SHAPING OUR FOOD

AN OVERVIEW OF CROP AND LIVESTOCK BREEDING

SECOND EDITION

Shaping our food – an overview of crop and livestock breeding, second edition

ISBN 978-91-981907-1-7 (PDF English)

ISBN 978-91-981907-2-4 (print Swedish)

ISBN 978-91-981907-4-8 (PDF Swedish)

Editor: Anna Lehrman

Authors: Anna Lehrman, Sevasti Chatzopoulou, Dennis Eriksson, Elisabeth Jonas, Fredrik Levander, Alessandro Nicolia, Lotta Rydhmer, Helena Röcklinsberg, Per Sandin and Li-Hua Zhu.

Layout: Viktor Wrange, Wrange Design AB

Illustrations: Fredrik Saarkoppel, Kobolt Media AB

Photo: Istockphoto.com (unless otherwise is specified)

Citation: Lehrman, A. (ed.). 2020. Shaping our food – an overview of crop and livestock breeding. Uppsala, SLU. ISBN 978-91-981907-1-7

www.slu.se/shapingourfood

MILJÖMÄRKT 341 271

FSC www.fsc.org MIX Papper från ansvarsfulla källor FSC® C011449

This button takes you to the content page!

Contents


0368
0365
0343
0343

Preface

The domestication of plants and animals is a long and on-going process that has shaped not only the domesticated species and the landscape, but also the humans who have domesticated them. For example, the evolution of our immune system has been strongly influenced by the close contact between humans and domestic animals. The changes in domesticated species have been dramatic, from the wild red junglefowl hen raising two clutches of 10 chicks per year, to today's laying hen producing more than 300 eggs per year. In one hundred years the average wheat yield has increased from two tonnes per hectare to six tonnes per hectare in many European countries. Although part of this increase is due to management techniques, fertilizers, and pesticides, the genetic component of such progress has been substantial.

With an increased knowledge of evolution, the understanding of heredity, and the discovery of chromosomes and genes, we have gone from unintentional selection to advanced breeding programmes. Our ever-increasing knowledge of the mechanisms behind different traits can be used to customize the sources of our food. Thanks to these breeding programmes, we now have access to healthier livestock and crops, and are producing milk, meat, and grain at levels our ancestors could only have dreamed of.

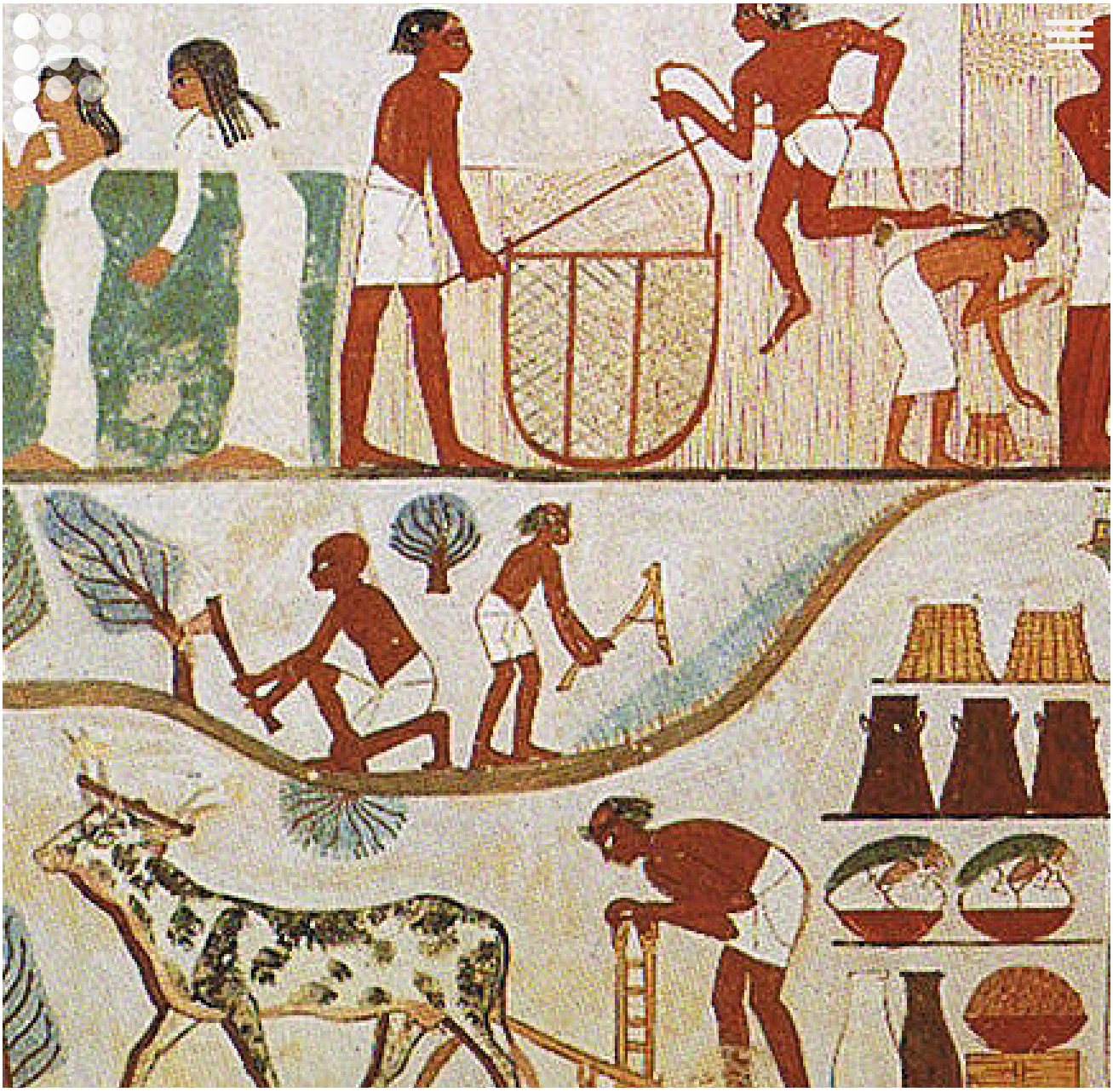
With this book we wish to provide an overview of the methods and techniques used in the domestication and development of new agricultural crop varieties and breeds of livestock. We also describe the legislation and discusses different ethical views on the use of biotechnology in crop and animal breeding.

This book is published within the Mistra Biotech research programme, financed by the Swedish Foundation for Strategic Environmental Research (Mistra) and the Swedish University of Agricultural Sciences (SLU). This second edition is a shortened version of the previous edition. We are grateful to Inger Åhman and Marie Nyman for helpful comments on the manuscript.

FUNDED BY

MISTRA
The Swedish Foundation for Strategic Environmental Research

SLU



Agricultural scene from the tomb of Nakht in Egypt (14th century B.C.).

7000-9000 BC

Start of collecting and sowing seeds, and domestication of large mammals

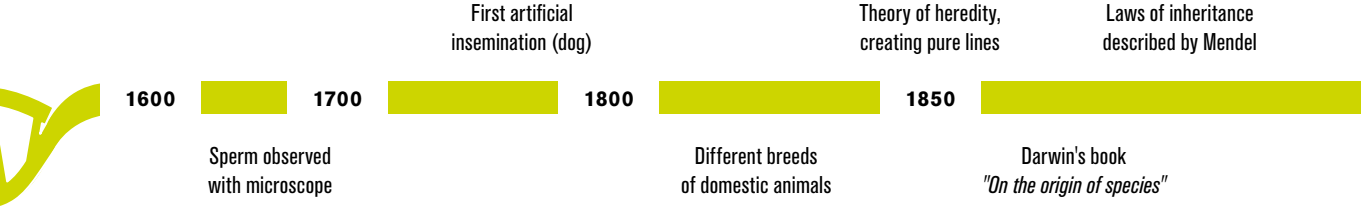
The history of breeding

Plant breeding began unintentionally about 7000–9000 B.C. when people began sowing seeds instead of just collecting seeds from wild plants. The plants that yielded a better harvest were the ones that were propagated year after year, and thus natural selection was replaced with artificial selection by human hands. With the change from a nomadic lifestyle to one of village life based on plant cultivation, hunting in the areas around the villages decreased the wild animal populations and motivated the husbandry of mammals and poultry. The first animal to be domesticated was the dog, some 10,000 years ago. Fear of humans and aggressive behaviour were probably the first traits to be selected against. As humans started to choose parent animals, traits such as body size could also be selected for.

One of the most problematic traits when domesticating a crop is seed shattering in which mature seeds drop to the ground or are dispersed by the wind or by animals. This trait is crucial for survival in the wild but is useless when trying to harvest the crop. Consequently, seed shattering was one of the first traits to be selected against in the early stages of crop domestication. Collection of seeds from superior plants continued and agriculture evolved. Irrigation, removal of weeds, and fertilization altered plants even further from their wild relatives because they no longer had to compete for water, sun, space, and nutrients. However, it would take a long time before we began to understand the mechanisms behind these changes.

In the middle of the 19th century, the theory of heredity was presented and it was discovered that "pure lines" of crops could be created by inbreeding (see page 33). At the same time, Gregor Mendel showed that traits such as seed shape, seed colour, and plant height are inherited in a specific pattern in peas. Unfortunately, it was not until 40 years after his discoveries that the importance of his work was realized. In contrast, the entire edition of Darwin's book *On the Origin of Species* was sold out shortly after it was printed in 1859. Darwin understood that traits important for survival and reproduction are inherited, that there is a variation in the ability to survive and reproduce, and that there is a limitation in resources so that not all individuals who are born will survive. By combining these three insights, he could explain the principles of evolution as well as the selection of domesticated species even though he did not know about genes.

As more controlled crossings between breeds or varieties were made, the phenomenon of heterosis, or hybrid vigour, was discovered.



Heterosis is when the progeny of a cross outperform both parents, and this effect is for example noticeable in traits related to disease resistance in animals and to biomass in plants. A decade after the discovery of heterosis, the fact that many traits depend on many genes, so called quantitative traits, was understood and statistical models were developed to account for such traits in livestock breeding.

As with evolution, breeding is dependent on genetic variation and the recombination of genes. However, genetic variation can be more or less limited, especially in crops. Also, a desired trait might be closely connected to undesirable traits and, therefore, selection for a desirable trait can result in selection for undesirable traits as well. The discovery that the mutation rate could be increased has become a useful tool in plant breeding. The use of X-rays and toxins can increase mutation rates by thousands of fold. Few of the plants will survive such treatment, but with a bit of luck one can get rid of bad traits or acquire new traits in those that do survive, hopefully without detrimental changes to the rest of the genome. Most of the barley varieties currently under cultivation have genes that have been changed by induced mutations, and today there are over 2,500 known plant varieties that have been developed by induced mutations. In animals, induced mutations of breeding is not possible due to both ethical and economic concerns.

The numbers of offspring are low among animals compared to plants, but with artificial insemination (AI) breeders found an effective way get many more offspring from one male than would be possible with natural mating. The first AI experiments were performed in dogs in 1780, but it was not until the beginning of the 20th century that the method was developed for practical use.

Spontaneous chromosome doubling, which often results in larger plants, had been noticed in

Gregor Mendel, known as the "father of modern genetics", cultivated about 29,000 pea plants during his studies on inheritance. Photo: Wikimedia Commons.

Commercial crop bred from a controlled crossing

Plant cell culture

Heterosis (hybrid vigour) discovered

Haber & Bosch's method for nitrogen fixation

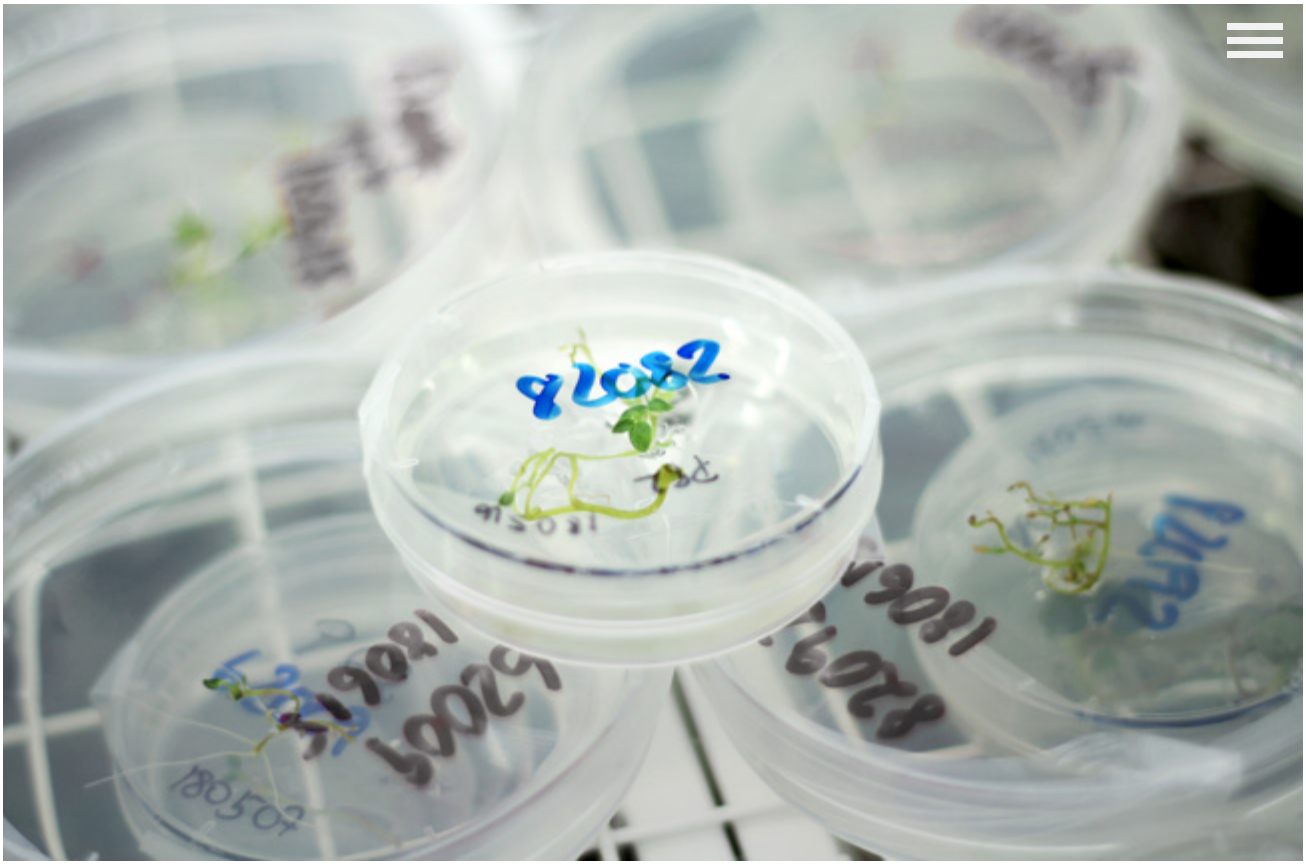
1900

1910

Crop bred for disease resistance though controlled crossing

Plant embryogenic cell culture

Role of chromosomes (carrying genes) described

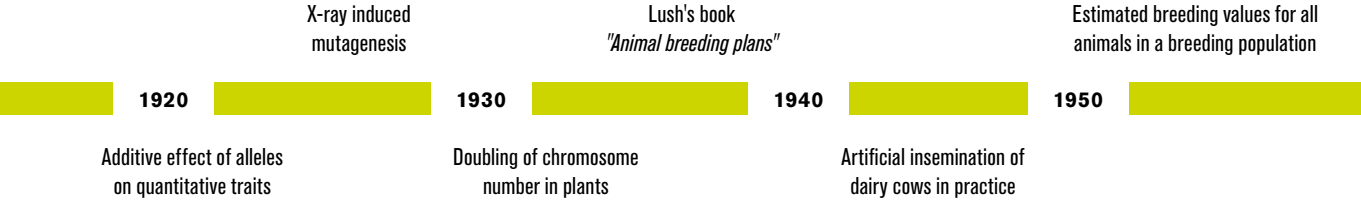


Plant tissue culture is a method where plant tissues can be induced to regenerate a new plant. This is an important tool in plant breeding.

wild species, but it was not until the beginning of the 1930s that a substance called colchicine was found to stop the chromosomes from separating prior to cell division. Now breeders had a tool with which they could increase the number of chromosomes in crops and thereby produce larger plants.

With colchicine it also became possible to produce sterile plants. By doubling the two sets of chromosomes (diploid) into a tetraploid (four sets), and then crossing the tetraploid with the original diploid, a triploid is created. A triploid is sterile and subsequently do not form any seeds. This is how we got for example seedless watermelons.

In parallel with the breakthroughs in genetic research, the first steps were taken toward growing plants from cells in a growth medium, i.e., tissue culturing, which has become an important technique in modern plant breeding.



Two major advances were test tube fertilization, which overcame barriers of sexual reproduction, and the ability to regenerate plants from single somatic cells (non-germ cells), which meant that small amounts of tissue could be used to raise thousands of plants.

Colchicine has been used to create for example seedless watermelons.

Because Mendel studied qualitative traits – such as colour and seed shape – that are governed by a few genes, and Darwin studied quantitative traits – such as growth rate – that are governed by many genes, their theories at first seemed to be in conflict. It was not until the 1930s that scientists began to understand how traits are inherited. With the discovery that genetic material is carried by deoxyribonucleic acid (DNA), and the structure of the molecule, pieces fell into place.

Years earlier, the ability to fixate nitrogen from the air was discovered, but to begin with the prioritized use of this knowledge was production of explosives during World War I, before it came to use for production of fertilizers on a large scale. Fertilizers had a huge impact on agriculture and plant breeding, and the green revolution with modern agricultural production techniques had begun. Norman Borlaug developed improved wheat varieties, and the increased use of herbicides also provided incentives to breed for herbicide tolerant crops.

In the beginning of the 1970s, cells from two tobacco species were fused and the first somatic hybrid plant was produced. The knowledge about cell functions and gene regulation increased, and with the ability to use restriction enzymes, the cell's built-in "scissors", came the ability to cut specific genes out of the DNA. This was one important tool that led to the construction of the first recombinant organisms, including the transgenic bacteria that still provide us with insulin today.

DNA molecule described

Barley with induced mutations

Description of the genetic code

Restriction enzymes discovered

First genetically modified organism (bacterium)

1960

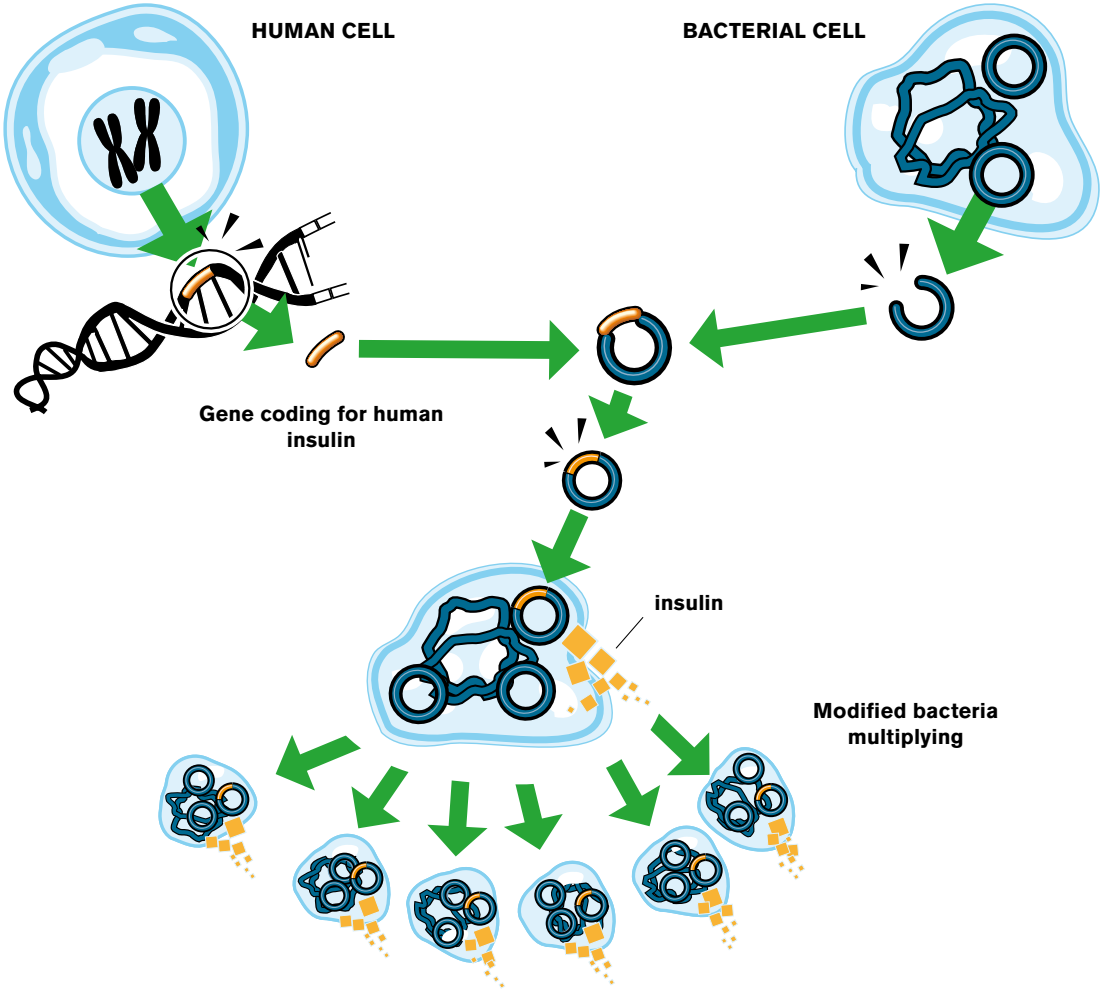
1970

Crops tolerant to herbicides developed

Test tube fertilisation between sexually incompatible plants

Somatic hybridisation (cells from different species in plants)

Embryo transfer in cattle



The first organism to be genetically modified was a bacterium. Today, insulin is produced by bacteria that have had the gene coding for insulin inserted into a plasmid (a small circular piece of DNA).

Site-directed mutagenesis developed

Calf born from in vitro fertilisation

First genetically modified animal

PCR technique for fast amplification of DNA

1980

Use of soil bacteria to insert new DNA into plants

Insulin producing bacteria commercialised

Gene maps for animals

In the beginning of the 1980s, researchers managed to create the first transgenic plant, a tobacco plant, with the help of the soil bacterium *Agrobacterium tumefaciens*. In nature, this bacterium causes plants to grow tumours by inserting its DNA into the plant's genome, but now those genes could be replaced by any other gene of interest. Not all plant species were susceptible to infection by the bacterium, so other methods were developed such as the gene gun with which the desired DNA could be shot into the plant. Soon genetically modified (GM) plants appeared around the world, first in field trials, then as commercial crops.

At the same time, AI of sows became routine and methods for embryo transfer were established in dairy cows to enable those with the best breeding values to produce more calves. The first GM animal was a mouse that received genes important for growth from a rat, but the application of GM technology for commercial breeding of farm animals has been limited, for ethical and economic reasons.

Although he was the man behind several inaccurate and questionable theories (like eugenics) Francis Galton (1822-1911) contributed to one of the most important breeding methods used on livestock today – genomics, a concept proposed one hundred years after his death. He was one of the pioneers in the use of statistics to calculate quantitative traits (traits often affected by several genes and environment) and the same statistics is used today. In genomic selection the breeder makes use of genetic markers covering the entire genome to select for the best individuals for further breeding. Since the beginning of this century the costs to analyse genetic material have dropped considerably. In parallel the developments in computer capacity have made it possible to analyse large data-sets, which is needed for the statistical analyses.

Because of the lower number of offspring and long generation times, animal breeders have had a different approach compared to plant breeders. The researchers and breeders have focused on estimation of breeding values and the use of gene maps that provide information about the location and arrangement of specific genes on a particular chromosome (see page 29). The gene maps contain genetic markers that (mostly) do not themselves govern any particular traits but can be used for selection if they are located close to genes that do affect important traits. Today, selection with the assistance of one or multiple genetic markers is used both in plant and animal breeding. Since the first farm animal (the chicken) had its full genome mapped (i.e., its entire DNA sequence was described), most of the domesticated livestock species have had their genomes mapped.

The term "proteomics" was not coined until in the early 1990s although mapping of proteins began in the mid-1970s. The beauty of proteomics is the same things that makes it difficult. Unlike the genome which is more or

First GM domestic animal (pig)

Biolistic "gene-gun" to insert new DNA into plants

Marker assisted selection in animal breeding

First animal born from cloning of a somatic cell (a sheep)

1990

Field trial with a GM crop

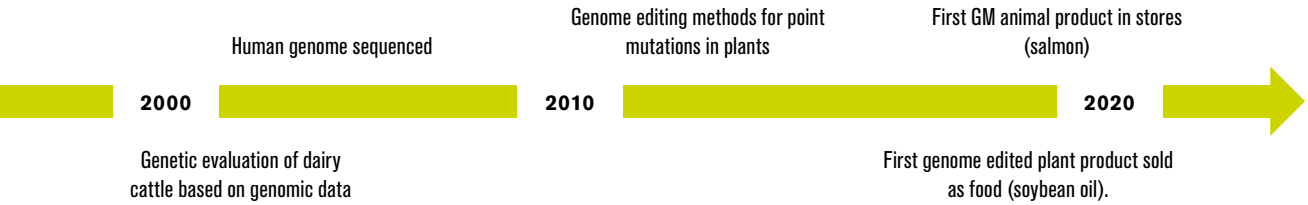
Commercial GM crop (virus resistant tobacco, China)

RNA interference in plants

The AquAdvantage® Salmon (background) is a GM Atlantic salmon that carries a growth hormone gene from the Chinook salmon that makes it grow faster during an early stage of life and reach full size one year earlier than non-GM Atlantic salmon of the same age (foreground).

less constant, the composition of proteins differ between cells and from time to time. Such information is used to find and understand functions of different genes, and can also be used in breeding (see page 39).

The development from the random mutation breeding by radiation or chemicals to precise alterations through site-directed mutagenesis, or genome editing, has taken about 40 years, but it has only been in recent years that these new methods have been sufficiently refined for use in commercial applications. The most famous genome editing techniques CRISPR/Cas9 was presented in 2012, but similar techniques had been developed earlier, zinc finger nucleases (ZFNs) and especially transcription activator-like effector nucleases (TALENs) in 2010. With these "genetic scissors" it is possible to target specific sites or genes, compared to the randomness of other methods (see page 44). The success of CRISPR/Cas lies in that it is less time-consuming and easier compared to ZFN and TALEN.



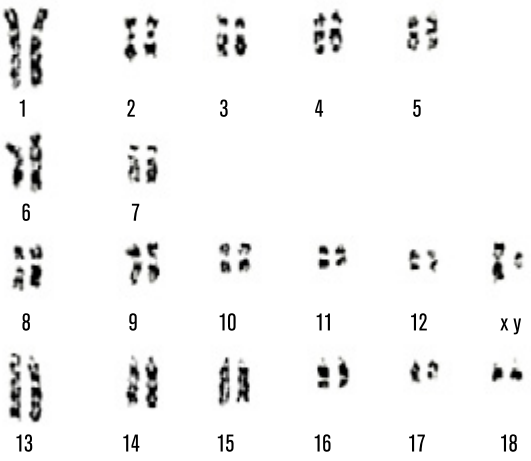
The building blocks of life

Why do we and all the plants, animals, and other organisms around us look and behave the way we do? In this chapter, we briefly explain the structures and mechanisms that are the basis for living organisms and focus particularly on plants and mammals.

Chromosomes

One can find flower seeds tinier than the period at the end of this sentence. In these seeds, just like in animal cells, one finds the genome, that is, all of the genes. They make the seed germinate and grow into a plant with a specific size and shape that thrives in a specific environment, flowers at a certain time, and has a certain scent. All of the information that is needed to regulate the plant's life has to be stored in that seed.

Genes are arranged in structures called chromosomes that, in mammals, come in pairs. Such organisms are known as diploids.



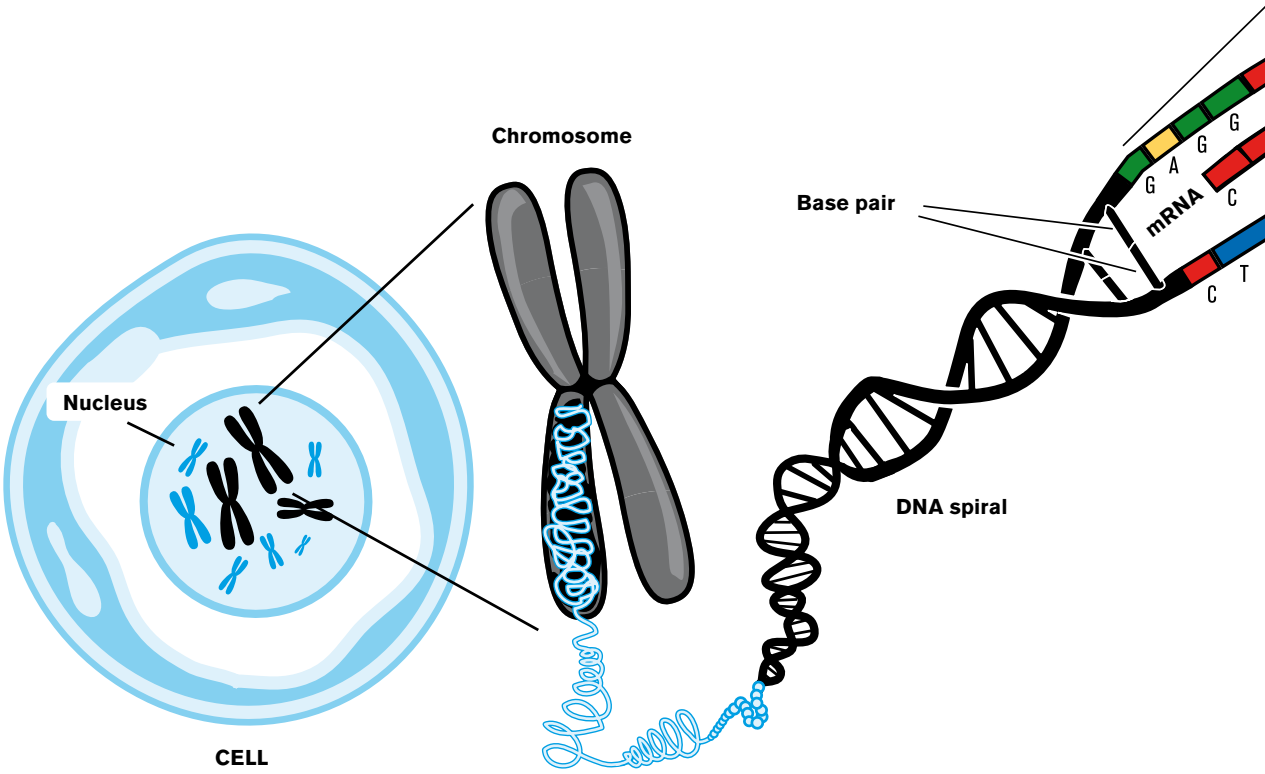
Pigs have 38 chromosomes, 19 from each parent.

For example, the domesticated pig has 19 chromosome pairs with each pair consisting of one chromosome from the father and one chromosome from the mother. Genes governing the same traits on corresponding sites on chromosome pairs are called alleles. In a homozygote, the alleles are the same on both chromosomes and in a heterozygote the two alleles are different. How the different alleles together affect a trait depends on whether the individual alleles are dominant or recessive (such as the case for brown or blue eyes) or if they have an additive effect (such as the case for height) (see also the section *Genotype and phenotype* on page 18).

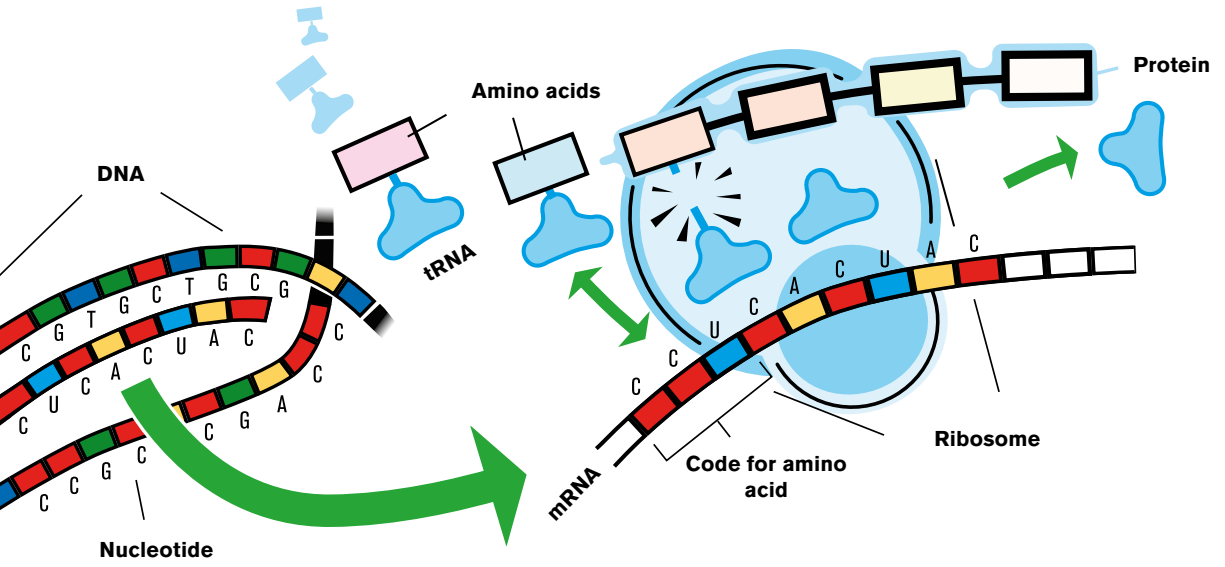
Many plants have more than two sets of chromosomes, that is, they are polyploids. Autopolyploids are the result of chromosome doubling within the same species, and an allopolyploid is a result of chromosome doubling through a combination of two different species. For example, durum wheat is allotetraploid (it has two sets of paired chromosomes) that originated from a hybridisation between wild grasses. A hybridisation between durum wheat and a wild diploid grass resulted in today's hexaploid bread wheat that carries three sets of paired chromosomes.

Genes and proteins

As stated above, genes are made up of DNA. DNA comes as a double helix and looks like a spiral-shaped ladder where every rung is made up of pairs of the four nucleic acids adenine (A), guanine (G), cytosine (C), and thymine (T). The nucleotides are often referred to as the "bases" of the DNA. The A nucleotide is always paired opposite a T and the C nucleotide is always



DNA is tightly packed into chromosomes by proteins. These proteins can unwrap the chromosome to expose the bare DNA strands and allow the transcription machinery to copy it in a very precise manner. Using one of the DNA strands as a template, an enzyme constructs a messenger ribonucleic acid (mRNA) molecule. The mRNA differs from the DNA in that it is single stranded and instead of thymine it contains uracil. The mRNA is translated into a protein by another set of protein molecules. Sophisticated modulations and regulations at this level are unique to complex organisms like animals and plants compared to simple organisms such as bacteria. The mRNA is translated, according to the genetic code, into a specific sequence of amino acids that are then folded into a functional protein.



paired opposite a G such that the two strands of the "ladder" are the mirror of each other. The order in which the bases appear determines which amino acid they code for. The code for an amino acid is made up of three bases. For example, the three bases AAG code for lysine.

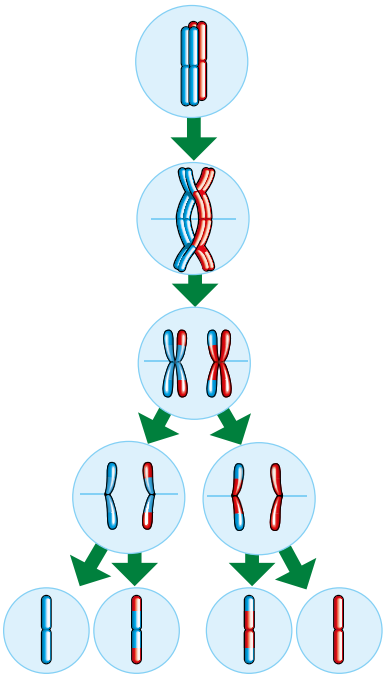
Proteins are responsible for almost all processes that occur in all living organisms, for example, enzymes and some hormones are proteins and muscles consist mostly of protein. Proteins are built of amino acids and it is the unique combination and sequence of the amino acids that determines the structure and properties of the protein such as its heat sensitivity, if it binds to other specific proteins, or if its shape and function are altered with changes in pH.

Genetic change

The success of all species in terms of survival and propagation depends on their ability to adapt to new and changing environments. High genetic diversity increases the probability that some of the individuals in a population will have characteristics that are advantageous in certain environments and, therefore, will be better at coping with changes in the environment than other individuals. Mutations and recombination between the chromosomes lead to the genetic diversity that is crucial for evolution.

REPRODUCTION

Genes code for the production of proteins and they transfer this information to the new cells when cells divide. Unicellular organisms like bacteria reproduce by a simple cell division. Bacterial genes are arranged on a single circular chromosome as well as on small rings of DNA or RNA called plasmids. Animals and plants, on the other hand, are built of many cells each having specialized functions, and some of these are specialized reproductive cells known as gametes. These cells are formed in two steps. In the first step, each chromosome is copied and the two doubled chromosomes (for a diploid organism) are lined up next to each other. At this stage pieces of the DNA strands on the corresponding chromosomes can switch place with one another in a process known as recombination. This crossover process results in offspring with a genetic makeup that is different from both of the parents. In the second step, the chromosome pairs move in separate directions after which the cell divides once and then a second time resulting in gametes with half the number of chromo-



Recombination of genes during meiosis. When the gametes (sperm and egg cells in animals) are formed, each chromosome pair exchanges some parts of their DNA before they separate. Which parts of the chromosomes recombine varies, and sometimes the exchange is imbalanced and this can have detrimental effects on the organism.

somes. In a diploid species, the gametes will have just one of each chromosome. When the female gamete is fertilized by the male gamete, the new individual receives half of the mother's genetic makeup and half of the father's to create a new individual with a unique set of genes.

There are examples of animals that can reproduce asexually, such as aphids, and many plants can multiply vegetatively (i.e., non-sexually) through bulbs (garlic), tubers (potatoes), or stolons/runners (strawberries).

MUTATIONS

Any of the bases A, T, C, or G can be exchanged for another base and this is known as a mutation. For this mutation to have any effect, the base has to be located in a gene or a region that is involved in the expression of a gene. In addition, the change in the base has to change the amino acid that is coded for. Also if the amino acid is changed, such a change must alter the protein's function in some way for the mutation to have an effect. Most mutations in the genome are repaired by the cell, but if a mutation occurs in a gamete and is not repaired the change will be inherited in the next generation.

Many mutations in gametes are harmful, and some are so harmful that the offspring never develops. Even so, mutations are crucial for the process of evolution, and a small portion of these mutations are beneficial for the individual organism's ability to survive and reproduce.

Depending on to what extent the mutation affects the individual's fitness, the new allele might become more and more common in the population with each generation. Established mutations in combination with the mixing of the parents' chromosomes and the recombination of genes, increases the variation in traits. This helps populations of organisms adapt to new conditions because these genetic variations often result in some individuals that manage better in the new environment. If a population is isolated, this development might eventually result in the establishment of a new species. In breeding, humans make use of such genetic variation to make selections based on which traits are preferred in crops and livestock.

Genotype and phenotype

An organism's genetic makeup is called its genotype. An organism's appearance is called its phenotype. The genotype can include many genes with "hidden" effects such as recessive alleles in a heterozygote, thus two individuals that look the same can have different genotypes. For example, two black sheep can have the same phenotype – they both have black wool – but one can have the genotype BB and the other can

The allele for black colour in sheep is dominant over the allele for brown colour. That is why black sheep are more common.

have the genotype Bb. In this case, the B (black) allele is dominant over the recessive b (brown) allele. This means that a sheep has to get the "b" allele from both its parents (it would need to have a bb genotype) in order to have brown wool. Most traits are more complex than this and are based on the interactions of several genes that can lead to a wide variety of phenotypes.

Many traits are governed by numerous genes with additive effects, and this results in offspring that have phenotypes that are combinations or intermediates between those of the parents. Most of the phenotypic traits are inherited in this way and it is not possible to distinguish specific genotypes by simply looking at an individual. Instead, one can only estimate the genotype based on the organism's phenotype and the phenotypes of its relatives. To make it even more complicated, the phenotype s often affected by environmental factors.

GENES AND THE ENVIRONMENT

The phenotype of an organism cannot be explained solely by the genotype of the organism. In fact, the observed phenotypic trait is the result of the expression of the genes influenced by a given environment. For some traits this expression is quite stable across a wide range of environments, but other traits show considerable variation with specific environments. When a genotype results in different phenotypes in different environments, this is called a "genotype by environment" interaction. For example, such interactions can be relevant for the maternal behaviour of sows. Perhaps the sow with the best genotype for maternal ability in an intensive indoor production system is not the best sow in a free-range system. In another example, when new spruce trees are planted in the forest the genotypes of the plants are chosen depending on the region of the forest the trees are planted in. For some traits it is easy to predict the offspring's properties based on the parents, but for other traits such predictions can be very difficult due to the influence of environmental effects.

EPIGENETICS

Epigenetics is a relatively new and flourishing research area. In the field of epigenetics, researchers investigate heritable, but reversible, changes in gene expression that are not caused by changes in the DNA sequence. In all living organisms, parts of the genome are switched on and off at specific times in different tissues and cells and during different developmental stages. This regulation is accomplished by an array of chemical reactions, and in some cases these changes are carried into the new cells after cell division and thus into the next generation. Such heritability of epigenetic regulation is considered to be an important mechanism by which many species can rapidly adapt to changes in their environment. Epigenetic changes can, for example, take place during the first steps of gene regulation during the unfolding of the packed chromosomal DNA or by the addition of methyl groups to the DNA. From a breeder's point of view, it is important to understand which alleles behave in this epigenetic fashion because they will not be inherited in the classic Mendelian manner and this will hamper the ability to link such alleles to different traits.

Breeding methods

As described in the first chapter, humans have been breeding plants and animals more or less intentionally for a very long time. With increased knowledge about how traits are inherited, a better understanding of molecular genetics, and the availability of powerful computers and statistical software, new breeding methods and technologies have been developed.

Animal breeding

There are two basic questions that farm animal breeders ask when choosing parents to breed. The first question is to define the breeding goals: "Which animal is the best animal?" Is it the cow that produces more milk, the one that lives longer, or the one that combines high milk production with good hoof health? Is it the sow that produces a larger litter, or the one that has more teats, the one with the best nursing behaviour, or maybe the most healthy and sustainable one? These questions are open to debate, and no one has all the answers, but they address the breeding goal and provide the direction of the selection that will affect the characteristics of the animals. The second question is, "How can we identify the best animals in order to improve future generations?". To respond to this question knowledge of animal breeding and genetic principles are needed. This chapter will give some answers to both questions.

Breeding of farm animals is a long-term, multi-step process that aims to improve future animal populations in order to sustain food production from animals or conserve the populations. For successful breeding it is important to study the genetics of traits and to address the question of to what extent the variation in a trait between individuals depends on the effects of various genes. This describes the "heritability" of the trait (see page 23). Another part of such a study is to determine the extent to which different traits relate to each other and to what extent such relationships can be explained by the different genes. This is the "genetic correlation" between traits. Both heritabilities and genetic correlations must be estimated in order to predict the consequences of the breeding programme. The next step is to record the traits that should be changed. It is important that individuals get unique identities so relationships among animals can be recorded (pedigree). This information can then be used to estimate the animals' breeding values. The best animals – those with the highest breeding values – are selected to become the parents of the next generation. The accuracy of the breeding value depends on the available information. In the following sections, these steps will be described in greater detail.

BREEDING GOAL

The first step is to decide on the breeding goal, for example, breeding pigs to be fast growing and healthy with low levels of aggressive behaviour. In cattle, the breeding goal could be robust cows that give birth to calves without any problems. Breeding goals are modified over time due to changes in the specific needs of the farmers or the market. The breeding goal can also differ among different organisations within and between different countries.

A breeding goal usually seeks the optimal combination of several traits. The weight given to each trait in the breeding programme depends on the heritability of each trait, on the genetic correlations between the traits, and

For breeding programmes to be successful one needs to keep track of each individual animal and measure several factors, both physiological and behavioural.

on the economic value of a change in each of these traits. Such weighing factors are called economic weights. Many breeding programmes include goals related to production traits (such as growth rate, milk production, and egg yield), reproductive traits, and health traits (such as robustness, udder health, and leg health).

BREEDING VALUE

A crucial part of every breeding programme is to record the traits that should be improved, together with the pedigree of the animals. This information is gathered in a database and used for the genetic evaluation to predict the animals' breeding values. The breeding value seeks to estimate the worth of the animal's offspring. For example crossing a cow and a bull with high breeding values for milk yield, will give a higher chance that their offspring have high milk yields. The breeding value can be expressed in monetary terms (such as the value of the meat produced in euros), in trait units (such as the increase in meat production in kilograms) or as a relative value (such as negative and positive derivation from an average).

The best animals – those with the highest breeding values – are selected to become parents of the next generation. Thanks to the database,

SPECIES	TRAIT	HERITABILITY low			moderate		high	
Cow	Hoof health	x						
Cow	Milk yield				x			
Dog, sheep & pig	Litter size	x						
Dog, sheep & pig	Growth rate				x			
Salmon	Flesh colour				x			
Fox	Fear of humans		x					
Honey bee	Honey yield				x			
Horse	Trotting speed, prize money				x			
Horse & cow	Stature (height)						x	
Human	Body height						x	
Human	Verbal ability			x				
Mouse	Ability to find the way in a maze				x			
Pig	Per cent lean meat, live animals					x		
Pig	Per cent lean meat, after slaughter						x	
Pig	Pubertal age				x			
Sheep	Lamb survival	x						

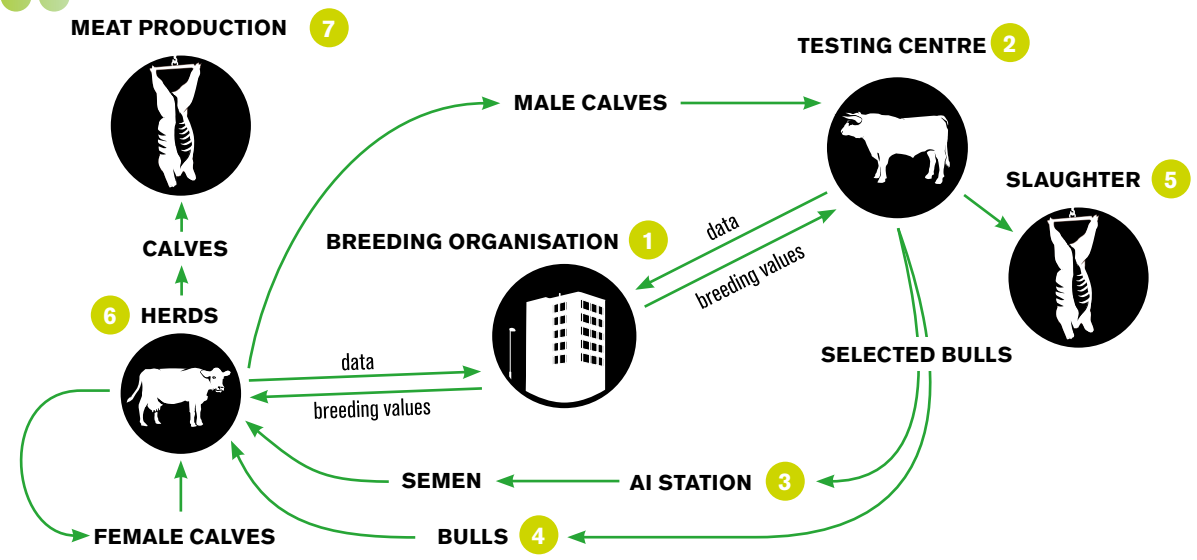
Heritabilities for different traits in different species. If the heritability is high, the rate of genetic change from generation to generation will be faster.

which covers all of the relationships between animals, it is also possible to estimate breeding values of animals that do not have individual records. Thus, a ram can have a breeding value for maternal behaviour, and a young stallion, too young yet for competitions, can have a breeding value for dressage.

In some breeding programmes, such as in dairy cattle, breeding values for different traits (for example milk production, growth, calf survival, or disease resistance) are combined with their economic weights to create the total merit index that describes the animal's total breeding value based on all of its traits.

This genetic evaluation is often performed by breeding companies. It is possible to combine the evaluation with the use of AI, superior males can then be mated to more females and even females world-wide. Thus genetic progress is assisted by AI.

The accuracy of the breeding value depends on the amount of information in the database. If more data is used for the estimation of the breeding value, the more reliable it is. Bulls used for AI have thousands of daughters, and the breeding value for the ability of their daughters to become pregnant can be estimated with a high level of accuracy. Some traits (like appetite

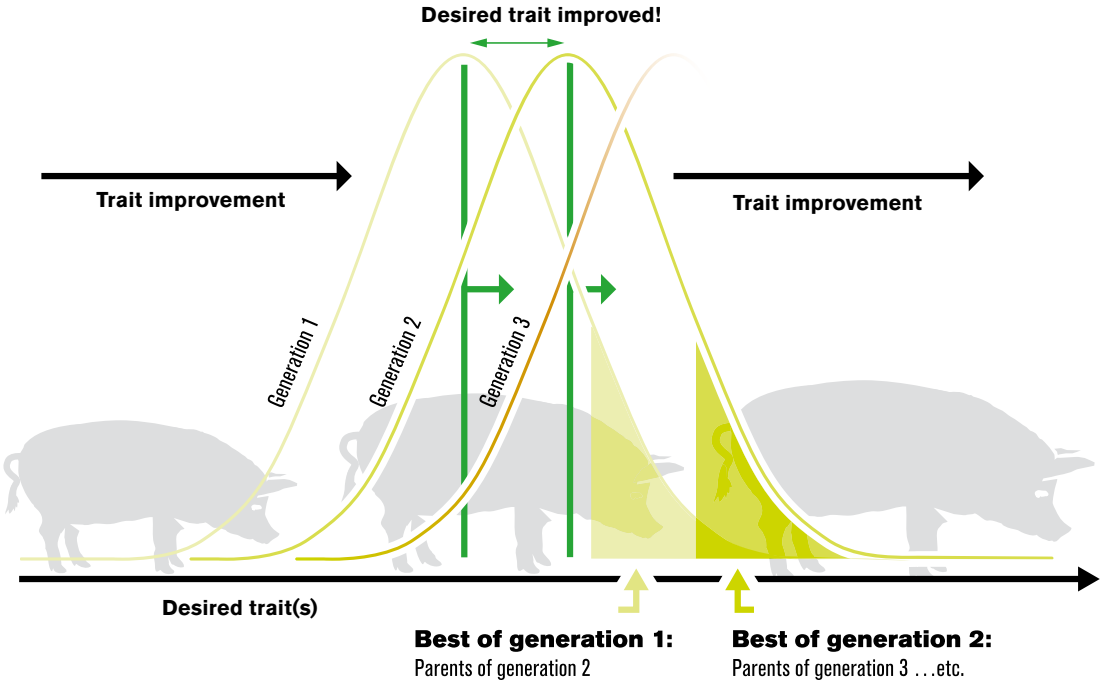


The breeding organisation (1) is the hub of this beef-cattle breeding programme. Here all phenotypic and genetic information is stored in a database and the genetic evaluation is performed. At the performance-testing centre (2), young bulls are tested for traits of interest (e.g. growth rate, etc). After these tests, the bulls with the highest breeding values are moved to an AI station (3) where semen is collected and distributed to many herds. Good bulls, but not the very best, are sold to farmers (4) and used for the natural service of cows. The bulls with the lowest breeding values are slaughtered (5). Cows (6) are either inseminated or mated. Most calves are raised for slaughter (7), but the best females are selected to become mothers of the next generation (6). Some male calves are sent to the performance-testing centre (2). The selection of breeding cows is based on breeding values for maternal traits (e.g. calf survival, etc.). Traits such as meat quality can only be measured after slaughter and thus cannot be measured in selection candidates. Instead, trait records and animal identities are collected on relatives of the selection candidates at the slaughterhouse (7).

or egg weight) can be recorded several times on the same animal, but others (like age at puberty or meat quality) can be recorded only once. Another important factor for the accuracy is the heritability. For traits with low heritability, including reproductive traits like the ability to become pregnant, litter size and piglet survival, it is especially important to collect as many records as possible. For traits such as leanness (or its opposite, fatness) which have a high heritability, selection can be successful without large breeding programmes and advanced statistical models. Simply choosing the leanest animals in the herd and using them as parents can result in rapid genetic progress. It would never be as easy to genetically improve a trait like piglet survival on the herd level.

CROSSBREEDING

The aim in crossbreeding is to boost hybrid vigour or "heterosis". In a trait with a pronounced heterosis effect, the quality of the trait in the offspring is better than the average of the trait in its parents. Heterosis is especially important for traits like survival, reproduction, and health. (You can read more about crossbreeding and heterosis on page 36 in the section about plant breeding.) For example, pig breeding has often a hierarchical structure. Genetic evaluation and selection is performed in a few special herds with purebred animals from "female" lines (selected for maternal traits such as reproduction) and "male" lines (selected for traits such as meat quality). Most of the sows in commercial herds are crossbreds of two female lines, which are



The animals with the best breeding values are used as parents for the next generation.

crossed with boars from a male line to produce slaughter pigs. Thanks to heterosis the crossbred pigs are more vital and healthier and grow faster than purebred pigs. Crossbreeding is also commonly used in many other livestock species.

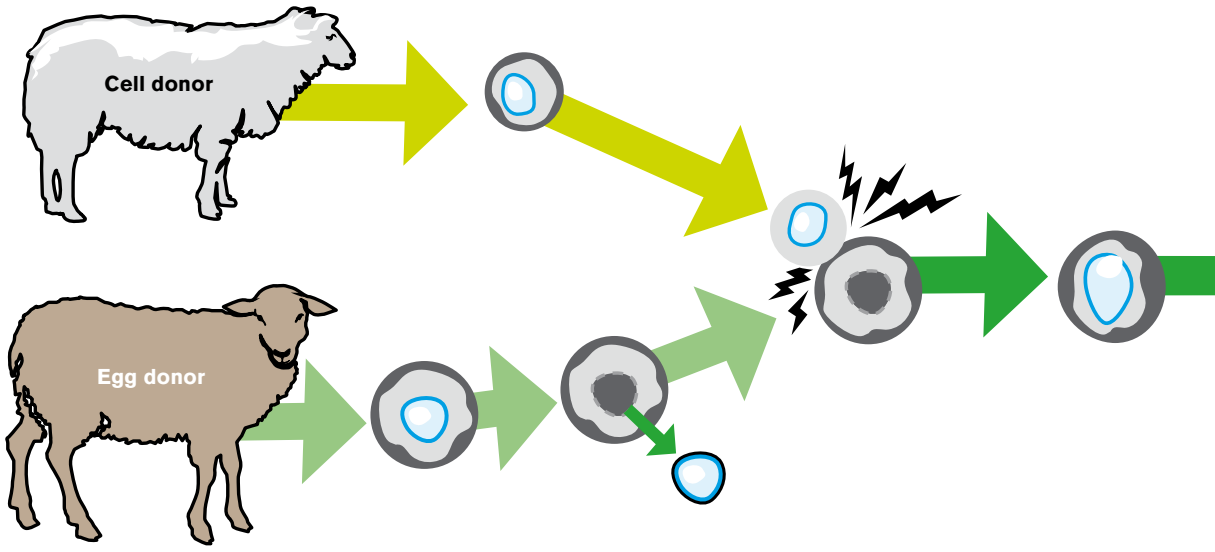
INBREEDING

Inbreeding is the mating of related individuals. Inbred organisms have an increase in homozygosity (acquiring the same allele from both parents) and exhibit more effects of recessive alleles, which are more likely to be detrimental. This phenomenon, known as inbreeding depression, can significantly decrease the performance of the organism. When relatives are mated, the total amount of genetic variation in the population decreases. A decrease in heterozygosity results in reduced production, survival, health, and reproductive efficiency.

Selection has dramatically reduced the genetic variation in some breeds. Today, for example, only a limited number of bulls in the Holstein dairy cow breed serve as fathers of highly influential bulls that are used for AI all over the world. In the short term, inbreeding can be avoided at the farm by never mating close relatives, but the setup of a long-term breeding programme depends on correctly selecting the young sires entering the test programmes and thus on the routines of the breeding organisations.

ARTIFICIAL INSEMINATION

When selecting the best animals for a breeding programme, one limitation has been that



In animal cloning, the nucleus from a somatic cell (non-germ cell) from the animal to be cloned (the cell donor) is fused with an egg cell that has had the nucleus removed. The cell divides and develops into an embryo that is then placed in the uterus of a surrogate mother.

one male can only mate with a limited number of females within a geographically limited area during a limited time period. With AI, one collects semen from the male and inseminates several females. This results in a more efficient use of bulls because a single bull can produce hundreds of doses from a single semen ejaculate. The semen is easy to transport and can be frozen allowing for insemination around the world and the ability to store it for long periods of time. One benefit from this is that improved genetics can be distributed to more farms. Another advantage is reduced disease transmission between males and females that can occur during natural mating.

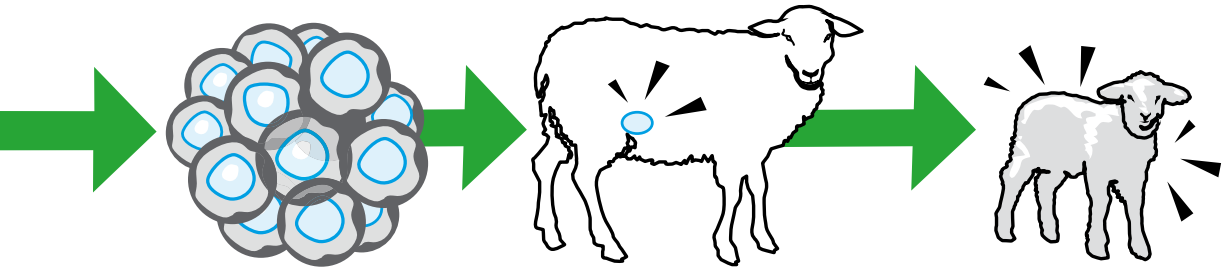
For the farmer, AI can also decrease costs and increase safety. Maintaining one or more males on a farm is often expensive and, depending on their size and level of aggressiveness, the males can be potentially dangerous to the farmer.

EMBRYO TRANSFER

The number of progeny born to a female can be increased through the use of embryo transfer (ET). This is a reproduction technique in which embryos are collected from a female with a very high breeding value (the donor female) and transferred to other females (the recipient females) that serve as surrogate mothers. ET techniques have been applied to almost all domestic animals as well as many wildlife and exotic species.

The MOET concept (multiple ovulation and embryo transfer) is mainly used to increase the speed of genetic change. The technique was taken up early in cattle breeding in order to circumvent the restricted number of calves a cow gives birth to. With MOET, the best cows are moved to a special herd, treated so that they ovulate many eggs, and inseminated with semen from the best bulls. The fertilized embryos are then collected and transferred to recipient cows. When the calves are born, they are raised, mated, and compared for traits like milk production. The best ones are used as parents for the next generation.

New technologies are constantly developed and existing techniques evolves, including



those for the freezing and long-term storage of valuable embryos. Such cryo-preservation can be a complement to the conservation of live animals in species and breeds that are at risk of extinction.

CLONING

Embryos from parents with very high breeding values have a high economic value. In species where only one offspring is born at a time, such as cows and horses, valuable embryos can be split to get two or even four new embryos.

Somatic cell nuclear transfer is a cloning process where genetic material is transferred within a generation, which is in contrast to normal reproduction where genes are transferred from one generation to the next. With this technique, an animal that is a genetic copy of another currently or previously existing animal is created. The sheep Dolly is the classic example. In practice, the nucleus (and its DNA) of a somatic cell (a non-germ cell) is transferred from a donor to an "empty" egg, that is, an egg from which the nucleus, and thus its genetic material, has been removed. For example, when Dolly the sheep was created the DNA was taken from an udder cell. The reconstructed egg containing the DNA from the donor animal must be treated with chemicals or electric current to stimulate cell division. Once the cloned embryo reaches a suitable developmental stage, it is transferred to the uterus of a recipient female where it continues to develop until birth.

Some famous competition horses have been cloned by somatic cell nuclear transfer. In this way, even genes from castrated horses can be propagated. It should, however, be remembered that the phenotype, in this case the success or failure of a jumping horse, is the result not only of the genes but how the horse is raised and trained (see page 19 about genes and environment). Thus, the buyer of a cloned horse might be disappointed with the new animal no matter how successful the donor was.

Animal cloning is also used both in the research on and application of therapeutic cloning. The goal is to create stem cells that can be used to study human development and to

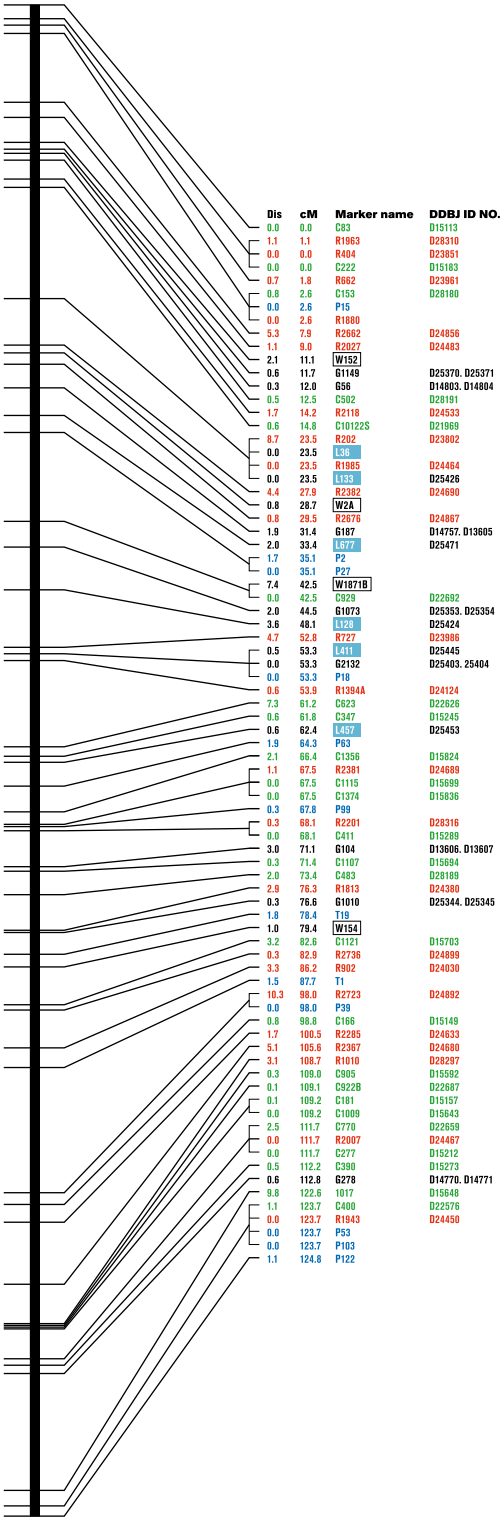
treat serious human diseases like heart disease, Alzheimer's disease, and cancer at the cell or tissue level.

MOLECULAR SELECTION

Most traits that are important in animal production seem to have a quantitative genetic background in which many genes, each with a small effect, influence the final result. Some traits, however, are governed by single genes. For example, in pigs the low ability to handle stress (Porcine stress syndrome) is caused by a mutation in a single gene. A similar example is a recently discovered mutation in horses that influences movements and, therefore, the horse's potential success as a trotter. If a gene with a large effect on an important trait is identified, individuals can be selected based on molecular analysis of their DNA. Even when the gene coding for the relevant characteristic is not known, DNA analysis is helpful if there is knowledge about gene markers – DNA sequences at known locations that are located closely to the genes of interest on the chromosome. The genetic material for such analysis can be provided by biological samples such as blood, hair follicles, or anything else with cells containing DNA. In 2005, the chicken was the first farm animal to have its full genome mapped, that is, its entire DNA sequence was described. Full mapping does not mean that the functions of all genes are known, but the map can be used to identify individuals with desired characteristics (see page 30-31). The amount of data in a genome is very large. For example, there are approximately 6 billion base pairs in the human genome, and these are stored on high speed computers with large storage capacities. The full sequence of a cow genome was completed after six years of work by more than 300 scientists in 25 countries. It was found that out of 22,000 genes in the cow genome, 14,000 were in common with all mammalian species, including humans. The list of species that have had their genomes fully sequenced is long.

In 2005 the entire genome of the chicken was sequenced. It was the first time a complete mapping of the DNA in an animal was made.

A genetic linkage map of rice chromosome number eight. Linkage maps show the positions of genes and genetic markers on a chromosome. The order and distance between the genes and markers on the map are based on their recombination frequency rather than their actual physical distance. If two genes or markers have a high recombination frequency (i.e., they segregate often), they are assumed to be far apart.
(With permission of the Nature Publishing Group)



Dis	cM	Marker name	DDBJ ID NO.
0.0	0.0	C83	D15113
1.1	1.1	R1963	D28310
0.0	0.0	R404	D23851
0.0	0.0	C222	D15183
0.7	1.8	R662	D23961
0.8	2.6	C153	D28180
0.0	2.6	P15	
0.0	2.6	R1880	
5.3	7.9	R2662	D24856
1.1	9.0	R2027	D24483
2.1	11.1	W152	
0.6	11.7	G1149	D25370. D25371
0.3	12.0	G56	D14803. D14804
0.5	12.5	C502	D28191
1.7	14.2	R2118	D24533
0.6	14.8	C10122S	D21969
8.7	23.5	R202	D23802
0.0	23.5	L36	
0.0	23.5	R1985	D24464
0.0	23.5	L133	D25426
4.4	27.9	R2382	D24690
0.8	28.7	W2A	
0.8	29.5	R2676	D24867
1.9	31.4	G187	D14757. D13605
2.0	33.4	L677	D25471
1.7	35.1	P2	
0.0	35.1	P27	
7.4	42.5	W1871B	
0.0	42.5	C829	D22692
2.0	44.5	G1073	D25353. D25354
3.6	48.1	L23	D25424
4.7	52.8	R727	D23986
0.5	53.3	L411	D25445
0.0	53.3	G2132	D25403. 25404
0.0	53.3	P18	
0.6	53.9	R1394A	D24124
7.3	61.2	C623	D22626
0.6	61.8	C347	D15245
0.6	62.4	L457	D25453
1.9	64.3	P63	
2.1	66.4	C1356	D15824
1.1	67.5	R2381	D24689
0.0	67.5	C1115	D15699
0.0	67.5	C1374	D15836
0.3	67.8	P99	
0.3	68.1	R2201	D28316
0.0	68.1	C411	D15289
3.0	71.1	G104	D13606. D13607
0.3	71.4	C1107	D15694
2.0	73.4	C483	D28189
2.9	76.3	R1913	D24380
0.3	76.6	G1010	D25344. D25345
1.8	78.4	T19	
1.0	79.4	W154	
3.2	82.6	C1121	D15703
0.3	82.9	R2736	D24899
3.3	86.2	R902	D24030
1.5	87.7	T1	
10.3	98.0	R2723	D24892
0.0	98.0	P39	
0.8	98.8	C166	D15149
1.7	100.5	R2285	D24633
5.1	105.6	R2367	D24680
3.1	108.7	R1010	D28297
0.3	109.0	C905	D15592
0.1	109.1	C822B	D22687
0.1	109.2	C181	D15157
0.0	109.2	C1009	D15643
2.5	111.7	C770	D22659
0.0	111.7	R2007	D24487
0.0	111.7	C277	D15212
0.5	112.2	C390	D15273
0.6	112.8	G278	D14770. D14771
9.8	122.6	1017	D15648
1.1	123.7	C400	D22576
0.0	123.7	R1943	D24450
0.0	123.7	P53	
0.0	123.7	P103	
1.1	124.8	P122	

QUANTITATIVE TRAIT LOCI

"Quantitative trait loci" (QTLs) are regions of the DNA sequence that are located close to genes that have a significant effect on a quantitative trait, for example, maternal behaviour or growth rate. Although many genes govern such traits, some of the genes might be more important than others. There are often several QTLs for a particular trait, and they can even be located on different chromosomes. Knowing the number of QTLs that have an influence on a trait, and the significance of each of these QTLs, provides information about the genetic architecture of that trait. It must, however, be remembered that the QTLs only give the approximate locations of interesting genes; they explain nothing about how traits are governed or the physiological background of the traits.

Finding a QTL is often the first step in locating one of the genes that is influencing a trait. The QTL points to a region of DNA on a chromosome, and this region can then be fully sequenced. This DNA sequence can be listed in a database and compared to other genes whose function is already known. Comparisons between species are very useful for this work because large parts of the genome have been conserved during evolution.

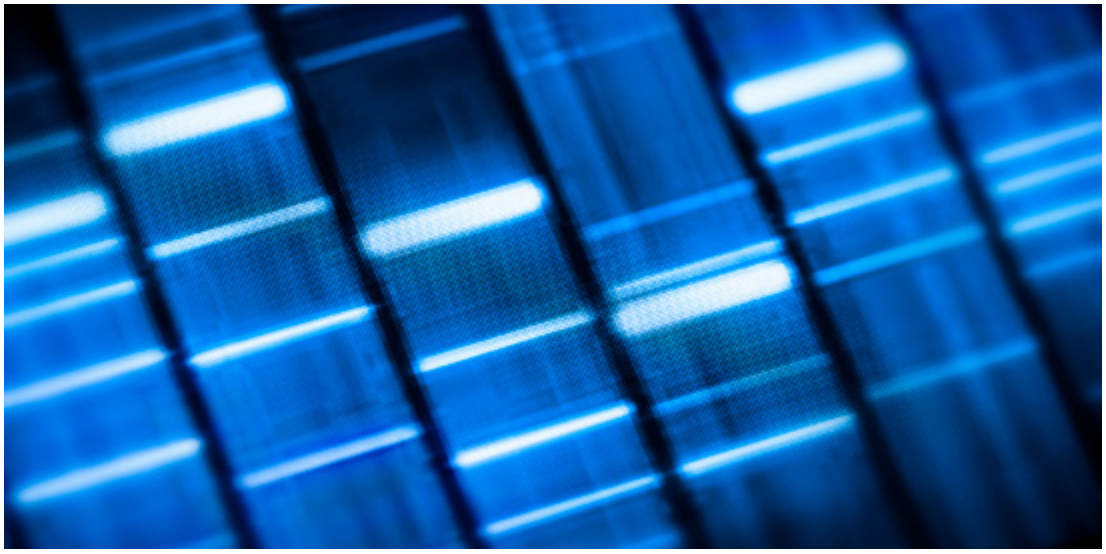
MARKER-ASSISTED SELECTION

Some parts of the DNA sequence show a large variation between individuals. Individuals with different values in a trait often have specific differences in their DNA sequences that co-vary with the differences in the trait. If a certain DNA sequence is found to be related to an important characteristic (such as an increased risk of a disease), individuals carrying that sequence can be culled. Such identified DNA sequences are called markers, and this selection method is called marker-assisted selection. The idea behind marker-assisted selection is that the marker can be used to identify individuals carrying a favourable allele (or to cull those carrying an unfavourable allele) even if the actual gene or genes coding for the characteristic itself have not necessarily been identified.

Marker-assisted selection can be used in breeding programmes as a complement to genetic evaluation. Due to the crossover between chromosomes when germ cells are created, markers that are useful in breeding for one breed are not necessarily useful in another breed. If a marker is located very close to the (unknown) gene of interest, however, it is unlikely that there will be a crossover between the marker and the gene. Thus the accuracy of marker-assisted selection is better the more markers (and fewer gaps between markers) there are.

GENOMIC SELECTION

Genomic selection makes use of genetic markers covering the entire genome. The markers used here are "single nucleotide polymorphisms" (SNPs) each consisting of single points in the DNA sequence where the nucleotide (A, T, C, or G) is highly variable between individuals. Genomic selection is a two-step process. First, the association of genetic markers with the trait of interest is established in animals along with phenotypic information, and a genomic breeding value is estimated for these animals. Such a group of animals with both desired phenotypes and genomic breeding values are called the training population or reference population. In the second step, genotypes of animals from the next generation, the selection candidates, are compared to those from the training population. This will then allow for an estimation of their genomic breeding value and will thereafter allow for selection of young animals for breeding based solely on their SNP marker information. If genomic selection can be successfully applied in a breeding programme, it will allow an early selection of the best breeding animals and im-



By gel electrophoresis DNA molcules can be separated depending on their size. You can then cut out the "band" of interest and sequence the DNA.

prove genetic progress due to the reduced time required for the selection process. Genomic selection has been highly successful in dairy cattle breeding populations, especially in the breeding of Holstein Friesian cattle. This approach allows for the selection of bulls for breeding as soon as their genomic information can be extracted. When only phenotype based methods are used the breeder has to wait with the selection until year 6, when data (like milk yield) would become available from the young bull's daughters.

For information about proteomics and metabolomics, see page 39 in the chapter about plant breeding.

GENETIC MODIFICATION IN ANIMALS

Genetic modification can be applied in animals. As in plants, the integration of DNA fragments via vectors and/or mechanisms based on the self-repair mechanisms of the DNA strand, have been used also in animals. The first transformation method developed for animals was based on microinjection of DNA into the nucleus of a newly fertilised egg. The egg cells that survived the process were then transplanted into the uteruses of recipient females. This technique was used to produce the first transgenic livestock in 1985, and since then several genetically modified (GM) animals have been bred. The microinjection technology is, however, rather inefficient and often leads to undesirable side effects caused by the random integration of new genes.

The list of alternative techniques is long. One method is sperm-mediated DNA transfer that makes use of the ability of spermatozoa to bind and take up DNA before fertilizing the egg. A promising tool is to use viruses as vectors for DNA injection into eggs. Some types of viruses – called retroviruses – have the ability to integrate their genomes into the genomes of other species. Humans, for example, have many such DNA elements in their genome that have been incorporated during evolution and have seldom negative effects. By transferring vectors derived from retroviruses into young embryos, DNA coding for specific proteins can be transmitted to animals. These founder animals are then used as parents of a population of GM animals with new traits, for example, animals that produce hormones for medical treatment in humans.

Brassica oleracea comes in many shapes and colours, for example cabbage, broccoli, cauliflower, and brussels sprouts.

Plant breeding

Just as with livestock, plants are bred to be resistant or tolerant to diseases, insect pests, or other organisms that damage the plants. Crops are also bred to allocate their resources to plant parts that give us a high yield of for example seeds and fruits. To make the most use of these crops, especially grains, the crops are bred to mature in time and to grow in such way that they are easy to harvest, and to resist pests and disease during storage.

With the availability of more efficient machinery and herbicides, there has been a reduced need to develop crops that can compete with weeds. Furthermore, crops with herbicide tolerance have been developed so that weeds can be controlled without harming the crop. Although efforts have been made to breed for resistance against pathogens, insect pests, and the diseases that they might spread, the use of fungicides and insecticides have offered an easy and quick solution in many cases. Also, cheap fertilizers and a lack of knowledge about the consequences of nutrient leaching have not encouraged breeding for more efficient nutrient uptake. However, modern environmental requirements for reduced use of fertilizers, herbicides, and pesticides have led to shifts in breeding goals.

Breeding of annual crops is not necessarily quicker than animal breeding, despite the shorter generation interval, because of the numerous breeding cycles that are required to get a crop

With molecular knowledge you can select the best individuals at an early stage and this will likely shorten the breeding process for trees.

of high quality. Breeding of trees involves even longer generation intervals than for animals like cattle and horses. There are several ways to improve plants depending on their mode of reproduction, breeding goals, and financial constraints.

PLANT BREEDING SYSTEMS

Plant species can be roughly divided into the following three groups based on their mode of reproduction: self-pollinators, cross-pollinators, and vegetative propagators. The majority of annual crops of agronomic importance are propagated by seeds and are self-pollinators. These are partially or fully self-fertilizing plants that can be easily used to create "pure lines" that are homozygotes carrying the same alleles for a gene on both chromosomes. Almost all cross-pollinators are biennial or perennial species that are not adapted to homozygosity to the same extent as self-pollinating species, which results in lost vigour if inbred. However, even strict cross-pollinators can be self-fertilized by various techniques. Many plants can also be propagated (multiplied) by tubers (like potatoes) or cuttings (like willows), which simplifies the breeding of these species. A large number of offspring and less time required for management enables plant breeders to work with larger populations compared to animal breeders.

MASS SELECTION

Mass selection is the oldest form of plant breeding and has been used by humans for millennia since we began collecting seeds to be sown. This method still finds use in certain species, especially in cross-pollinating plants. With this method, one collects the seeds from selected individuals in a population and the next generation is sown with the mixed seeds. An alternative method has been to remove all plants with undesired traits in the field prior to seed collection. Many old and traditional plant varieties have been improved this way, and the varieties have been passed down from one generation of farmers to the next.

PURE-LINE SELECTION

Pure-line selection is usually only practiced in self-pollinating plants, but it can sometimes be applied after crossing in cross-pollinating plants. With this method, one selects numerous superior

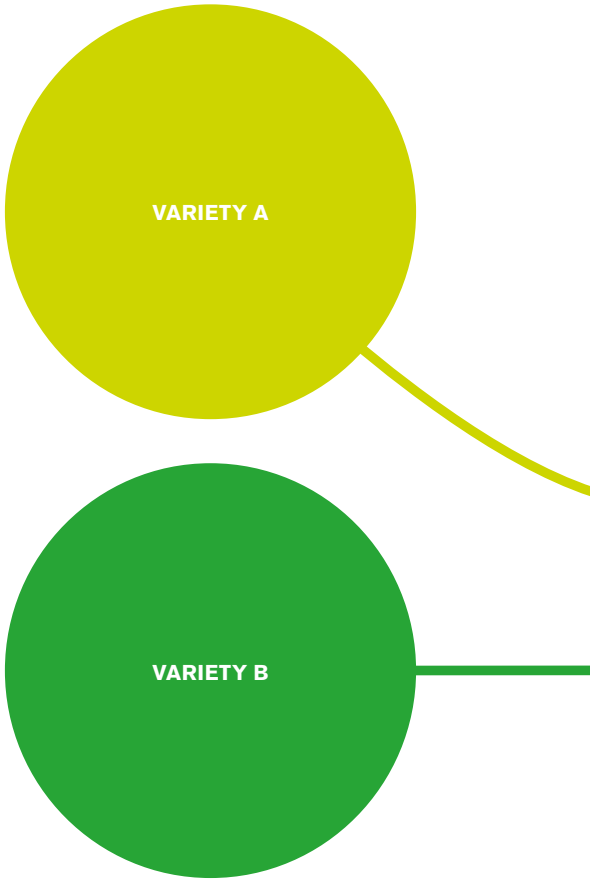
plants whose offspring are monitored separately, often for several generations. Promising lines are then further evaluated and the exceptionally good ones are released as new varieties. The early success with this method depended on the high genetic variability found in many of the landraces. For pure-line selection to be effective, one needs a population with high genetic variability which makes this method less important in the development of the major crops today. However, the method is still used in breeding less heavily selected species.

HYBRIDIZATION

This breeding method normally starts with the crossing of two lines with desirable alleles in order to produce progeny that are superior to the parents. Depending on how different the genetic makeup is between the two parents, billions of different genotypes are possible in the second generation (the first generation will all have the same genetic makeup consisting of half from each homozygotic parent). Depending on the reproduction system, among other things, the hybridization is followed by different selection schemes.

PEDIGREE BREEDING

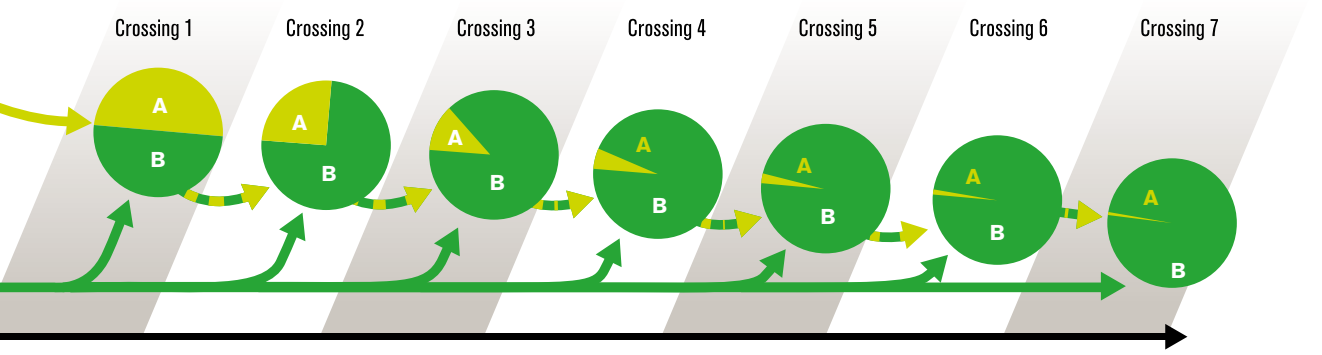
Pedigree breeding involves crossing two genotypes, each carrying one or more desirable traits that are lacking in the other. If the two parents do not provide all of the desired traits, a third parent can be included by crossing it with one of the progeny from the first generation (F1). Superior individuals are selected over several generations. The first selection in pedigree programmes is often made in the second generation (F2), which shows large variation because they are heterozygous for many genes. This step is usually focused on eliminating plants with undesirable alleles that have a clear effect on the trait such as low resistance against a specific disease. Self-pollinated plants enable pure-line selection until almost total homozygosity is achieved, usually in the fifth



generation (F5). At this stage, seeds from the selected lines are harvested in bulk to produce seeds for field trials, and at about the seventh or eighth generation the focus is on a more precise evaluation of plant quality and performance.

BULK POPULATION BREEDING

This method differs from the pedigree method primarily in the way the hybrid offspring are handled. In this method, the F2 generation is sown in a large plot and seeds are harvested all together. These are then sown in a new plot without keeping track of their ancestry. Plants with low survival rate are eliminated by natural selection, and plants with other undesirable traits are often removed as well. Sometimes seeds are harvested at an early stage to select for early maturing plants. These steps are followed by single



Backcrossing is used to introduce a specific trait into a plant line without ending up with other unfavourable characters. After the initial crossing, the best offspring are crossed with the original plant line (B) until a hybrid is produced with all of the desired traits.

plant selection and evaluation in the same way as in the pedigree method. The advantage of the bulk population method is that one can screen a very large number of individuals at low cost.

BACKCROSSING

Breeding commonly starts with a good variety that just lacks one specific trait, such as resistance to a specific pathogen. One way of introducing this trait is to use backcrossing. To start with, one needs to find a plant that carries the desired trait and that can be crossed with the variety that is lacking the trait. The chances for backcrossing to work are higher if the trait is coded for by just one or a few genes. After the first crossing and propagation, plants with the desired trait are selected and these are crossed again with a plant of the original good variety. This is usually repeated five or six times to produce a hybrid with all of the original traits and now also including the new desired trait. The advantages of this method are the small number of plants needed in each generation and that it is fast and predictable. The disadvantage of this method is that the desired genes might be tightly linked to less desired ones, and this lowers the probability of separating them no matter how many backcrosses are made.

HYBRID VARIETIES

Hybrid varieties are not the same thing as varieties produced by hybridization, and this is often confusing. In the process of producing hybrid varieties, selected plants are first inbred for several generations to create individually purebred lines. These purebred lines are then crossed. A cross between two specific homozygotes always results in the

Hybrid varieties – the maize example

The development of hybrid maize has had a huge impact on increasing its yield and is a prime example of the strong effects of hybrid vigour. Maize is pollinated by the wind that blows pollen from the tassels to the styles, and controlled crosses can, therefore, easily be made at the field scale by planting one row with parent plants providing the pollen and two or three rows of the seed parents from which the tassels are removed before they shed their pollen. To avoid the problem of low-producing inbred lines, most hybrid maize is produced by first crossing four inbred lines in pairs (A × B and C × D) and then crossing their offspring (AB × CD). In this way seed production becomes more efficient, which lowers the seed price. Instead of removing the stamens (the tassels at the top) by hand, one can use male-sterile plants that are unable to produce functional pollen.

Hybrid (left) compared to non-hybrid (right) maize.

same genetic makeup, which means that once the purebred lines that give the best hybrid have been identified the new variety can be continuously produced. Another advantage with hybrids is hybrid vigour, or heterosis, which can result in increased growth rate, earlier flowering, and increased yield. This is due to the fact that many disadvantageous characteristics are coded for by recessive alleles, and the high heterozygosity in the F1 generation decreases the probability of getting two unfavourable alleles for the same gene. However, if the seeds are re-sown, the next generation (F2) will consist of very diverse plants with average yields far below the F1 generation. This means that seed from hybrid varieties is poor as planting stock and farmers must buy new hybrid seed each year.

SYNTHETIC VARIETIES

A synthetic variety works like mass selection with the exception that all crosses are made between plant lines known to give superior offspring regardless of how they are combined. They give hybrid vigour and usable seeds for coming seasons. Many synthetic varieties are forage crops for which the production of hybrid varieties would be too costly.

SOMATIC HYBRIDIZATION

In somatic hybridization, or protoplast fusion, cells that are not germ cells are fused. First the cell walls are removed and the remaining protoplasts are then fused through electric shock or chemical treatment. Then the cell is treated

In Sweden, all sugar beet varieties produced in breeding programmes are hybrids.

with hormones so the cell wall grows back and the cell multiplies to a callus, plantlets and eventually into a full plant. This method makes it possible to overcome crossing barriers but is also used to combine varieties of the same species, for example non-flowering and flowering potato. If the plants cannot exchange genetic material through traditional breeding methods the resulting somatic hybrid is regulated as a GMO.

MUTATION BREEDING

Mutations are changes in the nucleotide sequence of an organism caused by errors in the replication process, radiation, or chemicals. Although mutations occur at a very low frequency in nature, they create sufficient genetic variation to drive evolution. Traits might change or disappear or new traits might be introduced. One way to increase genetic variation is to speed up the mutation rate. Chemical mutagenesis involves treating the seeds with a toxic chemical agent, for example, ethyl methanesulfonate (EMS) or dimethyl sulphate. Depending on the chemical, the changes in the DNA can be more or less specific. For example, EMS commonly leads to a change from a G-C base pair to an A-T base pair. Radiation can break chromosomes and produce a wide variety of altered nucleotides. The common type of radiation used are X-rays and gamma rays. Rapeseed, barley, cotton, and rice are examples of crops in which mutation breeding has been used.

One problem with mutation breeding is that mutations happen randomly. This makes the selection of the desired phenotypes difficult, time consuming, and expensive. Thousands of plants might be needed before a viable individual with the desired genetic changes is found. Another disadvantage with this method is that most of the mutations are undesired. Important genes can be mutated, and this requires additional breeding, for example by backcrossing, to restore the plant line to its original quality.

CHROMOSOME DOUBLING

As described earlier, many plants have more than two sets of chromosomes, that is, they are polyploids. Polyploids usually have more biomass or larger fruits and seeds than diploids, and this is often desirable. Potatoes and bananas are examples of autopolyploids (all of their chromosomes originate from the same species). Allopolyploids carries a combination of chromosomes from different species. For example, rapeseed is an allopolyploid from the crossing of a cabbage and a turnip.

If a diploid is crossed with a tetraploid, the

offspring will be triploid (one chromosome set from one parent plus two sets from the other). Triploids have to be propagated vegetatively because they are sexually sterile. Many banana varieties and seedless watermelons are triploids. Polyploids occur naturally but can also be created by the use of a chemical called colchicine that prevents the chromosomes from separating during the cell division process. Colchicine has been used to create autopolyploids and seedless triploids as well as to restore fertility in triploids like Triticale (wheat crossed with rye) by making it hexaploid (six sets of chromosomes).

PLANT TISSUE CULTURE

Plant tissue culture is a collective name for various laboratory techniques used for culturing parts of plants under controlled sterile conditions using either cells, tissues (pieces of leaves, flowers, or roots), anthers, microspores or meristems (with undifferentiated cells) from the plant.

Plant tissue cultures are used for vegetative mass production of plantlets in many species, especially woody horticultural species that are difficult to propagate by grafting. Plant tissue culture is a very useful tool for long-term preservation of genetic material from endangered species, and it also has important applications in plant breeding. For example, completely homozygous lines can be created by preventing the chromosomes from separating in the first cell division in immature pollen (which in a diploid only carries one set of chromosomes). The resulting plants are referred to as "double haploids". In addition, tissue culture is an important method used in genetic modification of plants.

The theoretical basis of tissue culturing is that every intact cell has the potential to grow and develop into a complete plant under optimal growing conditions, that is, the cells are totipotent. Plant tissue culture is also called *in vitro* culture ("in glass" in *Latin*) because the plants are often grown on a solid medium in a small glass jar. The growth medium normally consists of nutrients including sugars, salts, and vitamins that are necessary for the cultures to grow, as well as plant hormones that regulate growth and development. The medium is usually jellified with agar (a polysaccharide/pectin mixture from red algae) mainly to avoid abnormal growth by preventing the cultures from taking up too much water. However, there are also liquid cultures where plant cells or tissues are grown in a nutritional liquid medium in a specially designed container called a bioreactor. Bioreactors can be used for cultivating plant cells or tissues for extraction of important compounds with medical value.

There are several advantages with this technique, including disease-free (especially virus-free) plant material, mass production of high-quality plants within a short time in a limited area, year-round production, and no need for pesticides.

MOLECULAR SELECTION

If one has knowledge about which alleles (variants of a gene) result in a specific phenotype, which genes affect a trait, or just which regions of a plant's DNA are associated with a trait, the best individuals can be chosen without having to wait for the plant to fully develop, flower, set seed, etc. This saves both time and resources. The following molecular methods can be used as tools for selection in plant breeding.

QUANTITATIVE TRAIT LOCI

As described in the section about animal breeding (page 30), "quantitative trait loci" (QTLs) are regions of the DNA that have a significant effect on a quantitative trait, for example height. A single trait is often influenced by several QTLs that can be located on different chromosomes. QTL analysis has been an effective tool in allowing for the identification of genes that govern traits such as grain productivity, plant height, or resistance against pathogens.

MARKER-ASSISTED SELECTION

The majority of the selection markers used in plant breeding today are based on DNA, but such markers can also be morphological or biochemical markers. As described in the section on animals (page 30), the theory behind this method is that one can use a marker to select for, or against, a gene that is associated with a specific trait. To find DNA markers, one must compare individuals with a high degree of variability in the trait of interest. A good marker is so closely located to the gene of interest that the probability is very low that the marker and the gene will segregate during meiosis.

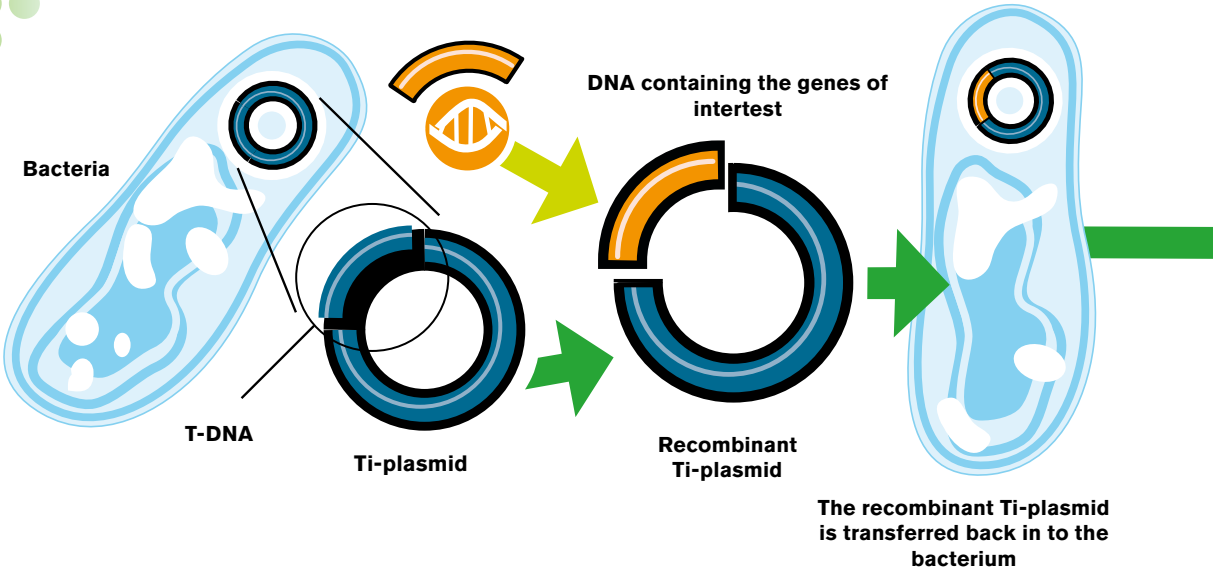
GENOMIC SELECTION

As described previously, genomic selection is an important tool in animal breeding, but genomic information is also used in plants, and this allows for an assessment to be made for thousands of genetic markers across the genome. However, the progress in acquiring knowledge of the entire genome has been slow in most species. One of the main reasons for this is that many crops are polyploids, that is, they have more than two sets of chromosomes and, therefore, more than two alleles per locus (see page 15). Additionally, many plants have complex genomes, including repetitive sequences and pseudo-genes (genes without function). Plant genomes are therefore often much larger compared to the genomes of animals, and thus, acquiring sequence information of a full plant genome is more difficult.

Due to decreased costs, the genomes of many crops like rice, maize, potato, and bread wheat have now been sequenced and techniques for genomic selection in plant breeding are being developed. The procedure, in principal, is the same as in livestock, but the genomic complexity in terms of polyploidy and the modes of reproduction differ greatly among plant species. Trees are examples of plants where breeding will benefit hugely from genomic selection because their reproductive period and time until harvest are very long. The ability to predict future wood or fruit quantity and quality at an early stage of development will, therefore, be very beneficial. Samples can be collected from trees at an early stage and their genotypic data can be compared to older trees with known phenotypic measures. Environmental effects play a major role in both crop and tree breeding, and methods are being developed to allow genomic selection to take such effects into account.

PROTEOMICS AND METABOLOMICS

Proteins make up the machinery of the cells, and they mediate signalling and chemical events by catalysing a vast array of chemical reactions. Measuring the levels of specific proteins can be used to predict the features that will occur in different crosses in breeding programmes and can be used as an alternative or complement to the use of genomic markers. One way to study the different proteins in a sample is to first digest them with a specific enzyme to obtain peptides (small proteins). The peptides in the mixture are then separated based on their hydrophobicity (how little attraction to water they have), and the levels of specific peptides are measured. Highly reproducible measurements can be achieved with targeted mass spectrometry techniques, such as Selected Reaction Monitoring that allows hundreds of peptides to be measured in large sample cohorts. Metabolites are small molecules such as various types of carbohydrates and amino acids in the cells. Those are usually analysed using mass spectrometry in a similar way as the proteins. The metabolites are often identified by comparing their spectra with known metabolite mass spectral fingerprint. There are still many technical challenges to be overcome before complete proteomic and metabolomic measurements can be made, but the use of these techniques in breeding is promising.



The most common method for the genetic transformation of plants is to make use of Agrobacterium tumefaciens' ability to insert DNA. The bacterium has a plasmid that carries tumor-inducing (Ti) genes that, together with other genes, are inserted into the DNA of the infected plant. Those other genes can be deleted and replaced by one or several genes chosen by the breeders.

GENETIC ENGINEERING

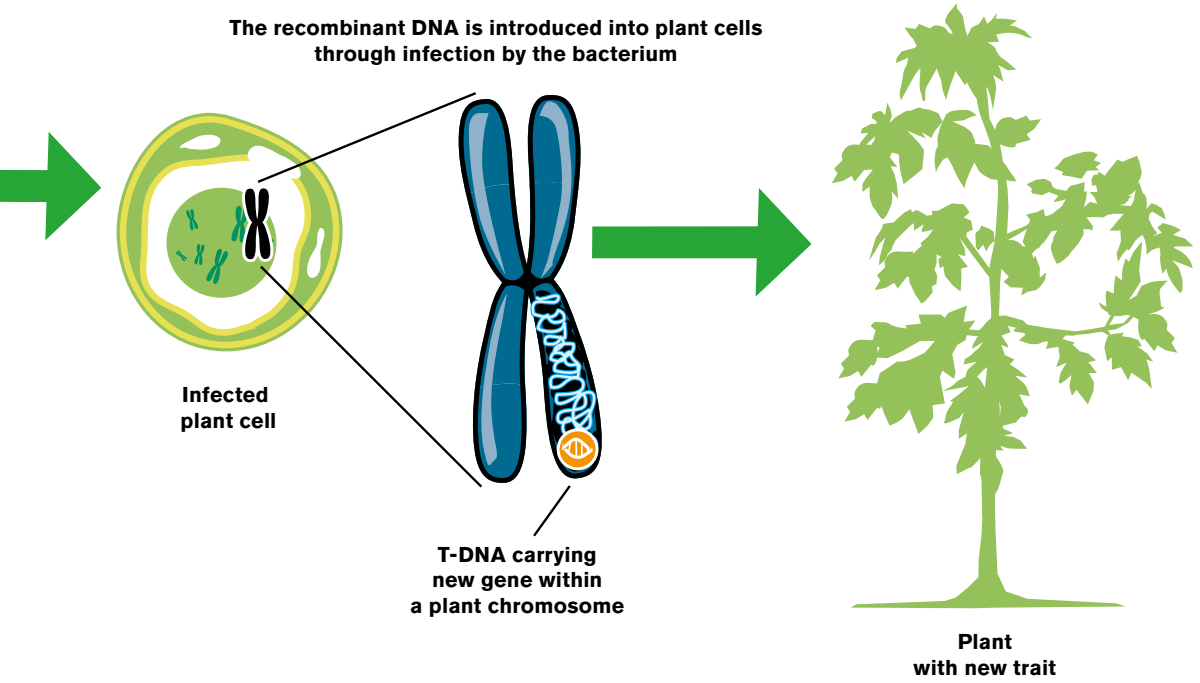
This section describes modifications of plant genes using molecular approaches. This includes breeding methods in which the expression of a target gene is altered or a foreign gene is introduced into the genome of a target crop for developing a desirable trait. Depending on which technology is used, the product obtained may or may not be defined as a genetically modified organism (GMO). The technologies that are in use in plant breeding today are explained along with some of the new methods that are expected to have broad applications in crop development in the future. For definition of GMO, see page 55.

GENETIC TRANSFORMATION

Even though one might change a trait or introduce a new one using the classical techniques described previously, the desired results can be difficult, and in some cases impossible, to obtain. These are cases where genetic transformation can prove useful. This technique is particularly advantageous for improving existing varieties that have just one or a few flaws or undesirable traits.

Genetic transformation involves the direct introduction of a piece of DNA or a whole gene into an organism's genome in order to express a foreign gene or to modify the expression of the organism's own genes. The crops modified using this technique are called genetically modified (GM) crops. Because the functions of the target genes to be modified are usually well characterized, the genetic transformation approach is more precise and straightforward compared to conventional breeding by crossing or mutation. These techniques also eliminate the disadvantage of traditional crossing methods in which several genes are added along with the gene of interest.

Genetic transformation in plants is normally carried out with the help of the soil bacterium *Agrobacterium tumefaciens*. In nature, the bacterium can infect wounded plants and cause tumour (also called crown gall) formation. Most bacteria have their DNA in the form of a main circular chromosome and several smaller circles of DNA called plasmids. *A. tumefaciens* has a tumour-inducing (Ti) plasmid that contains a



piece of DNA called T-DNA (transfer-DNA). T-DNA can be transferred into the plant cells and incorporated into the genome of the infected plant. The T-DNA carries the genes that stimulate the cell division without differentiation that leads to tumour formation.

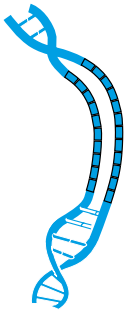
The part of the plasmid responsible for DNA transfer from the plasmid into the genome consists of only about 25 base pairs at the beginning and end of the DNA sequence to be transferred. The sequences in between these two bordering sequences can be replaced with any other DNA sequence without affecting the DNA transfer. The discovery of this natural gene transfer that works across species barriers has provided a powerful tool for the genetic improvement of plant properties.

Restriction enzymes, which function like scissors, can cut DNA into pieces, and ligases, which work like glue, allow the cut pieces of DNA to be put back together. These enzymes are used to remove the genes causing tumours from the T-DNA in the isolated plasmid and to replace them with the DNA sequence of interest. The modified Ti-plasmid, now called a recombinant plasmid or a transformation vector, is transferred back into *A. tumefaciens*. The bacterium is then propagated and the plant is infected. This method can be further divided into either tissue culture-based transformation (TCBT) or in planta transformation. For TCBT, a piece of plant tissue or organ (called an explant) is cultivated *in vitro* and the target gene is introduced into the explant by *A. tumefaciens*. A new and genetically modified plant carrying the target gene can then be grown from the explant. For *in planta* transformation, open flowers on a living plant are infected with *A. tumefaciens*. The infected plant will then produce seeds that can be harvested and sown. The individual plants that grow from these seeds will carry the new gene or genes into subsequent generations. This method tends to work very poorly for the majority of plant species and is mainly used in model plant species such as thale cress (*Arabidopsis thaliana*).

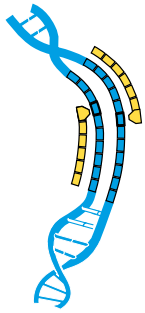
A. tumefaciens transformation has been used for genetic modification in many plants, especially

SDN 1

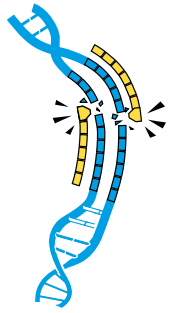
1. DNA to be edited.



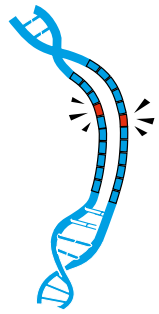
2. An introduced protein complex consisting of one binding domain (site specific) and one cutting domain (nuclease).



3. When the protein complex has found the pre-determined place in the genome, the nuclease creates a double strand break (DSB).

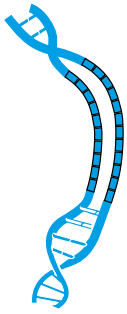


4. The cell responds to the DSB by repairing the DNA strands, often with some alterations in nucleotide sequence, thereby creating a random mutation at a specific site.

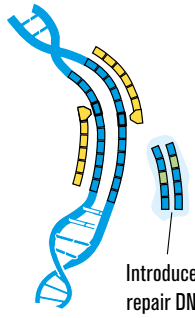


SDN 2

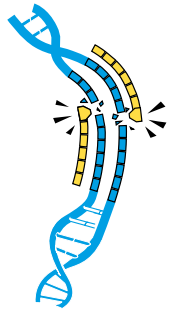
1. DNA to be edited.



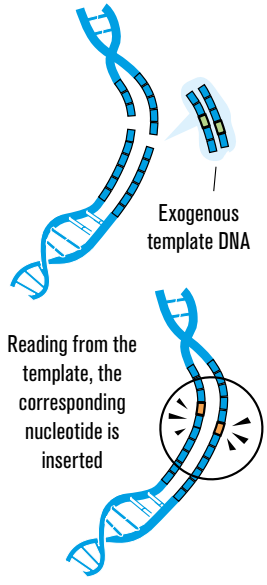
2. As in SDN1 an introduced protein complex consisting of one binding domain and one cutting domain binds to a specific site. But in this case a short DNA strand is also added. This template is homologous to the target area, with the exception of the specific base alterations to be introduced.

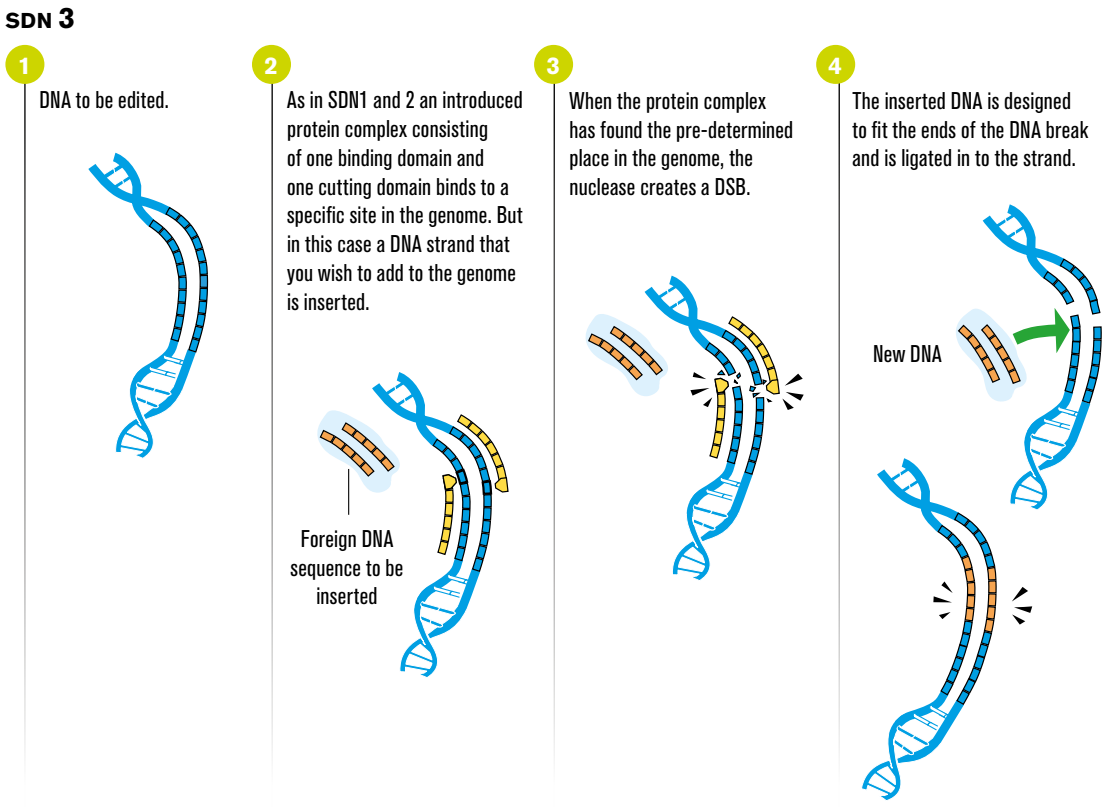


3. When the protein complex has found the pre-determined place in the genome, the nuclease creates a DSB.



4. As in SDN1 the DNA repair system responds, but in this case the introduced DNA will be used as a template for a specific nucleotide change.





Examples of geneom editing though Site Directed Nucleases (SDNs) such as CRISPR/Cas and Transcription Activator-Like Effector Nucleases (TALENs), which are based on the same principle. A cutting domain is combined with a designed binding domain that will determine where the cut will be made. In the first example the protein complex can be introduced via DNA, mRNA, or as a pre-made complex.

dicotyledonous[1] species, to improve various agronomically important traits such as disease or insect resistance. Compared to dicotyledonous plants, monocotyledonous species are in general less susceptible to *Agrobacterium* infection. To solve this problem, some alternative chemical and physical DNA transfer methods have been developed. Among these, the most commonly used is biolistics using a gene gun or particle bombardment. In this method, the target DNA is coated on the surface of gold or tungsten particles. The micro-particles are then introduced into the plant cells or tissues with a propelling force such as compressed gas (helium) or electrostatic discharge.

Note that also some variants of site-directed nucleases (SDNs), described in the next section, include genetic transformation.

SITE DIRECTED MUTAGENESIS – GENOME EDITING

Site-directed mutagenesis, or genome editing or gene editing, as the methods are also called, have been developed to overcome the problem of randomness that results from mutation breeding

1 Dicotyledons are flowering plants with two seed leaves and leaves with net veins (compare to monocotyledons that have one seed leaf and paralell leaf veins, for example cereals and maize)

as described in the previous section. These techniques allow particular sequences in a given gene to be modified in a specific manner. Site-directed mutagenesis can be achieved with different techniques including Oligonucleotide Directed Mutagenesis (ODM), Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), Homing Endonucleases (HEs), and recently, Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems. The ZFNs, TALENs, HEs, and CRISPR/Cas are collectively known as Site-Directed Nucleases (SDN).

SDN are synthetic restriction endonucleases (enzymes that cut DNA strands) consisting of a customized DNA binding domain fused to a non-specific nuclease domain. The technique enables the introduction of a double strand break in any DNA sequence, and the cell responds by repairing the break resulting in a random mutation at the target site. The ZFN technique has been used in maize and tobacco, but its efficiency of mutagenesis is low in most plant species. This introduction of a random mutation at a specific site is generally called or SDN-1 according to the specific class of nucleases.

The SDN-2 method works like SDN-1 with the difference that a repair template for the desired alteration is included. This template is used by the target cell's repair machinery to produce a DNA sequence that is modified at specific single nucleotides.

The SDN-3 method introduces genetic material at a specific site. The difference with this method compared to introducing DNA with *A. tumefaciens* or biolistic techniques is that the insertion is directed to a specific site in the genome.

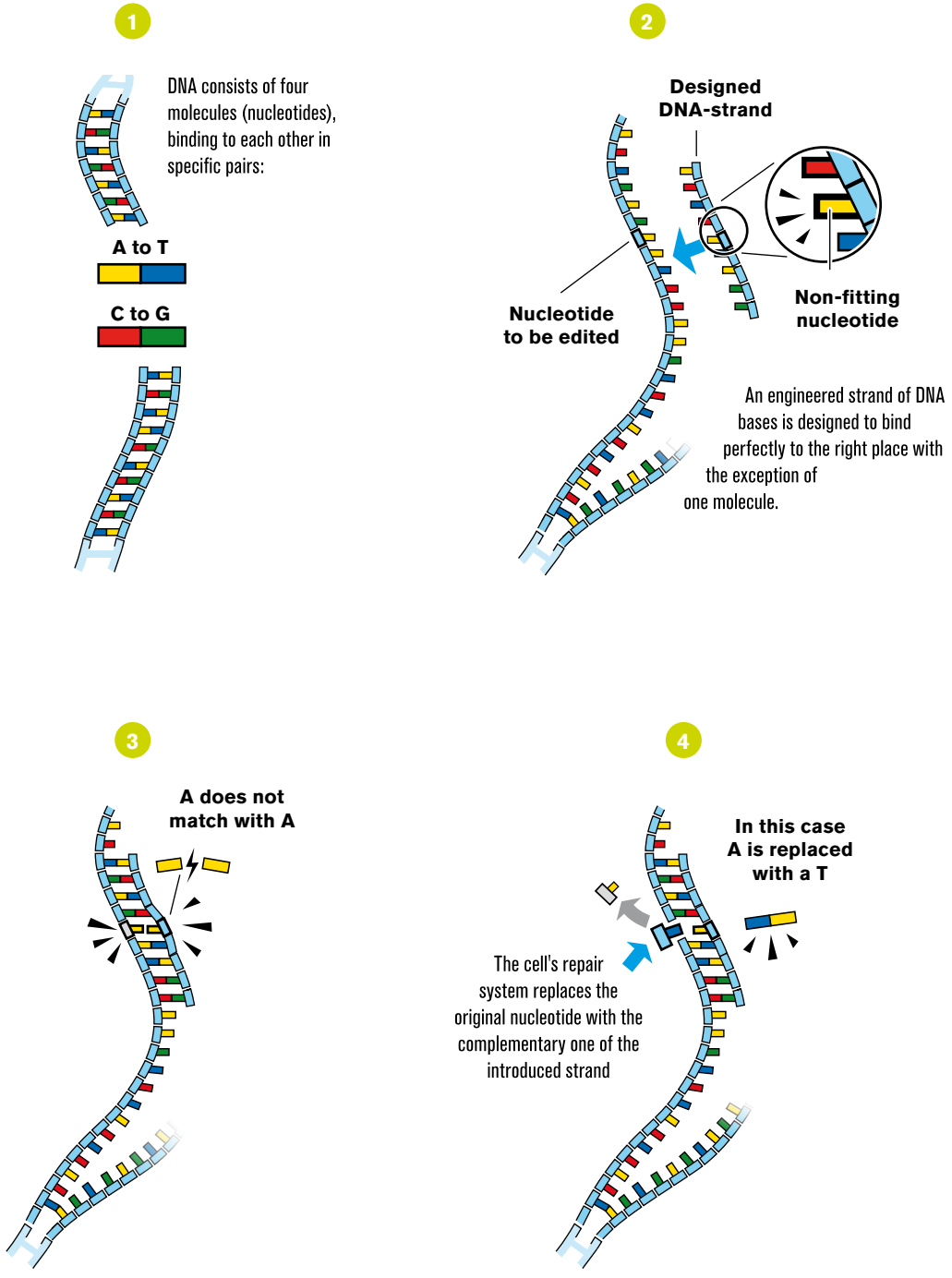
Similar to ZFNs, TALENs also have a customized DNA binding domain fused to a non-specific nuclease domain. Here the DNA binding domain consists of a longer modular structure derived from the bacterium *Xanthomonas*. The nuclease domain can cut the DNA strand at a single nucleotide and each module can be engineered to recognize DNA sequences up to 30 base pairs, which improves the targeting specificity compared to ZFNs. TALENs enable the introduction of double strand breaks into virtually any DNA sequence with high efficiency in plants. As with ZFNs, TALENs can be used either to introduce an error (to knock out a target gene) or to introduce a new DNA sequence into the target site (that is, to perform genetic transformation).

Homing endonucleases (HEs) are naturally occurring enzymes that recognize rare DNA sequences from 14 to 44 base pairs in length. This feature makes them suitable for site-directed mutagenesis. Both natural and engineered HEs have been used to introduce double strand breaks, mainly in mammals. The main limitation to the use of HEs is that the DNA binding domain is not clearly distinct from the nuclease domain, and this complicates the engineering procedure.

Similar to ZFNs and TALENs, the CRISPR/Cas systems also introduce double-strand breaks into almost any DNA sequence, but in this case specificity is achieved by pre-loading the nuclease with a small RNA molecule complementary to the target DNA.

Due to its simplicity CRISPR/Cas has represented a real breakthrough for both basic and applied research and not only in the plant field. Moreover it is constantly evolving due to the identification of novel enzymes found in microorganisms and artificial engineering approaches.

Common for the ZFNs, TALENs, HEs, and CRISPR/Cas systems are that they cause alterations at specific sites in the genome. They can be introduced into the plant cells by electroporation (a short burst of high-energy electrical discharge) or treatment with polyethylene glycol (PEG) that facilitates penetration of the molecule



In oligonucleotide directed mutagenesis (ODM), a short single strand of DNA complementary to the region to be edited, except for one nucleotide, is introduced into the cell. The cell's repair system recognizes the mismatch and replaces the nucleotide with the complementary one. The added single strand of DNA will then be degraded by the cell.

A soybean has been genome edited using transcription activator-like effector nuclease (TALEN) to produce an oil high levels of oleic acid, no trans fats, and less saturated fat.

through the cell membrane. With this method, integration of new DNA into the genome is much less frequent than in *Agrobacterium* transformation and in most of the cases only a temporary expression of SDNs is achieved. In this latter case, no new genes are left in the genome but the DNA modifications that the SDNs have introduced can be permanent. In the case where stable integration of the genes coding for SDNs occurs, it is still possible that the process of segregation can result in offspring that do not carry these new genes.

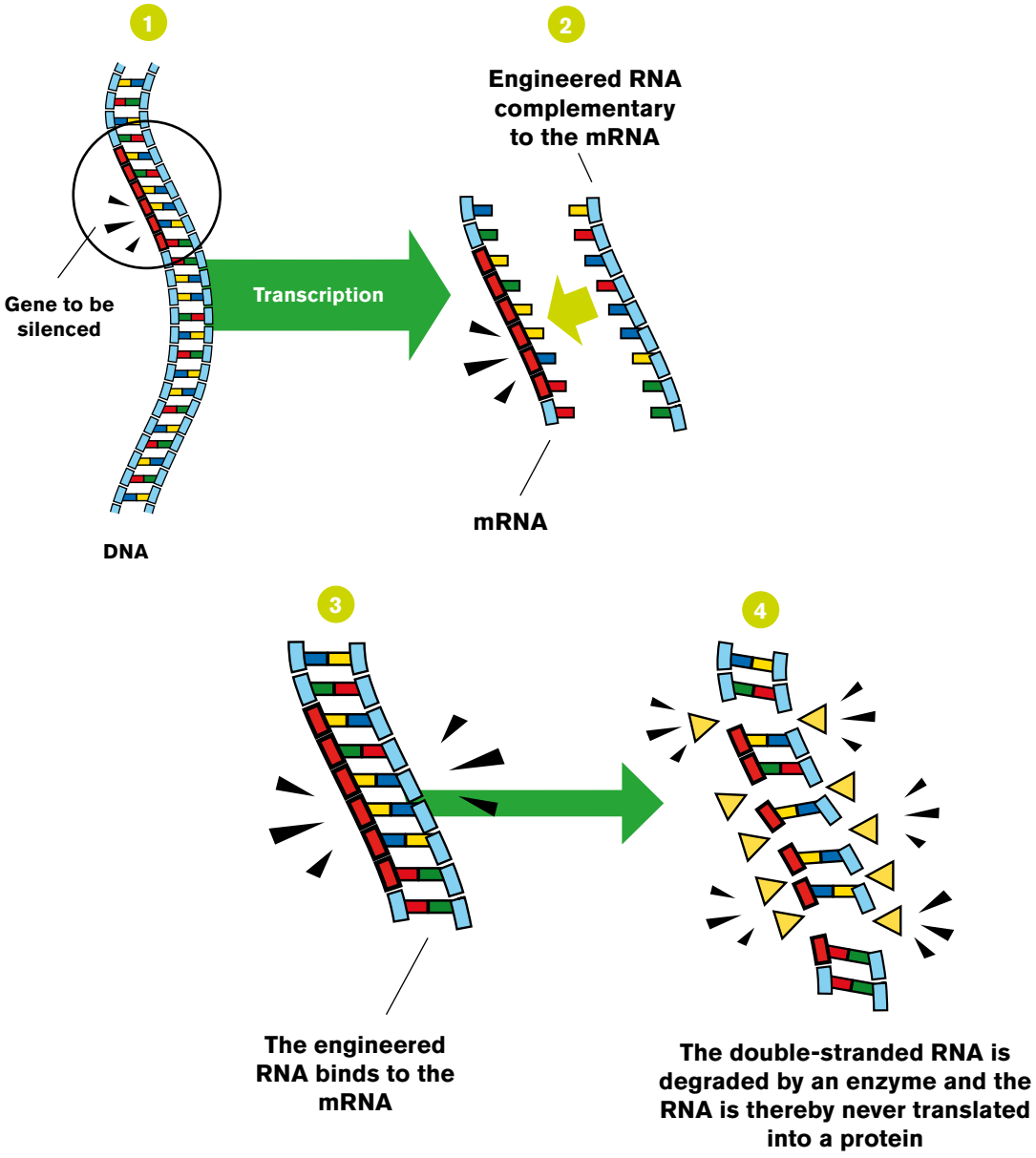
Other methods of delivery of SDNs are possible, for example, mammalian and insect embryos can be injected with mRNA encoding for SDNs. Direct delivery of SDN proteins would not include transfer of DNA, but such techniques will require further development if they are to be applied effectively in plants.

In conclusion, genome editing enables precise modifications of DNA sequences. Some countries classify genome edited products as non-GM if no inserted DNA is left, while others require that no insertion of DNA should have taken place in the process at all for the new genotypes to be classified as non-GM. In the EU all genome edited products are classified as GMO, regardless if DNA is inserted in the organism or not, according to a court decision in 2018.

The ODM technique involves targeting DNA with short sequences carrying the desired mutation, usually about 20–30 base pairs. These are introduced into the cell through the same processes of electroporation or PEG transformation used for SDN. ODM sequences are complementary to a region in the target gene and carry a desired modification but do not carry any nuclease domains. This technique is very simple but the efficiency is extremely low and other mutations can occur. The technique has been used in maize, rice, tobacco, and rapeseed to modify their herbicide tolerance traits.

MICRO-RNA AND RNA INTERFERENCE

Another expanding research area focuses on microRNA (miRNA). These are short RNA molecules that are not translated into proteins but instead regulate the levels of gene expression by interfering with the mRNAs of genes before they are translated into proteins. If a miRNA is complementary to a part of the mRNA sequence, it will pair with the mRNA resulting in a double-stranded RNA. This double-stranded RNA will be cut into small pieces by a specific RNA-cleaving enzyme (which normally functions in the cell to destroy double-stranded viral RNAs). This principle is used in genetic engineering for down-regulating the expression levels of target genes and is called RNA interference (RNAi). The miRNA is introduced through regular transformation techniques (*A. tumefaciens* or a gene gun). The method has been widely used in human disease studies and in animal and plant breeding. For instance, this technique has been used to increase the level of the beneficial plant oil oleic acid in soybeans to over 80 per cent of the total oil content.



This principle of RNA interference (RNAi) is used for down-regulating the expression levels of target genes by preventing mRNA from being translated into a protein. A gene that codes for an RNA strand complementary to the gene's mRNA is transferred into the genome. The two RNA strands pair up to form a double-stranded RNA. In plants, mRNA normally only exists as single strands, and double-stranded RNA is quickly degraded by the cellular enzymes that protect the plant against viruses.

Ethics of breeding

Breeding aims to refine plants, animals, or other organisms for particular purposes through a process of selection. This process sometimes raises ethical issues, such as when questions of animal welfare or environmental consequences are at stake or when traditional animal breeds or plant varieties with cultural significance are no longer available. Purposeful changes of genes have also been criticized with arguments that refer to religious or ethical bounds on what mankind is entitled to do with nature.

Ethics in animal breeding

The breeding of animals dates back to the first attempts to domesticate them and make them useful for human purposes. This process has generally led to changes of some animal behaviours and has produced animals that are less frightened by humans, less active, and have higher social tolerance. It has also produced animals with higher reproductivity or changes in phenotype such as body size and fur colour. Dogs are not only one of the first species to be bred, but are also the most evident example of large phenotypic changes; from a wolf to a Chihuahua. Breeding of dogs originally sought to achieve different capacities such as hunting, herding, and guarding and this has resulted in today's large variety of breeds. However, these dogs are seldom used for their original purposes today.

In livestock, on the other hand, increased production has been the single, overarching aim that has influenced most breeding programmes. This includes rapid growth in chickens, high milk yield in dairy cows, and a high number of offspring in pigs. Strivings for more efficient production per animal have occurred in parallel to industrialization after World War II, and this has often been described as a civilisation's victory over poverty and malnutrition. In many industrialised countries, having meat or sausages etc. on the table is no longer regarded as a luxury. However, the increased production of animals for meat has negative side effects, and can be seen as a threat to civilization. For example, the global spread of diseases such as African swine fever, BSE (Bovine spongiform encephalopathy, N5H1 (Asian Avian Influenza), and recently Covid-19, have turned out to be serious challenges to human health, and so has the increased prevalence of antibiotic resistant bacteria. Similarly, climate change and negative environmental impacts are related to efforts to reduce production costs, increased specialisation of production branches and internationalisation of animal production and consumption, which causes further challenges to 'modern' lifestyle. In this situation, genetic modification and genome editing are suggested to be valuable tools for both increasing yield per animal in a smarter way, and handling such negative side effects as diseases.

However, there are reasons to be cautious, and in any judgment of today's industrialized animal husbandry, including its potential use of genetic modification or genome editing a plethora of perspectives need to be taken into account. Examples of important questions to be addressed include which role animal breeding plays in the development of these methods what role it will play in the future, and how breeding programmes can contribute to reduced negative environmental impacts. The breeding of farm animals, therefore, is not an ethically neutral undertaking but rather builds on ethical values concerning what has been good so far, what

The average dairy cow in Sweden produces over 8000 kg of milk per year. Are there any ethical aspects on increasing the production even more?

needs to be improved, and how future global challenges are best met. These issues connect to the overall aim of farm animal breeding and need to be considered in relation to the narrower choices that are often defining the goals of breeding programmes today.

In all farm animal breeding programmes, defining the overall goal of the programme is crucial, along with a clarification of why such a goal is important. However, ethical aspects of animal breeding concern all steps of the process where choices have to be made (see figure on page 24) regarding methods, techniques, and variables for measurements, the choice of criteria for genetic evaluation, and an evaluation of the estimated genetic gain. As described on page 21, these steps all contain elements of choices made by the breeders and are thus dependent on their assessments of the offspring – "did we get what we wanted?". If not, was this due to a limited understanding of the genetic traits or to the use of the wrong methods? How should one decide when a limit is reached and when should one re-evaluate the breeding goal? These issues are of ethical relevance because sentient animals are used and there is a risk of causing them suffering or pain due to certain methods such as; hormone treatments in egg donors and surrogate mothers, artificial insemination, and welfare issues related to male animals that are often kept apart from other animals. If the evaluation of a goal is not properly undertaken, animals might be used unnecessarily or the use of a better treatment might be delayed.

In interdisciplinary research that combines breeding, animal welfare studies, and animal ethics, another core question is up for discussion. Should we breed for behavioural changes that will allow the animals to better cope with the environment, or should we change the environment to better suit the animal's behavioural needs and welfare? This relates to the fundamental ethical issues of our 'right' to use animals for our purposes, and their 'rights' to experience well-being and not to be reduced to instruments. In husbandry and its breeding procedure, animals are used as instruments, but this can of course still be combined with respect for their welfare. Hence, it has been argued that the appropriate solution is to create husbandry systems that are suitable for the animals, rather than breeding animals that are less frightened

or susceptible to stress, because low reactions to stress do not necessarily imply good animal welfare. Furthermore, additional ethical aspects of breeding are relevant at the farm level, that is, aspects related to the daily use of the breeds. In general, animal health is considered to be important, albeit often given limited consideration when connected with a concrete economic value, but broader aspects of animal welfare are also relevant. As mentioned previously, mastitis is a common welfare problem on dairy farms and any solution to this problem would be welcomed. On the other hand, mastitis is regarded to be related to production levels and to a general increase in yield, even if not each individual cow with high yield develops mastitis. Another example of an ethical dilemma is the litter size of sows that increasingly give birth to more piglets than they have teats. An interview study in Sweden shows that dairy and pig farmers would be likely to accept lower milk and litter size if this increased animal health and thus lowered the disease and mortality risk and, albeit indirectly, reduced costs.

Thus, from an ethical point of view, both the aim of breeding and the selection of traits have to be considered. But, more general ethical questions also need to be considered, such as: what is the role of high producing cows and sows in a global context, and the ethical justification for breeding them? For example, what are the consequences of increased production per animal in terms of e.g. food security, farmer income, global disease control, and animal welfare? And, what traits are necessary for a robust animal and how should such robustness be defined? Even if it might be economically sustainable to cull a high-producing cow at an age of four or five due to mastitis rather than to use a less productive but healthier breed, is such activity also environmentally sustainable? And what are the social aspects; what do farmers think and what do consumers know? From an ethical point of view, any current practice can be scrutinized and discussed with the aim of finding the most solid arguments for each position.

Another fundamental ethical issue is whether animals have an intrinsic value. Criticism of the genetic modification of farm animals has often been related to their intrinsic value, whereas genetic modification of mice for the purposes of medical research has become a self-evident necessity. Thus, other aspects such as the ethical value of the animal, our relation to it, or simply tradition strongly influences what we deem ethically acceptable in our dealings with animals. Also, given the different methods for changing the genetic makeup of an animal (as described on pages 25–31) it might be difficult to see a clear distinction between conventional breeding and genetic modification, and even more so considering genome editing. Does the most relevant ethical aspect lie in the choice of method, in the method itself, in the consequences of using a certain method or in the 'product' coming out of the method? There are a number of ethical issues to consider regarding the importance of the methods, and these will be shown in the following section about plant breeding. These issues also concern animal breeding.

Ethics in plant breeding

The breeding of plants has rarely been seen as involving controversial ethical issues. The genetic modification of plants, however, is often thought to involve such issues. It is a common belief that genetic modification is wrong, but what might such a claim amount to and what might it imply?

Some people have objections to the technology as such – they argue that there is something inherently wrong with genetic modification that sets it apart qualitatively from changing a genome through traditional

means such as selective reproduction. One such argument is that genetic modification is unnatural and, therefore, immoral or at least morally problematic. A representative of this position is the Prince of Wales, who in his commentary on the 2000 Reith Lectures on BBC Radio 4 argued that "above all, we should show greater respect for the genius of Nature's designs – rigorously tested and refined over millions of years. This means being careful to use science to understand how Nature works – not to change what Nature is, as we do when genetic manipulation seeks to transform the process of biological evolution into something altogether different" (emphasis added). This is a strong claim, and even if many people share the idea of genetic modification as "unnatural", it appears to be less of a moral problem in medical applications such as when genetically modified microorganisms produce insulin for treating diabetes. Other opponents might not be so much against GM technology as such, but more against different applications of it. This means that even people who do not have an objection in principle to the technology still can be critical to its use in agriculture in general or in food production in particular. This way of arguing is an appeal to the consequences of the technology and to its applications. Some people emphasize risks and uncertainties of this unfamiliar technology and argue either that there are risks to human health or the environment, or that there *might* be such risks. The argument in the latter case is that while risks with established technologies can be reliably estimated and managed, more recent inventions might involve surprises, and that for this reason some version of the precautionary principle should be applied.

Considerable efforts have been made by GM proponents to argue that the crops themselves are not riskier per se than any other type of agricultural plant by citing extensive evidence from risk assessments of GM crops. Opponents of GM crops are sceptical to such arguments. However, this focus on environmental or health risks might not be what the critics are aiming at. Many people who are critical of GM crops are critical not because they think they are dangerous, but for other reasons. GM crops are seen as perpetuating a particular economic, social, and cultural world order that includes large-scale industrial agriculture. Thus, criticism of GM crops might not be directed towards the technology as such but against its envisaged social consequences. Genetic modification, it is argued, is another way of transferring power from consumers and farmers to a small number of multinational corporations, from the poor to the rich, and from the developing countries to the developed.

Another reason to look beyond the risk discourse is that one critique of GM crops has nothing to do with risks but rather with a perceived absence of *benefits* to end users and society. First-generation GM crops mainly have agronomic traits (herbicide tolerance or pest resistance) that are useful to the grower but which make no difference in terms of the quality of the end product. Chocolate made from GM soy and sugar beet does not taste better than non-GM chocolate, so there appears to be no inherent reason for the consumer to buy it.

Whether these arguments are reasonable or not can, of course, be debated, and it is quite conceivable that some of them might lose their intensity as the technology and regulatory systems develop. As GM crops with perceivable consumer benefits – better tasting or healthier products – become available, the argument based on a lack of such benefits will no longer be valid. In addition, political reforms might loosen the connection between the technology and particular corporations. Such reform may include changed patent rules or increased public involvement in the development of new crops, thus diminishing the dominance of the corporations.

The legal framework for biotechnology-based breeding in the European Union

§

EU Directive 2001/18/EC on the deliberate release into the environment of GMOs defines a GMO as "an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination".

The general legal framework for genetically modified organisms (GMOs) in the EU constitutes part of the doctrine of the Food Law established by Regulation 178/2002 of the Council and the European Parliament. However, the first regulations on GM products appeared already in 1990 in Council Directive 90/220/EEC on the deliberate release into the environment of GMOs. This was followed in 1997 by the Commission Regulation (EC) No 258/97 of the European Parliament and of the Council concerning novel foods and novel food ingredients.

A series of critical food-related events in the 1990s, not related to GM products tough but including the mad cow disease (bovine spongiform encephalopathy, BSE), *E. coli*, and *Salmonella* outbreaks and the discovery of dioxin residues in foodstuff, resulted in a number of important changes in the EU concerning the regulation of food, and these regulations still apply today. One of the changes was the move from "vertical harmonisation" to "horizontal harmonisation". Vertical harmonisation means that the rules apply to a specific food at all production levels. The introduction of horizontal harmonisation refers to regulations for the entire food chain and all of the food and feed products or groups of products across sectors simultaneously.

The GMO legislation is developed according to the Ordinary Legislative Procedure after the Lisbon Treaty where both the European Parliament and the Council co-decide on new legislation. The EU legislation is founded on common directives and regulations that are applied in the national legislations and implemented by each member state. In addition, each member state can also introduce national laws on particular details, such as containment measures, field trials and co-existence, but always in accordance with the EU common legislation. The rules governing GMOs also make a distinction between contained use, deliberate release, and commercial use.

The purpose of the GMO legislation is to avoid negative effects on animals and human health, and the environment. Therefore, all GMOs are assessed through a case-by-case risk evaluation. The European Food Safety Authority (EFSA), located in Parma, Italy, is responsible for risk assessment and risk communication on scientific issues while risk management remains under the auspices of the European Commission, specifically the Directorate General for Health and Food Safety, DG Sante.

Depending on the use and type of organism, one or more of the different national governmental agencies are responsible for the application of the GMO legislation. In Sweden, for example, ten different authorities have responsibilities in relation to regulatory decisions concerning GMOs. Competent Authorities (CA) include: the Swedish Board of Agriculture, the Swedish Forest Agency, the Swedish Chemicals Agency, the National Food Agency, the Medical Products Agency, the Swedish Agency

for Marine and Water Management, and the Swedish Work Environment Authority. The Swedish National Environmental Protection Agency and the Swedish Gene Technology Advisory Board advise the Competent Authorities. The Swedish Civil Contingency Agency is consulted in questions regarding transportation of GMOs.

CONTAINED USE

Contained use refers to the use of GMOs under conditions where contact between the GMOs and the surrounding environment and the public is restricted. The uses of GMOs in approved laboratories and greenhouses are examples of contained use. It can also involve GM animals, plants, and microorganisms in research laboratories, as well as GM microorganisms used for production of for example enzymes for food production or pharmaceutical proteins. T-cells (type of lymphocyte) and viral vectors that have been modified for medical use are also classified as GMOs. In Sweden, contained use of GMOs is regulated by the Ordinance on Contained Use of Genetically Modified Organisms (SFS 2000:271). The Swedish Work Environment Authority is the competent authority in the case of GM microorganisms and cell cultures of higher organisms. Aquatic

§

EXAMPLES OF CURRENT EU REGULATIONS AND DIRECTIVES CONCERNING GMOS
Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms
Regulation (EC) 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
Regulation (EC) 1829/2003 concerning GM food and feed
Regulation (EC) 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs
Regulation (EC) No 1946/2003 concerning the cross-border movements of GMOs and transposing the Cartagena Protocol on Biosafety into EU law
Regulation (EC) 65/2004 establishing a system for the development and assignment of unique identifiers for GMOs
Directive 2009/41/EC concerning contained use of GM microorganisms

In addition, there are a number of regulations, Implementing Decisions, and Directives (including those amending earlier Directives) that refer to specific details concerning GMOs

GM organisms are governed by the Swedish Agency for Marine and Water Management. The Swedish Board of Agriculture governs contained use of all other GMOs including terrestrial plants and animals.

DELIBERATE RELEASE FOR FIELD TRIALS

The Swedish Environmental Code defines "deliberate release" as any intentional introduction of GMOs into the environment without containment. Examples are field trials with GM plants, clinical trials with GM microorganisms, and farm-based trials with GM animals. A trial of any GMO must comply with the requirements laid down in part B of Directive 2001/18/ EC of the European legislation, and such trials require a permit from the relevant national competent authorities. In Sweden, this is regulated by the regulation on deliberate release of genetically modified organisms (SFS 2002:1086).

In the case of field trials of GM animals and GM plants, permits are granted by the Swedish Board of Agriculture, or by the Swedish Forest Agency in the case of trees for wood production. Because field trials with GM plants occur outdoors, precautions such as fences, insect nets, seed traps, and minimum crop-dependent cultivation distances to related crops and beehives have to be implemented in many EU member states, depending on the crop, to limit the risk of dispersal of GMOs into the surrounding environment. As these precautions are not defined in the EU law, the member states may individually decide on the requirements on the field trials. In Sweden, a minimum cultivation distance, a protective edging around the field, and removal of residual plants may be requested. In the case of animal trials, all precautions must be taken to avoid the escape of GM animals and the mating of GM animals with wild animals.

COMMERCIAL USE

Commercial use of GMOs is referred to in the legislation as "placing on the market". Approval for placing a GMO on the market can include one or several possible uses such as cultivation, import and processing of living GMOs, or the use of GMOs as food and feed. Decisions on placing GMOs on the market are taken collectively by the EU member states. There are two ways to apply for placing a GMO on the market in the EU. One can follow either Directive 2001/18/ EC, which regulates deliberate release (both field trials and commercial cultivation) within the EU and the importation of a GMO from a country outside of the EU, or Regulation (EC) no. 1829/2003 that regulates the commercial cultivation, importation, processing, and use of GM food and feed. As of today, the majority of the applications have been filed according to Regulation (EC) no. 1829/2003 (see the flowchart of the approval process).

An application for placing a GMO on the market for use as food or feed is submitted to a national competent authority (NCA) in any EU member state. The NCA, in turn, sends the application to the EFSA. The EFSA GMO panel conducts a scientific risk assessment of the proposed GMO with respect to potential hazards to animal or human health and to the environment. The risk assessment is based on the available scientific literature and documentation handed in by the applicant that has to follow internationally agreed guidelines according to the CODEX *Alimentarius*[1]. Based on the risk assessment, the GMO panel issues a scientific opinion to the European Commission. CAs from the other members states are invited to comment on the application. The decision to approve, or not approve, the application is taken

1 Collection of internationally recognized standards, codes of practice, guidelines and other recommendations relating to foods, food production and food safety.

by the Standing Committee on Plants, Animals, Food and Feed if a qualified majority[2] (QM) among the Member States can be reached, or by the Appeal Committee if a QM cannot be reached in the first instance. If also the Appeal Committee cannot reach a QM agreement, the European Commission should, according to EU law, take the decision. Decisions of approvals are valid throughout the European Union.

Applications filed according to Directive 2001/18/EC (deliberate release into the environment) follow a similar route; the application is sent to a national CA for the environmental impact assessment. A proposed approval of the application is followed by an objections phase and an agreement phase. If there is no agreements the matter goes to EFSA, after which it follows a similar procedure as above (though the first vote takes place in the Regulatory Committee on directive 2001/18/EC). The decision is in principle valid in all EU countries, however, since 2015, the EU legislation (Directive 2015/412) allows individual Member States to prohibit the cultivation of GM crops within their own borders, even if the crop has been approved for cultivation at the EU level.

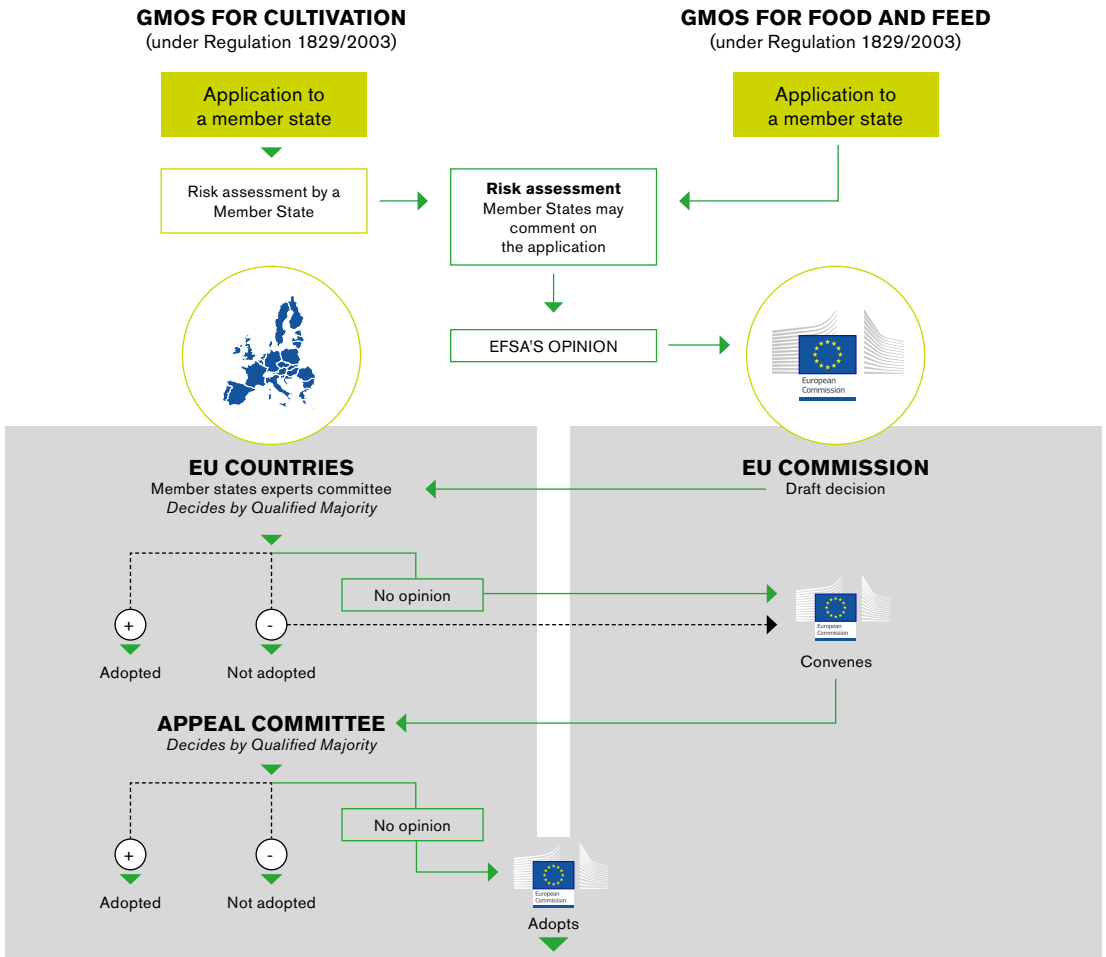
LABELLING AND TRACEABILITY

The labelling and traceability of GM food and feed are regulated through EU Regulation 1830/2003. Food or feed that contain, consist of, or are produced from GMOs have to have "genetically modified" or "produced from genetically modified x" clearly visible on the label[3] to ensure traceability and freedom of choice for the consumers. Also, processed food and feed that do not have detectable levels of DNA or proteins but that are made from GMOs, such as refined sugar and rapeseed oil, have to be labelled. Because large parts of the world's production of staple foods such as maize, soybean, rice, and rapeseed are currently derived from GM varieties, involuntary or technical intermixing of GMOs in conventionally produced food and feed is sometimes difficult to avoid. GM varieties or derived products that have been authorised within the EU approval system are allowed to occur up to a limit of 0.9 per cent of that particular ingredient in a product, without GMO labelling. Intermixing of unauthorised GMOs is in general not allowed in the EU although intermixing up to 0.1 per cent in feed is accepted under certain circumstances (see Regulation EU No. 619/2011).

Vitamins and enzymes produced from GMOs do not require labelling nor do textiles produced from GM cotton or oils from GM plants that are used for technical or cosmetic purposes such as skin care products. Meat, eggs, and milk produced from animals that have been fed GM feed do not require labelling because the animals themselves are not GMOs. In principal, the

2 A qualified majority (QM) is reached if two conditions are met: 1) 55% of member states vote in favour – in practice this means 15 out of 27, and 2) the proposal is supported by member states representing at least 65% of the total EU population.
3 Certain traces of GMOs in products may be adventitious or technically unavoidable. Such presence of GMOs should therefore not trigger labelling and traceability requierments.

EU decision-making process on GMOs



	Selection breeding	Hybridization (of species)	Mutation breeding (radiation/ chemical)	Genetic modification (gene transfer)	Genome editing (without gene transfer)
Number of genes affected	10 000 - 300 000[1]	10 000 - 300 000[1]	300 – 700 000[1]	1-3[2]	1-3[2]
Tests for effects on humans and the environment are required	X	X	X	√	√[3]
Needs to be labelled[3]	X	X	X	√	√[3]
Traceable	X	√	X	√	X
Allowed in organic farming	√	√	X[5]	X	X
Examples	Most plants we eat	Apples, wheat, rice, triticale, oilseed rape	Pear, grapefruit, oilseed rape, barley	Corn, oilseed rape, aubergine, soy, papaya	Champignon, apple, soy, oilseed rape, potato

1. Depending on species. 2. More genes can be affected. 3. In the EU. Several other countries do not regard those crops as GMO. 4. In the EU, products in which an ingredient contains more than 0.9 per cent GMO needs to be labelled. 5. According to the IFOAM, mutation breeding is not allowed but in practice, this cannot be controlled for and many of those crops are grown also on organic farms. Sources: Sikora et al. 2011. Int J Plant Genom doi:10.1155/2011/314829, Genetic Literacy Project.

legislation stipulates that food and feed should be labelled according to which ingredients the food contains rather than what it does not contain. Hence, labelling foods as "GM-free", which is commonly found in countries outside of the EU, is not supported by the current legislation, although this is interpreted differently in different EU member states.

The demand for traceability stipulates that a GMO or a product that contains GM ingredients or is made out of a GMO (except enzymes and vitamins made from GM microorganisms) should be followed by documentation that allows traceability through all stages of its production and placement on the market. In 2018 the Court of Justice of the European Union (ECJ) ruled that all organisms developed through mutagenesis are classified as GMOs, whereas only those developed through mutagenesis technologies that were available before 2001 are exempted from the provisions of the GMO directive. This means that products developed by site-directed mutagenesis techniques are regulated as GMOs. Since it is not possible to determine if a mutation is a result from site-directed techniques or randomly induced mutagenesis, or if it is a naturally occurring mutation, the demand of traceability and identification might be difficult to sustain in the future considering that several countries outside the EU are already de-regulating these crops and products.

COEXISTENCE

Because products that contain GMOs have to be labelled, involuntary intermixing between conventionally produced products and GM products

poses an economic risk to the farmers. Farmers using conventionally bred varieties might have to label their products if they contain over 0.9 per cent of a GM crop, and the farmers growing GM crops might face liability charges. Directive (EU) 2015/412 imposes to EU countries cultivating GMOs to put in place coexistence measures at their borders with non-cultivating EU countries. To minimize the risk of intermixing between GM varieties and conventionally bred crops, Swedish authorities have developed a regulatory framework for the cultivation of GM crops. The intention with the rules is to reduce intermixing to a level below the threshold of 0.9 per cent. The requirements include cultivation distances to neighbouring crops (which have been implemented for maize and potatoes), the duty to inform local authorities and neighbouring farmers about the cultivation of GM crops, and the cleaning of equipment used for GM crops.

OTHER ORGANISMS

Currently, no GM mammals or fish, or derived products are on the EU market, but the EFSA published environmental risk assessment (ERA) guidelines with the aim of assessing the possible direct, indirect, immediate, or delayed risks to human health and the environment by such organisms as well as related issues concerning animal health and welfare. This will support possible future applicants in submitting their applications for GM mammals, fish, insects, birds and derived products for entry into the European market.

Variety testing and plant breeders' rights

In Sweden, and many other countries, commercial crop varieties may be protected by plant breeders' rights (PBR) following the standard of the International Union for the Protection of New Varieties of Plants (UPOV). Patent law may also apply when a crop variety contains a biotechnological invention for which one or more patents are valid, but this depends to a large extent on the jurisdiction in question. In Europe, plant varieties as such are excluded from patentability, according to the European Patent Convention. However, a patent can be obtained, encompassing many plant varieties within the scope of the claim, if the invention is not technically restricted to a specific plant variety. PBR means that you need the owners' permission to propagate, promote, and sell the protected variety. Farm-saved seed, i.e. the production of seeds for use on one's own farm, is an optional exception to the PBR. In many countries, the plant breeder's royalty is significantly reduced if the size of the farm does not exceed a certain production volume.

For a variety to be protected and/or listed in Sweden, it needs to be approved by the Board of Agriculture. For variety protection, the variety needs to be distinguishable from other varieties, uniform, and stable, that is, it does not change when it is propagated. Agricultural crops also need to have a satisfying Value for Cultivation and Use (VCU). Varieties listed in other EU countries can be sold and grown in Sweden without additional testing.

All seeds of agricultural and horticultural crops sold in Sweden need to be certified. To be certified, the variety needs to be listed and of good quality in terms of germination rate, water content, pathogens and have a certain level of purity regarding weed seeds, other seeds, and debris.

The plant breeders' rights hold for a maximum of 25 to 30 years depending on the species. These rights do not, however, prevent others from using the protected varieties in research, trials, or as parents in further breeding.

The podcast Shaping our food

Shaping our food is a podcast produced by Lisa Beste and Anna Lehrman within the research programme Mistra Biotech. Many of the topics covered in the podcast overlap or relates to what is covered in this book. Each section is based on interviews with researchers and experts. You can find the podcast on several platforms such as Libsyn, Spotify, and iTunes..

1. **Why do we need plant breeding?** Guest: Inger Åhman, professor in plant breeding at SLU.

2. **Why do we need animal breeding?** Guest: Lotta Rydhmer, professor in animal breeding at SLU.

3. **Taming a wild plant.** Guests: Emelie Ivarson, PhD student, Sten Stymne and Li-Hua Zhu, both professors in plant breeding at SLU.

4. **Fighting the blight!** Guest: Erik Andreasson, professor in plant protection at SLU.

5. **Now it is time for oats.** Guests: Alf Ceplitis, senior breeder and technology manager at Lantmännen, Elisabeth Jonas, associate professor in quantitative genetics at SLU, and Olof Olsson professor in applied biochemistry and project leader at ScanOats, Lund University.

6. **One hundred per cent unnatural.** Guest: Per Sandin, associate professor in philosophy at SLU.

7. **Green fields, not green oceans.** Guests: Henrik Svennerstam, researcher at Umeå Plant Science Centre and Martin Weih, professor in plant ecology and eco-physiology of agricultural crops at SLU.

8. **Proteins take us closer to truth.** Guest: Fredrik Levander, associate professor in immuno-technology at Lund University.

9. **Noah's ark for real – gene banks save traits for the future.** Guests: Ulrika Carlson-Nilsson, senior scientist and Pawel Chrominski, *in vitro* lab responsible at NordGen, and Denise Costich, senior scientist and head of the germplasm bank, and Bibiana Espinosa research associate at CIMMYT, the International Maize and Wheat Improvement Center in Mexico (interviews in English).

10. **Creamy, greasy, but still stable – this is starch.** Guests: Mathias Samuelsson, sales and development director at Sveriges stärkelseproducenter (the Swedish potato starch cooperative), and Mariette Andersson, researcher in plant breeding and Roger Andersson, professor in food science, at SLU.

11. **Genes by all means – from genomics to traits.** Guests: Cecilia Gustafsson, geneticist in genomics-assisted plant breeding, and Dirk-Jan de Koning, professor in animal breeding, at SLU.

12. **Thousands and thousands of varieties – but only one GMO.** Guests: Jens Weibull, senior officer at the Swedish Board of Agriculture, Marie Nyman, chief secretary at the

Swedish Gene Technology Advisory Board, and Dennis Eriksson, researcher in plant breeding at SLU.

13. **Fifty per cent like mum, sixty per cent like a banana?** Guests: Fredrik Sundström, director of studies at the Biology Education Centre at Uppsala University, and Niclas Gyllenstrand, curator at the Swedish Natural History Museum.

14. **Consumers, food, and gene technology.** Guests: Minna Hellman, manager, consumer health and well-being, Stockholm Consumer Cooperative Society, and Carl Johan Lagerkvist, professor in business economics at SLU.

15. **GMO + organic = true?** Guests: Lars Hällbom previously standards director at KRAV, Minna Hellman, manager, consumer health and well-being, Stockholm Consumer Cooperative Society, Sara Sundquist, sustainability manager and industrial policy expert at the Swedish Food Federation, and Carl Johan Lagerkvist, professor in business economics, Klara Fischer, associate professor in rural development, and Per Sandin, associate professor in philosophy, at SLU.

16. **Artificial intelligence at the breeder's service.** Guests: Aakash Chawade, associate professor in plant breeding at SLU, and Tina Henriksson, senior breeder at Lantmännen.

17. **"Yes, and by the way no" How a whole industry changed their minds on GMO.** Guest: Bo Ekstrand, CEO at Bioconsult AB.

18. **Ethics, animal breeding and technology.** Guest: Helena Röcklinsberg, university lecturer in animal ethics at SLU.

19. **Methods and techniques in plant breeding.** Guests: Cecilia Gustafsson, geneticist in genomics-assisted plant breeding, Jonas Skytte af Sätra, PhD student, Emelie Ivarson, research engineer, and Mariette Andersson, associate professor in plant breeding, all at SLU.

20. **How cautious should we be?** Guest: Sven Ove Hansson, professor emeritus in philosophy at KTH Royal Institute of Technology, and programme director of Mistra Biotech.

Further reading

Comstock G. 2012. Ethics and genetically modified food. In Kaplan, D.M. (eds.) *The Philosophy of Food*. Berkeley: University of California Press

Curtin SJ, Voytas DF, & Stupar RM. 2012. Genome engineering of crops with designer nucleases. *The Plant Genome* 5:42-50

D'Eath RB, Conington J, Lawrence AB, Olsson IAS, & Sandøe P. 2010. Breeding for behavioural change in farm animals: practical, economic and ethical considerations. *Animal Welfare* 19: 17-27

Einsiedel EF. 2005. Public perceptions of transgenic animals. *Scientific and Technical Review of the Office International des Epizooties* 24: 149-57

Forabosco F, Löhmus M, Rydhmer L, & Sundström LF. 2013. Genetically modified farm animals and fish in agriculture: A review. *Livestock Science* 153: 1–9

Gaj T, Gersbach CA, & Barbas CF. 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology* 31:397-405

Gjerris M. 2012. Animal Biotechnology: The Ethical Landscape, in Brunk CG & Hartley S *Designer Animals: Mapping the Issues in Animal Biotechnology*. Toronto: University of Toronto Press, pp. 47-70

George EF, Hall MA, & De Klerk G-J. 2008. *Plant propagation by tissue culture*, 3rd edition, Exegetics, Basingstoke, UK

Gregorowius D, Lindemann-Matthies P, & Huppenbauer M. 2012. Ethical discourse on the use of genetically modified crops: A review of academic publications in the fields of ecology and environmental ethics. *Journal of Agricultural Environmental Ethics* 25: 265–293

Hansson SO. 2019. A science-informed ethics for agricultural biotechnology. *Crop Breeding, Genetics and Genomics* doi:10.20900/cbgg20190006

Jannink J-L, Lorenz AJ, & Iwata, H. 2010. Genomic selection in plant breeding: From theory to practice. *Briefings in Functional Genomics* 9: 166-177

Jorrín-Novo JV, Komatsu S, Weckwerth W, & Wienkoop S (eds.). 2014. *Plant Proteomics: Methods and Protocols* 2nd ed. Series: Methods in Molecular Biology 1072, Humana Press

Kingsbury N. 2009. *Hybrid, the history & science of plant breeding*. The University of Chicago Press.

Meuwissen T, Hayes B, & Goddard M. 2013. Accelerating improvement of livestock with genomic selection. *Annual Review of Animal Biosciences* 1: 221-237

Podevin N, Davies HV, Hartung F, Nogué F, & Casacuberta JM. 2013. Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. *Trends in Biotechnology* 31: 375–83

Primrose SB, & Twyman RM. 2006. *Principles of gene manipulation and genomics*. Blackwell Publishing

Riddihough G, & Zahn LM. 2013. What is epigenetics? *Science* 330: 611

Rollin BE. 1995. *The Frankenstein Syndrome. Ethical and social issues in the genetic engineering of animals*. Cambridge: Cambridge University Press

Slater A, Scott N, & Fowler M. 2003. *Plant biotechnology – the genetic manipulation of plants*. Oxford university press

www.gmo.nu

www.sjv.se

www.genteknik.se

www.slu.se/mistrabiotech

Glossary

Allele	Alternative form of a gene.
Allopolyploid	Polyploid with chromosomes derived from different species.
Autopolyploid	Polyploid with multiple chromosome sets derived from a single species.
Chromosome	A structure of DNA and associated proteins.
Cloning	Development of an organism from a single somatic cell or nucleus.
Diploids	Organism with two sets of chromosomes.
DNA	Deoxyribonucleic acid. The large molecule that stores the genetic information in all cells.
Endonuclease	Nuclease that cleaves within polynucleotide chains.
Exonuclease	Nuclease that cleaves polynucleotide chains one by one from the ends.
Gamete	Haploid reproductive cell produced by meiosis.
Genome	The complete set of genes carried by an organism.
Genotype	The genetic constitution of an organism.
Germ cell	Reproductive cell that give rise to a gamete.
GMO	Genetically Modified Organism. An organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.
Haploid	Cell only containing one set of chromosomes (comp. diploid).
Heritability	Proportion of phenotypic variation in a population that depends on genetic variation.
Heterosis	Hybrid vigour, superiority of the offspring in one or more characters over the parents.
Heterozygous	Diploid organism with two different alleles at a given locus.
Hexaploid	Organism with six sets of chromosomes.
Homozygote	Organism with identical pairs of genes (or alleles) for a specific trait.
Locus	Location of a gene.
Meristem	Tissue in plants containing undifferentiated cells.
Metabolites	Small cell molecules with various functions.

Microspore	Plant spore that develop into male gametophyte and then sperm cell.
miRNA/ micro RNA	Small non-coding RNA molecules regulating gene expression.
Meiosis	Cell division resulting in gametes (egg or sperm) with half the number of chromosomes.
Messenger RNA (mRNA)	RNA molecules that transport genetic information from DNA to the ribosome, where they specify the amino acid sequence of the protein produced.
Mitos	Cell division resulting in two cells with identical sets of chromosomes.
Mutation	A change in the nucleotide sequence in an organism.
Nuclease	Enzyme that cleaves the bonds between nucleotides.
Nucleotide	The basic subunits of DNA; adenine, thymine, cytosine, and guanine. And RNA where the thymine is replance by uracil.
Oligonucleotide	Short, single-stranded DNA or RNA molecules.
Phenotype	The result from the expression of an organism's genes + environmental factors and the interactions between the two.
Plasmid	Short DNA, most commonly found as circular, double-stranded DNA in bacteria.
Polyploid	Organism containing more than two paired (homologous) sets of chromosomes.
QTL	Quantitative trait loci, DNA sequences containing or linked to the genes coding for a quantitative trait.
Ribosome	The large and complex molecule where mRNA is translated into proteins.
RNA	Ribonucleic acid, a family of molecules that perform coding, decoding, regulation, and expression of genes.
Somatic cell	Cell other than a gamete, germ cell or undifferentiated stem cell.
Totipotent	A cell with the ability to divide and produce all of the differentiated cells in an organism.
Transcription	When DNA is copied to messenger RNA (the first step of gene expression).
Transformation	Introduction of exogenous DNA into the genome.
Transgenic	Organism into which genes from another species have been deliberately introduced though genetic modification.
Translation	Decoding of messenger RNA into an amino acid chain that later is folded into a protein.

SHAPING OUR FOOD

AN OVERVIEW OF CROP AND LIVESTOCK BREEDING

You may not have thought about why tomatoes look the way they do, why our pets and farm animals are so calm and friendly, or why seedless watermelons are possible. Although the breeding of plants and livestock have shaped more or less everything we eat, few people know about the scientific achievements and the extensive work that resulted in the food we see on our plates every day.

This book provides an overview of domestication and breeding, from the beginning of farming more than 10,000 years ago to the molecular work of today. We present the basics of the structures and functions of genes, describe why and how different breeding methods are applied, and give some insight into legislation surrounding the use of biotechnology in breeding in the EU and in Sweden. We also discuss ethical issues related to breeding in general and to genetic modification in particular.

This book is published within the research programme Mistra Biotech that is financed by the Swedish Foundation for Strategic Environmental Research (Mistra) and the Swedish University of Agricultural Sciences (SLU).

SLU

ISBN 978-91-981907-1-7