 126

Nutrient uptake The content of a nutrient element measured in the whole plant or in parts of it. 126

Oligonucleotides a linear sequence of about 10-20 linked nucleotides, natural or synthetic. 38

Open pollination There is no human influence on the seed formed, selfing may occur; seeds from **open-pollinated families** have been collected from individual female trees following wind or animal pollination. 142

Ortet Original plant, the plant or the tree which is the founder for vegetative propagation. 147

Outbreeding The opposite to mating between related individuals. 89

Outbreeding depression The reduced vitality of a hybrid progeny. 89

Partial diallel mating *see* **Mating design** 150

PCR *see* **Polymerase Chain Reaction** 38

Pedigree A record of ancestry, often shown as pedigree diagrams representing the familiar relationships among relatives, such as full-sib and half-sib families. 137

Phenology Timing of periodic phenomena such as bud burst, budset, flowering, especially related to seasonal changes in temperature and photoperiod. 85

Phenotype The observable properties of an individual; the sum of the characteristics of a certain genotype at a certain occasion. The phenotype is determined by the interaction between genotype and the environment: phenotype = genotype + environment. 11

Phenotypic plasticity the amplitude for a trait of a genotype studied in at least two different environmental conditions. 73

Phenotypic variance The variance of the assessed values of a trait; phenotypic variance = genotypic variance + environmental variance. 61

Photoperiod The length of the period with daylight. 82

Photoperiodic response A type of change initiated by changes in the relation between daylength and nightlength. 82

Phytotron A series of growth chambers in which several environmental factors such as temperature, photoperiod, and air humidity can be regulated. 125

Plasmid An extrachromosomal DNA molecule replicating independently of the host cell genome. 40

Plasticity *see* **Phenotypic plasticity** 73

Plot The smallest research unit *e.g.* in a field or a nursery trial consisting of for example a single provenance. Plots are assembled into blocks. 158

Plus tree Selected tree with superior phenotype. 141

Pollen contamination Pollination with alien pollen either in stands or seed orchards. 159

Pollen mother cell A cell that gives rise to the male gamete. 12

Polycross *see* **Mating System** 152

Polyembryony Occurrence of more than one embryo in each seed. 132

Polygenic inheritance *see* **Quantitative inheritance** 57

Polymerase Chain Reaction (**PCR**) A technique that results in exponential amplification of a specific region of double-stranded DNA. 38

Polymorphism The occurrence in a population of two or more alleles at the same locus; the most common allele has a frequency of less than 0.95. 11

Polypeptide A chain of amino acids linked together by peptide bonds; a protein consists of one or more polypeptide chains. 24

Polyploidy Occurrence of more than two complete sets of chromosomes. 90

- **Allopolyploidy** Polyploidy as result of species hybridization. 90
- **Autoploidy** Polyploidy that has arisen after chromosome doubling within a species. 90

Population Usually a collection of individuals from a limited area that have a certain degree of adaptedness to that area.

Breeding population The collection of trees that will carry the advancement of breeding into future generations. 136

Gene resource population the seeds, acorns, nuts, plants, or trees that are included in the gene conservation. 136

Production population A population intended to produce human utilities. 136

Propagule population The plants or trees utilized in sexual or vegetative propagation. 136

Population genetics Studies of gene frequencies in populations and their changes. 53

Preconditioning See **After-effects**. 144

Production population *see* **Population** 136

Progeny trial A trial in which different families are tested. 61

Promoter A sequence of double-stranded DNA upstream of the start of transcription at which RNA polymerase binds and initiates transcription of the structural gene. 30

Propagule population *see* **Population** 136

Proteomics The study of all the proteins produced in a cell, tissue or organism. 35

Prothallial cell The sterile cell or cells found in the male gametophytes of gymnosperms but not in angiosperms; believed to be remnants of the vegetative tissue of the male gametophyte. 15

Provenance One definition of provenance is a population or group of individuals of the same species occurring within or originating from one more or less rigorously defined geographic area. 98

Provenance hybrid seed orchard *see* **Seed orchard** 143

Pseudogene A non-functional gene with sequence homology to a functional gene elsewhere in the genome. 26

Purine A nucleotide base with two carbon-nitrogen-rings; adenine and cytosine are purine bases. 21 Fig. 2-1

Pyrimidine A nucleotide base with one carbon-nitrogen-ring; guanine, thymine and uracil are pyrimidine bases. 21 Fig. 2-1

Q_{ST} an estimate of population differentiation of quantitative traits. 56

Quantitative trait locus (**QTL**) The genes in such loci participate in the regulation of quantitative traits. 58

Qualitative inheritance One gene strongly influences the phenotype. 57

Quantitative inheritance Genes in many loci influence a trait, the influence of each gene is usually small. 57

Ramet An individual obtained from vegetative propagation; a member of a clone. 147

Random genetic drift *see* **Genetic drift** 72

Random Amplified Polymorphic DNA (**RAPD**) A DNA marker that is based on the polymerase chain reaction (PCR) technique for amplifying specific DNA fragments by using arbitrary 10-base oligonucleotides as primers; RAPDs are usually dominant *i.e.* the heterozygote cannot be distinguished from the homozygote. 94

Real-time PCR A real-time PCR machine follows the amplification of the DNA sequence in real time, using fluorescent markers.This allows accurate quantification of *e.g.* gene expression. 39

Receptivity The stage of female flower or strobilus at which success of pollination is expected. 80

Recessive An allele that is phenotypically expressed only when homozygous. 11

Reciprocal crosses Two crosses in which each parent serves as female in one of the crosses and as male in the other; female A x male B and the reciprocal cross Female B x male A. 122

Recombinant DNA DNA created by bringing together DNA segments often from different species. 136

Recombination The creation of new combinations of genes in F_2 through segregation of chromosomes and crossing-over at meiosis *e.g.* $a_1a_1b_2b_2$ and $a_2a_2b_1b_2$ may be obtained in F_2 following the original cross $a_1a_1b_1b_1$ x $a_2a_2b_2b_2$. 13

Recurrent selection Selection repeated over several generations to obtain progressive change. 136

Repetitive DNA Certain sequences are repeated many times in the haploid genome, even up to one million times. It comprises 70-80% of total DNA in conifers. 26

Replication *see* **DNA replication** 21

Rest Budrest or bud dormancy is 'the temporary suspenspension of visible growth of any plant system containing a meristem'; a meristem is a tissue where new cells are formed by cell division; budrest is built up in the buds soon after budset and prevents an untimely budburst; budrest is broken by temperatures a few degrees above zero or by long nights. 16

Restriction enzymes restriction endonucleases are site-specific enzymes each recognizing specific DNA sequences and cleaving DNA at these sites producing DNA fragments. 33

Restriction Fragment Length Polymorphism (**RFLP**) A DNA marker in which the size of the fragments varies within a genetic entry, such as population; RFLPs are codominant (both alleles are expressed) and selectively neutral. 94

Retrotransposon or retroposon Transposable element that is transcribed into an RNA copy, and than back into DNA by a reverse transcribase enzyme. The DNA copy is inserted elsewhere into the genome. 27

Retrospective early test Studies of young siblings of the families studied in field trials. 154

Ribosome A cellular organelle on which the translation of mRNA into amino acids in protein synthesis occurs. 25 Box 2-1

Ribosomal RNA (**rRNA**) RNA molecules that constitute part of the structure of ribosomes. 24

RISC complex RNA-induced silencing complex; a multisubunit cytoplasmic structure that interacts with siRNA or miRNA leading to the breakdown of matching RNA sequences and to gene silencing. See small RNA for information on miRNA and siRNA. 36 Fig. 2-9

RNA, ribonucleic acid It consists of a chain of nucleotides linked through the phosphate groups. Each nucleotide contains the sugar ribose, and one of the four bases adenine, cytosine, guanine, and uracil. RNA is typically single-stranded unlike DNA. 28

RNAi (RNA interference) A process initiated by a double-stranded RNA that leads to the breakdown of an mRNA with a similar sequence. The mechanism proceeds through siRNAs. Often exploited to inactivate a gene in transgenic plants and investigate gene function. 36

Satellite DNA Short DNA sequences tandemly repeated hundreds or thousands of times. Typically present at centromeres and chromosome ends as well as other sites. See also microsatellite and minisatellite. 26

Secondary embryo sac The merged two nuclei in the centre part of the embryo sac. 15

Seed orchard An establishment for production of genetically superior seeds. 142

- **Clonal seed orchard** Grafts or cuttings are used. 143
- **Seedling seed orchard** Seedlings of full-sib or half-sib families are used. 142

Seed orchards can further be classified as:

- **Biclonal seed orchard** Two clones are used, its main implementation is for clones with high specific combining ability.143
- **Monoclonal seed orchard** One clone is used mainly aimed for seed production after artificial mass pollination. 143
- **Interprovenance seed orchard** The genetic entries originate from two or more provenances. 143
- **Interspecific seed orchard** The genetic entries originate from two species. 143
- **Intraprovenance seed orchard** The genetic entries originate from one provenance. 143

Seed tree stand A stand within the best provenances; in many countries approved for seed harvests by a federal organisation. 98

Seedling seed orchard *see* **Seed orchard** 142

Segregation Separation of the two alleles of a gene into different gametes at meiosis. 48

Selection *see* **Artificial selection** and **Natural selection** 72

Selection backward Selection of parents using data from progeny tests. 157

Selection differential, S The difference between the mean of the selected part of the population and the overall mean of that population. 68

Selection forward Selection of trees in progeny trials for generating a new breeding population. 157

Selection intensity, i the selection intensity is obtained by dividing the selection differential by the phenotypic standard deviation, *i.e.* the standardized selection differential. 68

Selective Environmental Neighbourhood, SEN An area within which there is no genotype x environment interaction as regards fitness which means that there is a large homogeneity within an SEN. 82

Self-fertility Ability to form viable offspring by fusion of female and male gametes from the same individual. 14

Self-sterility Inability to form viable offspring by fusion of female and male gametes from the same individual. 14

Self-sterility alleles Alleles that prevent selfing which means that a tree with the self-sterility alleles s_1 or s_2 does not form any seeds if the pollen grains contain s_1 or s_2. It does not matter whether the pollen originates from the same tree or another tree; the female tissue prevents fertilization with pollen containing these alleles. Conifers do not seem to have self-sterility alleles. 14

Selfing Fusion of female and male gametes from the same individual. 14

Selfish DNA Sequences use their host for propagation only, apparently without being of any use for the host. 27

Semiconservative replication After replication of DNA the newly generated double helices consist of one old strand and one new strand. 21 Fig. 2-2

SEN *see* **Selective environmental neighbourhood** 82

Severity index The expected plant mortality in per cent of the local population 20 years after establishment of the test plantation. The reason for using such a high age as 20 years for establishment is that the results have shown that it may take 20 years before knowledge about hardiness of *Pinus sylvestris* is complete. 101

Single Nucleotide Polymorphism, SNP It is caused by the change of a single nucleotide. Most genetic variation between individual humans is believed to be due to SNPs. 94

Small RNAs. 36 These include:

- **siRNAs (small interfering RNAs)** Double-stranded RNA sequences of 21-24 nucleotides that inhibit gene expression by directing destruction of complementary mRNAs. 36
- **miRNAs (microRNAs)** RNA sequences 21-24 nucleotides long, produced by the processing of RNA transcripts encoded by specialized genes, that regulate gene expression by pairing with complementary regions of mRNA. This leads to destruction of the mRNA or block in its translation. 36

SNP see **Single Nucleotide Polymorphism** 94

Somatic embryogenesis A process of asexual reproduction where an embryo is derived from a single somatic cell or group of somatic cells, usually growing in vitro. The somatic cells can be part of a zygotic or somatic embryo. 146

Speciation The differentiation between two populations has gone so far that they have become reproductively isolated from each other and therefore gene flow between them is prevented; two main types of speciation are distinguished (148):

- **Allopatric** The speciation takes place in geographically separated populations. 88
- **Sympatric** The speciation takes place in geographically common area. 89

Specific combining ability *see* **Combining ability** 64

Spermatogenous cell The cell of the male gametophyte of gymnosperms, which divides mitotically to form two sperm nuclei. 15

Stalk cell One of the two cells produced by the division of the generative cell in developing pollen grains of gymnosperms; it eventually degenerates. 15

Status number An estimate of the size of a population comprised of unrelated trees. A breeding population of 50 trees may have a much lower status number than 50 owing to various degrees of relatedness among the 50 trees. 140

Stop codon Nucleotide triplet, UAA, UAG or UGA, within messenger RNA that signals where translation stops. 29 Fig. 2-4.

Strobilus (plural **strobili**) Reproductive structure in *Pinaceae*; the pollen cone consists of microsporophylls with microsporangia containing pollen grains; the seed cone consists of ovule-bearing scales, the ovules contain egg cells. 14

Sublining The breeding population is divided in smaller populations, sublines, so that inbreeding is avoided in the production population, but permitted in each subline; from each subline one clone is selected for establishment of seed orchards for production of commercial seed. 140

Synergids Usually the two cells located adjacent to the egg cell in the embryo sac. 15

Synonymous substitution A nucleotide base is replaced by another base in a codon of the genetic code without changing the amino acid encoded; this replacement usually occurs only in the third position of the codon, *e.g.* when the base cytosine is found in the positions 1 and 2 in the codon, the amino acid proline is always formed irrespective of which base is located in position 3. 29

Synteny Partial conservation of gene order among species. 31 Fig. 2-6

Target species A species given priority in gene conservation for scientific reason, threat, charisma, or economic reason.171

Telomere The DNA sequence at the end of a chromosome that provides stability to the chromosome. 10

Terminator region Includes the stop codons for termination of the polypeptide (protein) synthesis.

Tetraploid Species or individuals with four chromosome sets (denoted 4x). 10

Thymine A pyrimidine base, one of the four nucleotide bases of DNA; thymine is paired with adenine in the DNA double helix. 22 Fig. 2-1

Transcription The synthesis of an RNA transcript on a DNA template. 28 Box 2-1

Transcription factor A protein that activates the initiation of eukaryotic transcription, either at all loci (general transcription factor) or at specific loci (specific transcription factor). 30

Transcriptome The set of all the RNAs, particularly mRNAs and small RNAs, produced in a cell, tissue, or organism. 35

Transcriptomics The study of transcriptomes. 35

Transfer RNA, tRNA A small RNA molecule that serves in protein synthesis; it binds an amino acid, specified by tRNA anticodon which pairs with a codon on the mRNA, and tRNA delivers its amino acid to the growing polypeptide during translation of mRNA. Box 2-1

Transformation, stable The incorporation of a new gene(s) into the host cells' genome using genetic engeneering. 40

Transgenic plant Plants into which genes have been transferred using genetic engineering. 40

Translation The synthesis of a polypeptide whose amino acid sequence is determined by the codon sequence of an mRNA molecule. 28 Box 2-1

Translocation Change of chromosomal segments between non-homologous chromosomes. 14

Transposon A piece of DNA that can move spontaneously from one position to another within the same chromosome or between chromosomes; also called **jumping gene**. 27

Triplet The three nucleotide pairs that constitute a codon. 29

Triploid Species or individuals with three chromosome sets (denoted 3x). 10

Tube cell In male gametophytes, the cell that develops into the pollen tube. 15

Uracil One of the two pyrimidine bases found in RNA, it is replaced by thymine in DNA. 28

Vector, cloning A DNA molecule capable of replication in a host cell, into which a gene or DNA segment is inserted by recombinant DNA techniques and can serve as a vehicle for transfer of DNA to a host cell. Fig. 2-8

Wahlund's principle The frequency of homozygotes decreases in the progeny after matings among individuals of two previously isolated populations. 54

Water use efficiency The ratio of carbon gain to water losses. 125

Zygote the cell formed by fusion of two gametes. 15

The rarely occurring and endangered orchid, *Cypripedium calceolus*, growing in forests in Sweden. Photograph Inger Ekberg

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