Spontaneous Chlorophyll Mutations in Scots Pine *(Pinus silvestris L.)*

Tallens (Pinus silvestris L.) spontana klorofyllmutationer

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Introduction

Chlorophyll deficient individuals are not unknown among the conifers. At seedling stage they are to be found in the nurseries, and they are also to be met with in the germination tests of the seeds. Variations in the degree of deficiency in such plants are relatively wide. White individuals, completely void of chlorophyll, serve to make the utmost limit of these variations and, on the other hand, slight chlorophyll deficiencies render it difficult to set such individuals apart from normal ones.

The occurrence of the chlorophyll deficient plants is, however, low, and their life short. Most of them perish already in the course of the first few weeks at seedling stage. For this reason they very often remain either unnoticed, or have very little attention paid to them. Older individuals with traces of chlorophyll deficiencies are rather a rarity, and even then the indications of these deficiencies are quite trivial.

A similar phenomenon is known and has been described in several other species. The genotypical character of such chlorophyll deficient individuals has been well investigated in barley, maize, and other plants (NILSSON-EHLE, 1922; GUSTAFSSON, 1938, 1940, 1952, 1954; STADLER, 1932, 1952; LINDSTRÖM, 1925; DEMEREC, 1935). They are designated as chlorophyll deficient mutants in so far as heredity, and not environmental factors, which in certain combination may interfere with the normal chlorophyll activities, is the cause of the existence of such individuals.

The origin of chlorophyll mutations is explained by changes in the genes, that is, by the alterations in the atomic structure of the gene molecule, or by rearrangements of the chromosome structure, for instance, chromosome deficiences. It has been proved that chlorophyll mutants bear recessive traits. Consequently, mutations in the heterozygous plants may appear already in the next generation, or they may be hidden in the genotype. In the latter case mutations can segregate and manifest themselves in the recombinations of genes in the progeny. To make the recessive mutant visible it is necessary that the chlorophyll deficient factor be present in both gametes.

In cases of cross-fertilisation, in this case pine, there is a very slender chance of the above-mentioned hereditary defects from the gametes of both parents being paired and of the mutation segregating from a heterozygous

recessive state into homozygous. If this, nevertheless, takes place and the mutation makes itself apparent even if it be within the limits of insignificant frequencies, this can only mean that the chlorophyll deficient factors are represented in the genetic constitution of the parents on a very large scale.

It is from this aspect that the chlorophyll mutations in Scots Pine should be estimated, even if they appear at very low frequencies. They reveal part of the recessive, deleterious characters of the individual stands which otherwise might have remained invisible.

Contrary to the great majority of other mutations which are difficult to notice and define, chlorophyll mutations are visible. They reveal themselves in their phenotypical effect, that is, by complete or partial lack of chlorophyll, and they are of great importance not only in solving the mutation problems of individuals, but also in solving mutation problems in stands.

Hardly any research work has been done dealing with chlorophyll mutations in Scots Pine. The investigations into these mutations meet with several difficulties. The most important and common are the cross-fertilising and distinctly heterozygous nature of Scots Pine, the life-time of each generation and the low rates of mutations in general.

The possibility of carrying out investigations on chlorophyll mutations in Scots Pine arose in connection with the great number of pine seed samples which our Genetic Department collected for research work on the provenience of pine. The source of origin of these seed samples embraces all Sweden and Norway.

I. Subject and Scope of the Investigation

Seed material for research work was obtained from 77 localities in Sweden an 11 localities in Norway from 1948 to 1950 (fig. 1). These localities cover a diversity of latitudes as well as different altitudes. The hereditary variability of the pine in connection with the climatic divergencies of the numerous localities was amply represented.

The collection of cones was based on the principle of individual trees, that is, cones were collected from each tree separately. Thus every stand was represented by a certain number of trees, usually from 25 to 30. The trees in the stands were chosen at random, with the view to having different types of trees represented. Cones were mostly collected from growing trees, which made it possible to replicate the experiment. In several stands mixed cone samples, originating from different localities in Sweden, were gathered, the number of trees always exceeding 25. It should, however, be added that eight samples came from Germany and one from Holland.

As regards the preparation of seeds for planting, which might possibly have a certain connection with the research problem, the following can be mentioned. The seeds were extracted from the cones at a temperature never higher than 52° C. The dewinging was made by hand in canvas bags. Seed samples were preserved in hermetically closed test tubes at a temperature of + 5° C.

The whole seed material was sown in the nurseries of the two experimental fields at Bogesund and Sundmo (fig. 1), in 1951. In both nurseries the edaphic conditions were uniform, which provided equal possibilities for the germination of the seeds from all samples as well as for the development of plants. The properties of the soil completely corresponded to the ecology of conifers, and not even the least disorderly metabolic changes were observed in the coloration of the plants. Climatic conditions at Bogesund and Sundmo were dissimilar on account of their geographic positions.

Four replications of each sample, each replication containing one hundred seeds, served to determine the germinating capacity of the seeds. Both the total number of the seeds sown and their germinating capacity being known, it was possible to compute the total number of the germinated seeds for each sample. The total number of plants used in the investigation, the number of

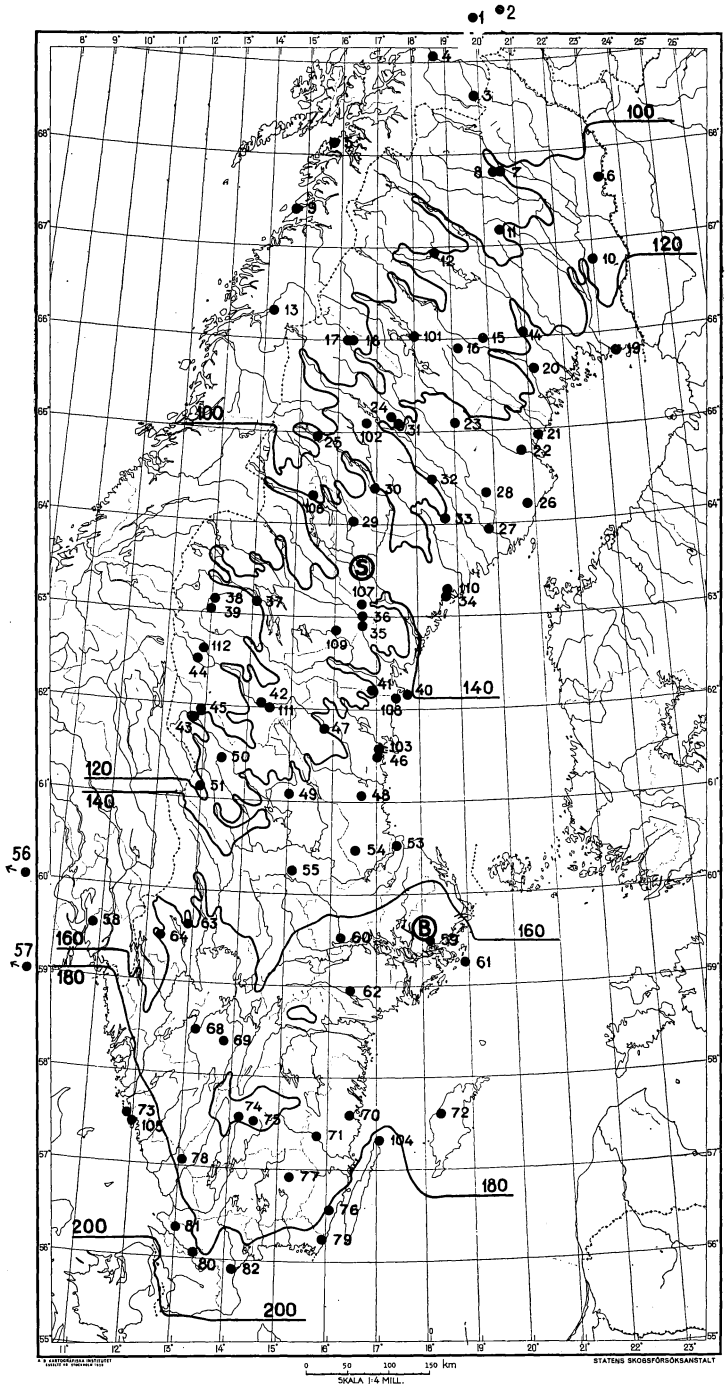

Fig. 1. Localities in Sweden and Norway with numbers of stands from which cones were obtained. Isotherms showing number of days in a year with normal average $t°$ of $\geqq +6°$ C (Langlet, 1936). B—field trial at Bogesund and C—at Sundmo.

Orter i Sverige och Norge med numrerade bestånd från vilka kott har insamlats. Isotermer som visar antalet dygn med normal medeltemperatur av $\geqq +6°$ C (Langlet, 1936). B — Fältförsök vid Bogesund och C — vid Sundmo.

mother trees as well as the number of stands where the mother trees originated are as follows:

Bogesund experimental field	1,012,000 individual plants, originating from 1,016 trees in 43 stands;
Sundmo experimental field	757,000 individual plants, originating from 1,015 trees in 43 stands.

It should be mentioned that cones were collected from a larger number of stands than above mentioned. It is to be regretted, however, that the germination of seeds from some of the stands was so low, and on account of that the progeny of mother trees were represented by so negligible a number of plants, that they were not included in the investigation. This refers to stands located in mountainous and northern regions.

In the Bogesund experimental field the number of progeny plants from mixed seed samples obtained from 15 stands was 87,000; at Sundmo their number was 98,000 plants from 18 stands.

As soon as chlorophyll mutants appeared they were marked by means of tooth-picks and coloured rings for corresponding mutation types. Notes were made regarding each mutant and every change was written down. Since several persons registered and marked mutants there was the danger of possible subjective approach. However, "personal equation" was equalized by making a provisional marking of all mutation cases and even of all doubtful cases. Later the decisive choice was, however, made by only one person. It has been possible to eliminate the methodological difficulties quite satisfactorily in the Bogesund experimental field, which also was the chief base of the investigation. Tests with chlorophyll mutations are being continued there.

The subsequent tests carried out in the greenhouse in 1953 and 1954 were replications with the same selected seed samples as those in the nurseries. These tests contained 6,400 individuals (seedlings and plants) originating from 23 seed samples in 1953 and 8,700 individuals from 28 seed samples in 1954.

The classification of chlorophyll mutations in the present investigation is made in accordance with the scheme in table 1. This scheme is based on the system of chlorophyll mutations in barley elaborated by GUSTAFSSON (1940). It was, however, necessary to introduce a few additions on account of the divergencies in the morphology and biology of the pine as compared with barley.

The classification of the mutants into groups is based on the phenotypical colour effect in the cotyledons, in the hypocotyl, in the primary needles and in the needles themselves. Mutations comprise two principal groups—seedlings and plants. The latter group contains all plants that have outgrown the

Table 1. Classification scheme of chlorofyll mutants in pine used in the experiments from 1951 to 1954

(C. d. C. — colours measured according to »Code des Couleurs», KLINCKSIECK and VALETTE, 1908).

Klassifikationsschema av tallens klorofyllmutanter.

Mutation types	**Seedling mutants**
1. *Albina*	Cotyledons white, but assuming a slightly yellowish colouring when withering. Hypocotyl 1—10 mm at the base reddish violet. C.d.C.—(541) 551, 556, 557; 561—562 (587). Size of mutants somewhat lower than that of normal seedlings, dwarf-sized 5 mm.
2. *Xantha*	Cotyledons yellow. C.d.C.—186, 191, 196, 203 D, 216, 228 C—D, 261, 266. Hypocotyl lightly brownish orange at the base. C.d.C.—78 D, 53 D (66); higher up—slightly orange yellow. Individuals representing *xanthoalba* subtype orange violet at the base. Size somewhat normal or normal.
3. *Xanthoviridis*	Cotyledons greenish yellow. The presence of chlorophyll more or less marked. C.d.C. 252—258. Hypocotyl orange, lightly brown at the base and higher up greenish yellow. C.d.C. 107, 112, 113, 117. Size normal. Dwarfs occur, but seldom.
4. *Viridis*	Cotyledons yellowish green, light green or slightly different in colour from normal seedlings. C.d.C.—276—292. Hypocotyl at the base yellowish brown or brown, lighter than that of normal seedlings. C.d.C.—153—154 (104, 113). Higher up yellowish green, still invariably lighter than in normal seedlings. Size normal or slightly shorter than normally. Dwarfs occur, but seldom.
	Plant mutants
1. *Albina*	Primary needles white, shorter than in normal plants. Needles—not produced.
2. *Xantha*	Primary needles yellow, shorter than in normal plants. Needles yellow but very seldom produced.
3. *Alboviridis*	Primary needles whitish green or green in the first pairs. The upper pairs white. Needles contain chlorophyll defects in different shades of white, green and yellow.
4. *Xanthoviridis*	The first pairs of primary needles green or partly green, the upper yellow. Needles yellow.
5. *Viridis*	A most heterogeneous type. Primary needles and needles similar to the cotyledons of seedling mutants. Traces of chlorophyll defects in different shades.

seedling stage of development. In the pine a characteristic and not seldom decisive mark of indication for the mutants at seedling stage is their hypocotyl.

The extensive scope of this investigation made it necessary to simplify, and consequently, when classifying mutants, detailed grouping was avoided. Special attention was paid to four or five mutation types respectively and these types quite satisfactorily represented the variation of chlorophyll mutations.

Only very few cases fell outside the range of the scheme. They were mostly surviving mutants with slight chlorophyll deficient traits, which are now the subjects of further investigations in the experimental field at Bogesund. These cases also contained chimeras.

The sub-classes of the different mutant types were not taken into consideration, although it was found that pine displays mutation nuances analogous to those met with in GUSTAFSSON's system in *viridis* and *alboviridis* groups. It seemed, however, reasonable to make up a separate *xanthoviridis* type which was numerously represented.

When working with chlorophyll mutations attention should be given to phenotypic variations caused by environmental conditions. These phenotypic variations at seedling stage often resemble certain mutation types. Such "phenocopies", to use GOLDSCHMIDT's term (1937), come nearest to the *viridis* type mutants and they mostly occur among the progeny of seeds coming from mountainous and northern regions. In these cases the slow and weak chlorophyll development in the seedlings should be associated with deficient ripening of the seeds and it is a modification called forth by climatic conditions. To distinguish seedling mutants from modifications caused by external factors requires most careful approach, but it is merely a matter of routine. Accuracy and biological erudition are required to set apart modifications of phytological nature in both seedlings and plants (which, for instance, may be produced by the damages caused by *Melampsora pinitorqua*) from chlorophyll mutants having similar coloration.

One of the most important measures in the experiments with chlorophyll mutations in pine was to secure the soil properties corresponding to the ecology of the pine. As an example might be mentioned that a high pH value of the soil as well as high lime contents calls forth metabolic changes in the coloration of the pine seedlings, so that difficulties arise in distinguishing them from "real" mutations. In the present investigation metabolic changes caused by the properties of the soil were completely eliminated.

II. Chlorophyll Mutations in the Progeny of Heterozygous Mother Trees

1. Mutant Rates of the Individul Trees

The sum total of all kinds of chlorophyll deficient individuals in the progeny of the individual heterozygous trees which were registered and submitted to the investigations in the experimental field at Bogesund, comprises 1,368 mutant individuals (seedling and plant mutants). This figure was obtained from 1,012,000 plants, which originated from 1,016 individual trees

representing 43 natural stands (fig. 1). The average mutant rate of these trees is thus 1.351 per thousand, which means that one mutant individual per 740 plants in the nursery is endowed with more or less expressed chlorophyll deficient traits. At the same time from the total number of 757,000 plants in the Sundmo experimental field 1,317 mutants were observed. The above mentioned plants were the progeny of 1,015 individual trees from 43 stands. The average mutant rate here was 1.740 per thousand.

The scale of the fluctuations in the mutant rates of the individual trees is very wide. In the experimental field at Bogesund no traces of mutations were found in the progeny of 606 trees, that is, in 60 % of the total number of the trees. The maximum mutant rate of the remaining 410 mutated trees is 83.06 per thousand. This originated from a mother tree in the stand No. 57 (Bergen, Norway) and is revealed in 75 seedling mutants of exclusively *xantha* type. The mutant rate nearest in rank to the above mentioned maximum mutant rate is 59.55 per thousand, comprising 24 seedling and plant mutants (stand No. 76, Kalmar). In the Sundmo experimental field no mutations were observed in the progeny of 720 trees, which makes 71 % of the total number of trees. The highest mutant rate of the 295 mutated trees was 25 % (stand No. 106, Harrsjön).

High spontaneous mutant rates in the progeny of individual trees command attention, but are not at all surprising. The ability of heterozygous individuals to absorb and preserve deleterious traits in the genotype in a concealed way is well known (DOBZHANSKY, 1952). Leaving the interpretation of the very essence of high mutant rates to be discussed later in this paper, the material aspect of research work should be ventilated first.

The frequency distribution of all the 410 mutated trees in the experimental field at Bogesund as well as their mutant rates are graphically expressed in fig. 2. The graph shows at the same time the number of the stands from which these trees have originated. Fig. 3 with similar contents refers to 295 mutated trees in the Sundmo experimental field.

The data are presented in the form of frequency polygons, no attempt being made to smooth the curve. The similarity of the polygons in figures 2 and 3 is obvious. The kind of frequency distribution which these polygons display corresponds to truncated logarithmico-normal distribution. The assumption can be made that this kind of frequency distribution is a common occurrence in the individual mutated pine trees and, consequently, analogous variation of mutant rates might be expected in all experiments with a sufficient number of trees. That, actually, this is not only the question of the variation of the mutation magnitude alone, but that the very character of mutations is also connected with the frequency distribution mentioned above, will be discussed later.

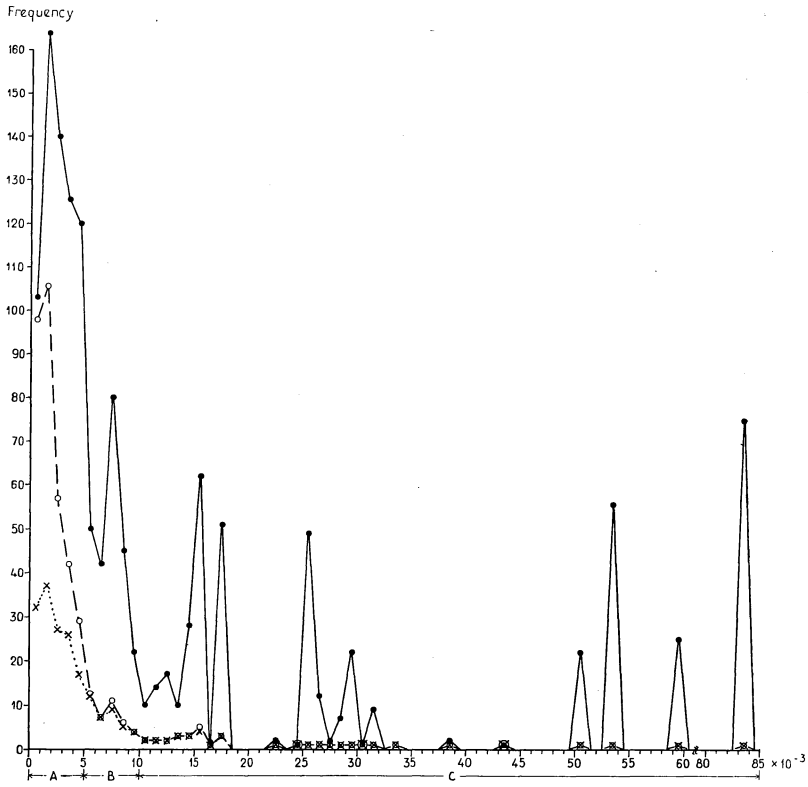

Fig. 2. Distribution of 410 heterozygous mother trees (— ○ —) and of 1,368 chlorophyll mutants (— ● —) found in the progeny of these trees in the field trial at Bogesund and also the distribution of 43 stands (. . . × . . .) from which the mother trees originated, all classified on the basis of mutant rates (class interval = one 10^{-3}).

Fördelningen av 410 heterozygota moderträd '— ○ —) och av 1 368 klorofyllmutanter (— ● —) som registrerats bland avkomman av dessa träd i fältförsöket vid Bogesund samt fördelning av 43 bestånd (. . . . × . . .), från vilka träden härstammade i mutantfrekvensklasser (klassbredd = en 10^{-3}).

The frequency polygon in fig. 2, containing data obtained in the experimental field at Bogesund, might be regarded as consisting of three parts. The first part (*A*) comprises frequency classes ranging from 0 to 5×10^{-3}; the second part (*B*) contains 5×10^{-3}—10×10^{-3} and the third part (*C*) embraces all the remaining frequency distribution spread from 10×10^{-3} to 84×10^{-3}. Part A contains the variation of lowest mutant rates as well as the polygon culmination. The statistical base of this part are 653 mutants in the progeny of 332 mother trees. On the average within the bounds of part A one stand has several mutated trees in the corresponding class. Part B is the descending part of the frequency polygon where the statistical base is 239 mutants and 41 mutated trees. Part C contains a considerable dispersion of mutation

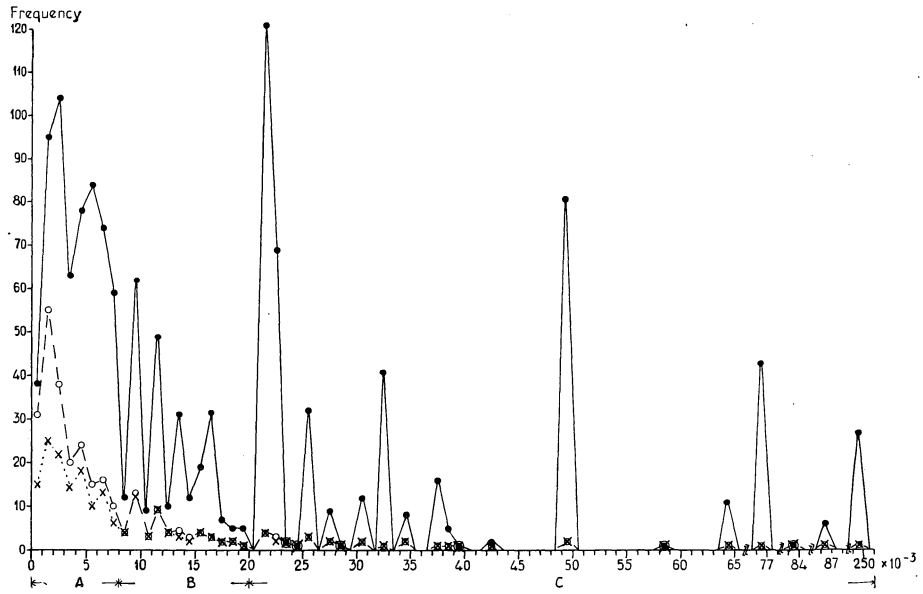

Fig. 3. Distribution of 295 heterozygous mother trees (— ○ —) and of 1,317 chlorophyll mutants (— ● —) found in the progeny of these mother trees in the field trial at Sundmo, and also the distribution of 41 stands (. . . × . . .) from which the mother trees originated, all classified on the basis of mutant rates (class interval = one 10^{-3}).

Fördelningen av 295 heterozygota moderträd (—○—) och av 1 317 klorofyllmutanter (—● —) som funnits i avkomman av dessa träd i fältförsöket vid Sundmo samt fördelning av 41 bestånd (. . . × . . .) från vilka träden härstammade i mutantfrekvensklasser (klassbredd = en 10^{-3}).

rates. The character of this dispersion is random. Statistically this part is represented by 476 mutants and 37 mother trees. The contents of this part of the frequency polygon are biologically most interesting.

The frequency polygon in fig. 3 containing data obtained in the Sundmo experimental field is divided into three parts similar to those in fig. 2. More detailed information regarding the contents of this frequency polygon is found in table 3. The analysis of the variation of mutant rates in parts A, B and C of frequency polygons is continued in the following chapter.

2. Types of Chlorophyll Mutations

In comparison with the species more closely investigated, a greater diversity is brought into the intricate problem of chlorophyll mutations in the pine by one factor, that is, time. Here time produces effect in the sense that the chlorophyll mutations can appear not only at the seedling stage or during the rest of the same vegetation period, but repeatedly within several vegetation periods, year after year. The scheme of the chlorophyll mutations in pine has

Table 3. Number of mutated trees and mutants in parts A, B, C of frequency polygons in figs. 2 & 3.

Antal muterade träd samt mutanter i A, B, C delar av frekvenspolygon i fig. 2 & 3.

Field trial at Bogesund (fig. 2)

	A	*B*	*C*	*Σ*
	$0—5\times10^{-3}$	$5\times10^{-3}—$ $—10\times10^{-3}$	$10\times10^{-3}—$ $—84\times10^{-3}$	
Number of heterozygous trees	332 *(81.0 %)*	41 *(10.0 %)*	37 *(9.0 %)*	410 *(100.0 %)*
Number of mutants	653 *(47.7 %)*	239 *(17.5 %)*	476 *(34.8 %)*	1 368 *(100.0 %)*

Field trial at Sundmo (fig. 3)

	A	*B*	*C*	*Σ*
	$0—8\times10^{-3}$	$8\times10^{-3}—$ $—20\times10^{-3}$	$20\times10^{-3}—$ $—250\times10^{-3}$	
Number of heterozygous trees	209 *(70.8 %)*	51 *(17.3 %)*	35 *(11.9 %)*	295 *(100.0%)*
Number of mutants	596 *(45.2%)*	251 *(19.1 %)*	470 *(35.7 %)*	1 317 *(100.0 %)*

been worked out accordingly (table 1). The classification of the chlorophyll mutations into types is based on the phenotypical effect of these mutations. The differences in this effect are at the same time the gauge for the segregation of the chlorophyll deficient factors and for the transition from the heterozygous into homozygous state. Depending on the changes in the environmental conditions at different stages of the development of the individual, the phenotypical effect of the mutations also becomes considerably subject to alterations. The environment as well as the diverse morphology and biology of the pine at different stages of development entangle the relationship between phenotype and genotype.

What are plant mutations in pine and what is the relationship between their types and those of the corresponding mutations at seedling stage? What kind of segregation in chlorophyll deficient factors of the heterozygous mother trees occurs, and what is the relationship between the different mutation types? The answer to these questions might be found in investigations where other methods, such as controlled pollination, inbreeding etc. could be used, and which would take several years of research work. The inferences reached in this paper are exclusively based upon the statistical data of the investigation regarding the mutants originating in the progeny of individual trees, and on the observations of the changes in the phenotype of each mutated individual.

From the total number of mutated trees in the Bogesund experimental field, mutations appeared in the progeny of 67 % of individual trees at seedling stage only. In 11 % of the mutated heterozygous trees mutations were observed at the seedling stage as well as in both or one of the plant stages, and in 22 % of the trees they appeared only at the plant stage (table 2). Only 12

Table 2. Number of trees with either seedling, plant mutants or with a combination of both mutant groups in their progeny

Antal träd med groddplant-, plant- eller groddplant- och plantmutanter i sin avkomma·

	Σ	Only Seed-lings	Seed-lings & plants 1951	Seed-lings & plants 1952	Seed-lings & plants 1951 & 1952	Plants 1951	Plants 1952	Plants 1951 & 1952
			Field trial at Bogesund					
Number of stands	43	41	16	7	8	20	18	21
Number of hetero-zygous trees...	410	275	24	8	12	26	33	32
	100.0 %	*67.1 %*	*5.9 %*	*2.0 %*	*2.9 %*	*6.3 %*	*8.0 %*	*7.8 %*
			44 (*10.7 %*)			81 (*22.2 %*)		
			Field trial at Sundmo					
Number of stands	41	40	2	10	11	5	15	17
Number of hetero-zygous trees...	295	213	2	15	12	5	26	22
	100.0 %	*72.2 %*	*0.7 %*	*5.1 %*	*4.1 %*	*1.7 %*	*8.8 %*	*7.4 %*
			29 (*9.9 %*)			53 (*17.9 %*)		

mother trees, that is 3 % of the total of the mutated trees, consistently repeated mutations throughout the three stages of development of their progeny, i.e., they produced seedling mutants, plant mutants of the first and plant mutants of the second year. It is, however, remarkable that the mutation rates of these trees are by no means higher than those of other trees. The data obtained in the Sundmo experimental field are similar to those mentioned above.

The fact that mutations appear continuously first at the seedling stage and then subsequently in the first and second year plants clearly shows the consistency of this process, which is even more definitely confirmed when observing the cases of individual mutations. As an example might be mentioned those mutants which, at the seedling stage, show very slight traces of chlorophyll deficiency, and which, not infrequently, take on a completely normal green appearance. However, at further stages of development, as plants, these mutants repeatedly and even strikingly show their mutation character. This metamorphosis can be repeated in these mutants when passing into the second year and also during the course of further development. The variation of the phenotypic effect in the above mentioned mutants is very wide and arouses attention. In any case the environment greatly modifies this variation.

The genetics of seedling and plant mutants should be connected with the mutability of certain definite alleles. It might be assumed that the kind as well as the number of the mutated genes controlling the activity of chlorophyll determine whether the mutation emerges at seedling stage, in the first, or in

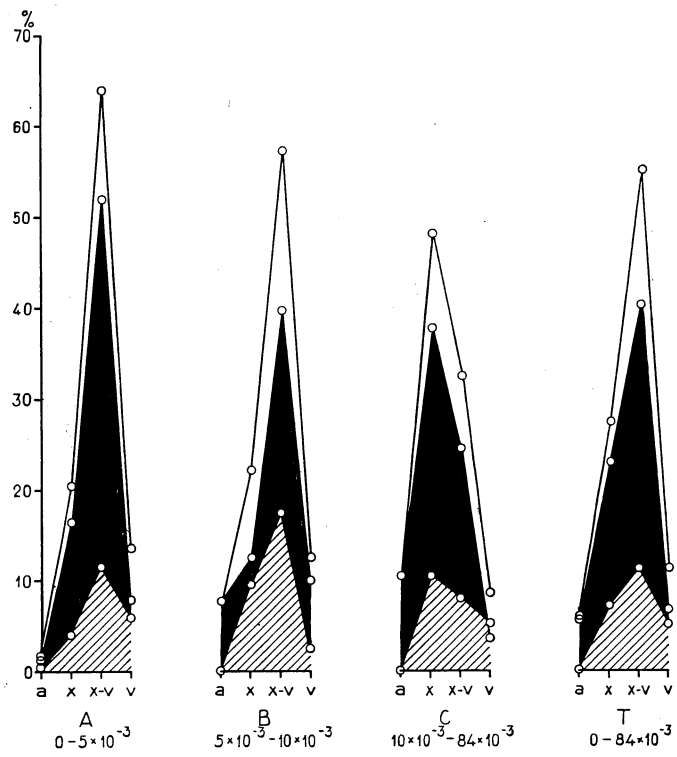

Fig. 4. Relative frequencies of seedling mutants ( ), of plant mutants ( ) and of the total of both mutant groups ( ) and also their distribution into mutation types (a—*albina*, x—*xantha*, x-v—*xanthoviridis*, v—*viridis*) in parts A, B, C as well as in the whole (T) of the frequency polygon in fig. 2, in the field trial at Bogesund.

Relativa frekvenser av groddplantmutanter ( ), plantmutanter ( ) och summan av bägge mutantgrupperna ( ) och deras fördelning på mutationstyper (a-*albina*, x-*xanaht-* x—v-*xanthoviridis*, v — *viridis*) i frekvenspolygondelar A, B, C samt av hela frekvenspolygonet i fig. 2 i fältförsöket vid Bogesund.

the second year plants. Accordingly chlorophyll mutations in pine seedlings and plants should be associated with continuous, or polygenic, variability. Polygenic variability, the mechanisms of gene action in the development of individuals as well as environmental influencies during the development, might be the factors causing the strikingly wide variation of the phenotypic effect in seedling and plant mutations.

The distribution of chlorophyll mutants into seedling and plant mutants and their subsequent distribution into types are expressed in figures 4 and 5 and in table 4, showing their relative frequencies. In order to make the graphical expression more conspicuous the plant mutants of 1951 and 1952 are com-

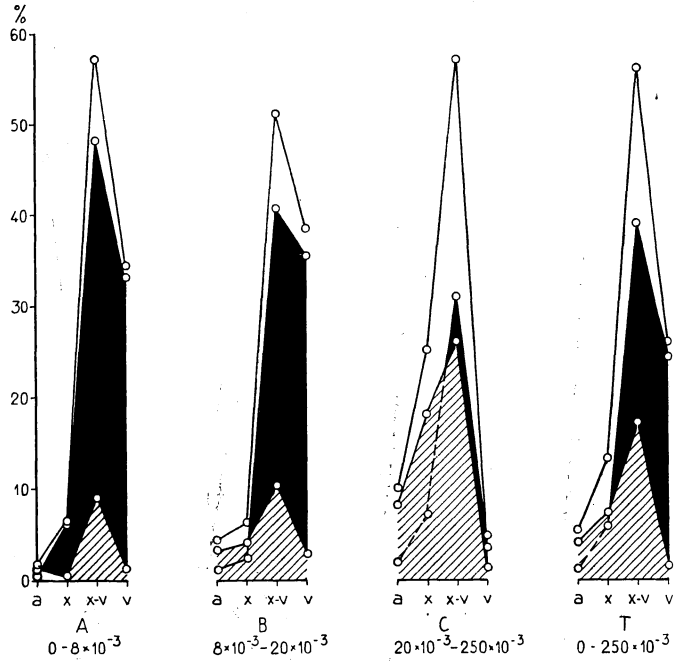

Fig. 5. Relative frequencies of seedling mutants (▲), of plant mutants (▨) and of the total of both mutant groups (▲) and also their distribution into mutation types (a—*albina*, x—*xantha*, x-v—*xanthoviridis*, v—*viridis*) in parts A, B, C as well as in the whole (T) of the frequency polygon in fig. 3., in the field trial at Sundmo.

Relativa frekvenser av groddplantmutanter (▲) av plantmutanter (▨) och summan av bägge mutantgrupperna (▲) och deras fördelning på mutationstyper (a — *albina*, x — *xantha*, x-v — *xanthoviridis*, v — *viridis*) i frekvenspolygondelar A, B, C samt av hela (T) frekvenspolygonet i fig. 3 i fältförsöket vid Sundmo.

bined. The graphs A, B, C and T agree with the corresponding parts of frequency polygons (figs. 2 and 3) designated by the same letters. The distribution of the relative frequencies within the graphs T in both figures impresses one by its uniform character.

From the biological point of view it is important to state that the maximum of the relative frequencies in the polygons of both seedling and plant mutants coincides in both graphs. These frequency distributions are, of course, only an average result of the whole investigation mass, and the variation of mutations in the progeny of individual trees actually gives a dispersion and a deviation from this average result. Nevertheless, even as a general estimate, this result once again calls to mind the mutual relationship existing between seedling and plant mutants.

The segregation of chlorophyll deficient factors into mutation types in the

Table 4. Distribution of relative frequencies of chlorophyll mutants into types in field trials.
Fördelning av klorofyllmutantfrekvenser i olika mutationstyper i fältförsöken.

Mutation types	Part *A*		Part *B*		Part *C*		Σ	
	of the frequency polygons i figs. 2 & 3							
	Seedlings	Plants	Seedlings	Plants	Seedlings	Plants	Seedlings	Plants
				Field trial at Bogesund				
Albina	8	3	19	—	50	—	77	3
	Σ = 11	(1.6 %)	Σ = 19	(7.9 %)	Σ = 50	(10.5 %)	Σ = 80	(5.9 %)
Xantha	107	16	30	19	180	22	317	57
	Σ = 123	(20.4 %)	Σ = 49	(22.2 %)	Σ = 202	(48.3 %)	Σ = 374	(27.4 %)
Xanthoviridis	343	89	95	46	117	66	555	201
	Σ = 432	(64.0 %)	Σ = 141	(57.4 %)	Σ = 183	(32.6 %)	Σ = 756	(55.4 %)
Viridis	52	35	24	6	16	25	92	66
	Σ = 87	(14.0 %)	Σ = 30	(12.5 %)	Σ = 41	(8.6 %)	Σ = 158	(11.3 %)
Σ	510	143	168	71	363	113	1041	327
	Σ = 653	(100.0 %)	Σ = 239	(100.0 %)	Σ = 476	(100.0 %)	Σ = 1368	(100.0 %)
				Field trial at Sundmo				
Albina	3	8	3	8	9	38	15	54
	Σ = 11	(1.8 %)	Σ = 11	(4.4 %)	Σ = 47	(10.0 %)	Σ = 69	(5.2 %)
Xantha	36	3	6	10	34	84	76	97
	Σ = 39	(6.6 %)	Σ = 16	(6.4 %)	Σ = 118	(25.1 %)	Σ = 173	(13.1 %)
Xanthoviridis	287	54	102	26	122	145	511	225
	Σ = 341	(57.2 %)	Σ = 128	(51.0 %)	Σ = 267	(56.8 %)	Σ = 736	(55.9 %)
Viridis	198	7	89	7	32	6	319	20
	Σ = 205	(34.4 %)	Σ = 96	(38.2 %)	Σ = 38	(8.1 %)	Σ = 339	(25.8 %)
Σ	524	72	200	51	197	273	922	395
	Σ = 596	(100.0 %)	Σ = 251	(100.0 %)	Σ = 470	(100.0 %)	Σ = 1317	(100.0 %)

progeny of heterozygous individual trees displays a wide variation. The low mutant rates hardly give any support for conclusions. Likewise, the trees having the highest mutation rates show most varied possibilities. On the other hand, the average data (graphs T in figs. 4 and 5, table 4) manifest tangible tendencies.

The low relative frequencies of the *albina* type are a common occurrence in the chlorophyll mutations in pine. In the Sundmo experimental field the relative frequencies of this type in the seedling mutants are even lower than those in plant mutants. This particular case should be accounted for by the low germinating capacity of the seeds possessing *albina* factors (figs. 6 and 7). In the like manner, there are only a few conspicuous examples of high *albina* frequencies in the individual trees. Two trees attain prominence on account of their high frequencies of *albina* seedlings and a few trees on account of the high frequencies of *albina* plants. As regards low mutation frequency

of the *albina* type, pine stands in contrast to barley, but it shows analogy with the pea species (GUSTAFSSON, 1951).

Mutation frequencies of the *xantha* type are considerably higher than those of the *albinas*. They have a shifting value in the different parts of the frequency polygon. In fig. 4 graph C the *xantha* frequencies are even higher than those of all other types. On the other hand, *alboviridis* mutations are rare and only in the second year plant mutants had it been possible to set them apart from the *xanthoviridis* type.

In both experimental fields the *xanthoviridis* type dominates the other types. The maximum of the relative frequencies is to be found in this type. The *viridis* type is only slightly represented in the Bogesund experimental field, contrary to the Sundmo field, where the relative frequencies of this type are very high. It would be rash to attempt to give a definite answer to the question whether this phenomenon is of a fundamental character or only accidental. Replications with the corresponding seed samples in the greenhouse confirmed the high mutant frequencies of the *viridis* type obtained at Sundmo, which is shown in fig. 8. This leads to the conclusion that mutations of the *viridis* type arise more often in northern localities (represented in the Sundmo experimental field) than in southern.

When considering the frequency distribution of chlorophyll mutation types, it is hardly possible to ignore the relationship existing between this frequency distribution and the value of mutant rates of the individual trees. One might allege that the increasing mutant rates have a very definite tendency to advance the degree of chlorophyll deficiency. Generally low values of mutant rates do not furnish a satisfactory statistical basis for expressing the relationship between the two variables in a kind of correlation. On the other hand, if the frequency polygons (figs. 2 and 3) are divided into parts A, B, and C and the relative frequencies of the mutation types are computed for each part of the polygon separately, the relationship assumes a very conspicuous shape (figs. 4 and 5). The relative frequencies of the *xantha* and partly also those of the *albina* type in parts B and C of the polygons, increase at the expense of the *viridis* and *xanthoviridis* types. A striking example is found in fig. 4, graph C where the *xantha* type even reaches the maximum. In any case, this phenomenon is most conspicuously manifested in graph C, in both figures. The contents of these graphs, as already mentioned above, are obtained from the trees having highest mutant frequencies (tables 2 and 3).

The problem regarding the relation existing between the values of mutant rates and the distribution of mutation types should be regarded as consisting of two parts. In the first place comes the question as to what causes high mutant rates, the answer to which should be found in the reproductive mechanism of mother trees producing such high mutant rates. The simplest and most

plausible interpretation here is the self-fertilising capacity of these trees. Complete or partial inbreeding seems to be the only explanation of high mutant rates, at least in most of those trees which are found in part C (figs. 2 and 3) of the frequency polygons. One particular tree (stand No. 106), whose mutant rate is 25 per cent, illustrates most conspicuously the segregation of the recessive chlorophyll deficient factors in the process of inbreeding.

The second part of the problem is the increase in the mutations of the *xantha* and partly also of the *albina* type in trees having high mutant rates. The cause of this phenomenon can be important destructive changes in the genotype. A hypothesis that might be assumed is an increased number of the mutated alleles. It should also be stated that in pine these deviations from the average segregation ratio in the cases of high mutant rates are similar to those mutational changes which are obtained in irradiation experiments and which also occur in spontaneous mutations in barley (GUSTAFSSON, 1951). In the absence of other tests on the genotype of mother trees these inferences have only a conjectural value.

3. Rates of Chlorophyll Mutation Types

In the preceding chapter the segregation of chlorophyll deficient factors into mutation types was examined from the point of view of the relative frequencies of individual mutants. Simultaneously the segregation ratio served as the average value obtained from the number of all mutants in each of the field trials. But in order to get an idea of how frequently each of the chlorophyll mutation types appears and in what combination mutation types emerge in the progeny of individual trees it is necessary to view the problem from another aspect.

The statistical basis of this chapter consists of two series of figures. The first of these series shows the number of mutation cases, i.e. how often each of the mutation types appears, and in the progeny of how many individual trees each mutation type emerges, single or in combination with other types. This series is expressed in table 5 for the Bogesund field trial and in table 6 for that at Sundmo. The second series shows the variation of the number of individual mutants in each mutation case according to mutation types, shown in table 7 for the Bogesund field trial and in table 8 for that at Sundmo.

As regards the number of mutation types in the progeny of individual trees no significant deviations appear in either of the trials (tables 5 and 6). It is a common occurrence that chlorophyll mutations emerge as one single type. 74 % of all mutated trees in the Bogesund field trial and 61 per cent at Sundmo confirm this observation. Two mutation types appear in the progeny of 21 per cent of the mother trees in the Bogesund field trial and in 34 per cent in that at Sundmo. On the other hand, three mutation types manifest

Table 5. Mutation types in the progeny of individual trees in the field trial at Bogesund.
Förekomst av mutationstyper i enskilda träds avkomma i fältförsök vid Bogesund.

Mutation type	Number of mutation types				
	1	2	3	4	Σ
		Number of mutations			
Albina (A)	A 6 *(1.1 %)*	A + X 2 *(0.4 %)* A + X-v 2 *(0.4 %)* 4 *(0.8 %)*	A + X + X-v 2 *(0.4 %)* A + X + V 2 *(0.4 %)* 4 *(0.8 %)*	A + X + X-v + V 1 *(0.2 %)*	15 *(2.8 %)*
Xantha (X)	X 53 *(9.8 %)*	X + X-v 53 *(9.8 %)* X + A 2 *(0.4 %)* X + V 2 *(0.4 %)* 57 *(10.5 %)*	X + X-v + V 16 *(2.9 %)* X + A + X-v 2 *(0.4 %)* X + A + V 2 *(0.4 %)* 20 *(3.7 %)*	X + A + X-v + V 1 *(0.2 %)*	131 *(24.2 %)*
Xanthoviridis (X-v)	X-v 197 *(36.5 %)*	X-v + X 53 *(9.8 %)* X-v + V 28 *(5.2 %)* X-v + A 2 *(0.4 %)* 83 *(15.4 %)*	X-v + X + V 16 *(2.9 %)* X-v + A + X 2 *(0.4 %)* 18 *(3.3 %)*	X-v + A + X + V 1 *(0.2 %)*	299 *(55.4 %)*
Viridis (V)	V 46 *(8.5 %)*	V + X-v 28 *(5.2 %)* V + X 2 *(0.4 %)* 30 *(5.6 %)*	V + X-v + X 16 *(2.9 %)* V + X + A 2 *(0.4 %)* 18 *(3.3 %)*	V + A + X + X-v 1 *(0.2 %)*	95 *(17.6 %)*
Σ	302 *(55.9 %)*	174 *(32.2 %)*	60 *(11.1 %)*	4 *(0.8 %)*	540 *(100.0 %)*
		Number of mutated trees			
Σ	302 *(73.7 %)*	A + X 2 A + X-v 2 X + X-v 53 X-v + V 28 X + V 2 87 *(21.2 %)*	A + X + X-v 2 A + X + V 2 X + X-v + V 16 20 *(4.9 %)*	1 *(0.2 %)*	410 *(100.0 %)*

Table 6. Mutation types in the progeny of individual trees in the field trial at Sundmo.
Förekomst av mutationstyper i enskilda träds avkomma i fältförsök vid Sundmo.

Type of mutation	Number of mutation types				
	1	2	3	4	Σ
		Number of mutations (cases)			
Albina (A)	A 10 *(2.3 %)*	A + X 1 *(0.2 %)* A + X-v 2 *(0.5 %)* 3 *(0.7 %)*	A + X + X-v 5 *(1.2 %)* A + X-v + V 2 *(0.5 %)* 7 *(1.6 %)*	A + X + X-v + V 5 *(1.2 %)*	25 *(5.8 %)*
Xantha (X)	X 11 *(2.6 %)*	X + X-v 12 *(2.8 %)* X + A 1 *(0.2 %)* X + V 2 *(0.5 %)* 15 *(3.5 %)*	X + A + X-v 5 *(1.2 %)* X + X-v + V 3 *(0.7 %)* 8 *(1.9 %)*	X + A + X-v + V 5 *(1.2 %)*	39 *(9.1 %)*
Xanthoviridis (X-v)	X-v 101 *(23.5 %)*	X-v + X 12 *(2.8 %)* X-v + V 83 *(19.3 %)* X-v + A 2 *(0.5 %)* 97 *(22.6 %)*	X-v + X + A 5 *(1.2 %)* X-v + X + V 3 *(0.7 %)* X-v + A + V 2 *(0.5 %)* 10 *(2.3 %)*	X-v + A + X + V 5 *(1.2 %)*	213 *(49.5 %)*
Viridis (V)	V 58 *(13.5 %)*	V + X-v 83 *(19.3 %)* V + X 2 *(0.5 %)* 85 *(19.8 %)*	V + A + X-v 2 *(0.5 %)* V + X-v + X 3 *(0.7 %)* 5 *(1.2 %)*	V + A + X + X-v 5 *(1.2 %)*	153 *(35.6 %)*
Σ	180 *(41.7 %)*	200 *(46.5 %)*	30 *(7.0 %)*	20 *(4.7 %)*	430 *(100.0 %)*
		Number of mutated trees			
Σ	180 *(61.0 %)*	A + X 1 A + X-v 2 X + X-v 12 X + V 2 X-v + V 83 100 *(33.9 %)*	A + X + X-v 5 A + X-v + V 2 X-v + X + V 3 10 *(3.4 %)*	5 *(1.7 %)*	295 *(100.0 %)*

themselves in only 4.9 and 3.4 per cent of the trees respectively. All four mutation types reveal themselves in only one of the mother trees (0.2 %) at Bogesund and in that of 5 mother trees at Sundmo (1.7 %). With regard to the latter case it is interesting to note that mutant rates of these trees are also high, although they are not the highest of all mutated trees. This shows that mutant rates of individual trees increase when several chlorophyll mutation factors coincide. However, as stressed before, in the case of high mutant rates of individual trees, the number of chlorophyll mutation types is by no means of decisive importance.

Nor are there any significant deviations in regard to the frequency of mutation cases or in regard to the combination of mutation types in the progeny of individual trees (tables 5 and 6) in either of the field trials. Only the sequence of the total values (Σ) of mutation frequencies 56 %, 32 %, 11 %, 1 % which corresponds to the mutation cases containing one, two, three or four of the mutation types in the Bogesund field trial deviates from that at Sundmo, where the sequence is 42 %, 46 %, 7 % and 5 %. As far as it is not a matter of chance, the deviation of these values in the Sundmo trial might be associated with the divergent germination of seeds at Sundmo, which is dicussed later in the paper (figs. 6, 7 and 8). This particularly refers to individual trees whose mutant rates are low. Each of the mutation types displays a similar falling sequence of mutation frequencies. Only the *xantha* type does not follow this rule. Its sequence of mutation frequencies is as follows:

At Bogesund — 9.8 %, *10.5* %, 3.7 % and 0.2 %.
At Sundmo — 2.6 %, *3.5* %, 1.9 % and 1.2 %.

The distribution of all mutation cases (Σ) into types is as follows:

	albina	*xantha*	*xanthoviridis*	*viridis*
Bogesund trial.....	3 %	24 %	55 %	18 %
Sundmo »	6 %	9 %	49 %	36 %

These relative values differ from the values of relative frequencies which show the distribution of mutants into types (table 4):

	albina	*xantha*	*xanthoviridis*	*viridis*
Bogesund trial.....	6 %	27 %	55 %	11 %
Sundmo »	5 %	13 %	56 %	26 %

The explanation of the divergency between the two series of values is found in the average number (M) of individual mutants per mutation (tables 7 and 8), which shows the following sequence of mutation types:

Table 7. Number of mutations with 1, 2, 3 and > 3 individuals per mutation in the progeny of trees in field trial at Bogesund.

Antal mutationer med 1, 2, 3 och > 3 mutanter/mutation i enskilda träds avkomma vid Bogesund.

Mutation type	Number of individual mutants per one mutation					Σ
	1	2	3	> 3	Mean	
Albina	9 (60.0 %)	1 (6.7 %)	2 (13.3 %)	3 (20.0 %)	5.3	15 (2.8 %)
Xantha	88 (67.2 %)	23 (17.5 %)	11 (8.4 %)	9 (6.9 %)	2.9	131 (24.2 %)
Xanthoviridis	144 (48.2 %)	65 (21.7 %)	27 (9.0 %)	63 (21.1 %)	2.5	299 (55.4 %)
Viridis	66 (69.5 %)	20 (21.0 %)	3 (3.2 %)	6 (6.3 %)	1.6	95 (17.6 %)
Σ	307 (56.8 %)	109 (20.2 %)	43 (8.0 %)	81 (15.0 %)	2.5	540 (100.0 %)

	albina	*xantha*	*xanthoviridis*	*vididis*	the mean
Bogesund trial	5.3	2.9	2.5	1.6	2.5
Sundmo »	2.6	4.5	3.5	2.2	3.1

The tendency shown by these figures is obvious. This sequence of mutation types is similar to "the hypothetical sequence in the genotypical size of the different types" (GUSTAFSSON, 1936), which was obtained in the progeny of X-rayed seeds in the experiments with barley. In the *albina* type the average number of individual mutants per mutation is higher than in all other types, the only exceptions being the low values of the *albina* type in the Sundmo field trial. According to GUSTAFSSON the cause of this fact probably is that the *albina* mutations involve smaller changes in the genotype than the other mutations.

The distribution of all mutations, grouped as the total of all mutation

Table 8. Number of mutations with 1, 2, 3 and > 3 mutants per mutation in the progeny of trees in field trial at Sundmo.

Antal mutationer med 1, 2, 3 och > 3 mutanter/mutation i enskilda träds avkomma i fältförsöket vid Sundmo.

Mutation type	Number of individual mutants per one mutation					Σ
	1	2	3	> 3	Mean	
Albina	14 (56.0 %)	5 (20.0 %)	3 (12.0 %)	3 (12.0 %)	2.6	25 (5.8%)
Xantha	17 (43.6 %)	10 (25.6 %)	3 (7.7 %)	9 (23.1 %)	4.5	39 (9.1 %)
Xanthoviridis	86 (40.4 %)	50 (23.5 %)	24 (11.3 %)	53 (24.8 %)	3.5	213 (49.5%)
Viridis	79 (51.6 %)	28 (18.3 %)	21 (13.7 %)	25 (16.4 %)	2.2	153 (35.6 %)
Σ	196 (45.6 %)	93 (21.6 %)	51 (11.9 %)	90 (20.9 %)	3.1	430 (100.0 %)

types (Σ) into classes with 1, 2, 3 and > 3 individual mutants shows the following series of values:

Bogesund trial.....	57 %	20 %	8 %	15 %
Sundmo »	46 %	22 %	12 %	21 %

This means that slightly more than half of all mutations manifest themselves by only one mutant and approximately only 25 % to 30 % of all mutation cases contain $\geqq 3$ mutants per mutation. It should be stressed, however, that these series of values show a striking similarity to the results obtained in the above experiments with barley, although the biological differences between the two species are well known.

Those mutations which manifest themselves by only one mutant per mutation are regarded by GUSTAFSSON (1936) as "probably associated with great structural alterations within the genome (macromutations)". On the other hand, mutations which manifest themselves by $\geqq 3$ mutants per mutation should be considered as minor mutations. If these conclusions might be applied to spontaneous chlorophyll mutations in pine, which seems to be justified, then the origin of most mutations of the *viridis*, of the *xanthoviridis* and of the *xantha* type should be associated with the structural changes in the genotype (tables 7 and 8).

The data of the present investigation alone do not afford the possibility to penetrate deep enough into the genetic background of chlorophyll mutations in pine. Other methods would have to be used before a final conclusion might be drawn. However, when the results of the present investigation are compared with those obtained in the experiments repeatedly carried out with barley, several important analogous cases are seen. At the same time there is a considerable divergency in the segregation ratio of mutation factors in the mutation types of the two species, as stated in the previous chapter. The remarkably low mutation rates of the *albina* type and the high rates of the *xanthoviridis* and *viridis* types in pine stand in striking contrast to the corresponding values in barley.

It is difficult to associate this phenomenon with another fact already mentioned, that is, that the average number of mutants per mutation in the *albina* type is consistently greater in both species than in other mutation types. This contradiction is left unexplained for the time being, although some observations discussed later in the paper (figs. 6 and 7) regarding the germination of seeds might suggest that one of the conjectural explanations of the above mentioned phenomenon could be zygotic sterility. At least a certain decrease in the germinating capacity of homozygotes, which varies in different mutation types, might be expected.

4. Impact of Environment on Chlorophyll Mutations

Replications in greenhouses under controlled light and temperature conditions were made in the winters of 1953 and 1954 with several mother trees whose progeny had previously been put to trial in the experimental fields at Bogesund and Sundmo (see chapter 1). The same seed samples, preserved under similar conditions, had been used in these replications as in the field trials of 1951. In the tests of 1953 mother trees with considerably high mutant rates were chosen, whereas in mother trees employed in the trials of 1954 these rates were comparatively low. In both tests a few common samples were used as connections, and some of the samples which in the field conditions had not produced any mutations were also tested.

During the four months (from December to April) the temperature was kept from + 13° to + 14° C with occasional variations down to + 12° and up to + 16° C. Seedlings were exposed to the light of four and six 40-W daylight lamps respectively, mounted on a white board at a distance of 70 cm from the lamp bulbs. This illumination supplemented the natural light from 4 a.m. to 10 p.m.

The difference in the climate of the field trials and of that in the greenhouses might be characterised in the following way. In field conditions the temperature fluctuated in the course of the day and night from a few degrees above zero to about + 35° C and the periodical changes of light were more marked at Bogesund than at Sundmo, where in summer the days are longer but the temperature somewhat lower.

The trials in the greenhouses showed a definite and general tendency to give higher mutant rates than in the field conditions. In order to obtain reliable mathematically statistical evidence the results of the trials in the fields as well as in the greenhouses were compared by using the analysis of variance according to SNEDECOR's "The Method of Proportional Subclass Numbers Using Expected Sums and Numbers". This analysis

Table 9. Analysis of variance of mutant rates between the field trials of 1951 and the greenhouse tests of 1953.

Variansanalys av mutantfrekvenser mellan fältförsök 1951 och växthusförsök 1953.

Source of variation	Df	Sum of squares	Mean squares
Individual trees	22	5.71828	0.25992
Field—greenhouse	1	0.13772	0.13772
Interaction	22	0.66674	0.03031
Individuals (Error)	38,137	344.23989	0.00903

Test for interaction, $F = 3.357^{***}$ ($P<0.001$).
Test for field—greenhouse, $F = 4.544^{*}$ ($P = 0.05$).

Table 10. Analysis of variance of mutant rates between the field trials of 1951 and in the greenhouse tests of 1954.

Variansanalys av mutantfrekvenser mellan fältförsök 1951 och växthusförsök 1954.

Source of variation	Df	Sum of squares	Mean square
Individual trees	27	0.8013	0.02968
Field—greenhouse	1	0.8201	0.82010
Interaction	27	1.6359	0.06059
Individuals (Error)	41,377	233.2330	0.00564

Test for interaction, $F = 10.821^{***}$ ($P<0.001$).
Test for field—greenhouse, $F = 13.533^{***}$ ($P = 0.001$).

shows that the difference between the field and greenhouse mutation rates is quite significative ($P < 0.001$) for those mother trees whose mutant rates are relatively low (table 10, fig. 7). On the other hand, in the greenhouse replication of 1953 containing high mutant rates of individual trees (fig. 6, table 9) this difference exists, but with less evidence ($P = 0.05$).

The increase of the mutant rates in greenhouse trials can be explained by a greater germinating capacity of the seeds than in the field trials (figs. 6 and 7, table 11). This circumstance is particularly revealed in the greenhouse tests of the winter of 1954 (fig. 7). The increase in the germination of seeds and the increase in mutant rates have a close reciprocal relationship. Unfortunately the number of variations in the experimental data was not sufficient to give the phenomenon the expression of a statistical correlation.

From the biological point of view this phenomenon calls for some consideration. The phenomenon is by no means explained by merely stating that mutant rates in greenhouse trials increase on account of the divergency in the germination of seeds in greenhouse and field conditions respectively. The answer to the question as to whether the seeds endowed with the chlorophyll deficient factors germinate less than the others should also be found. Actually, chlorophyll mutants appear all through the germination period simultaneously with normal seedlings, as early as on the tenth day. A slight tendency, however, to give relatively more mutants can be discerned in the seeds with delayed germination than in those germinating sooner, that is, in the seeds whose germinating energy is greater. The circumstance that dwarf chlorophyll mutants in the greenhouses are by no means a rarity (particularly *albinas* and *xanthas*), whereas they are hardly ever found in the field trials, also proves that the seeds with dwarf and chlorophyll deficient traits have a lower germination capacity. Most of the seeds that do not germinate in the field sprout in greenhouses stimulated by regular temperature and moisture conditions, revealing their chlorophyll deficiencies. Regarding the low germination of seeds and low germination energy, the double character of these

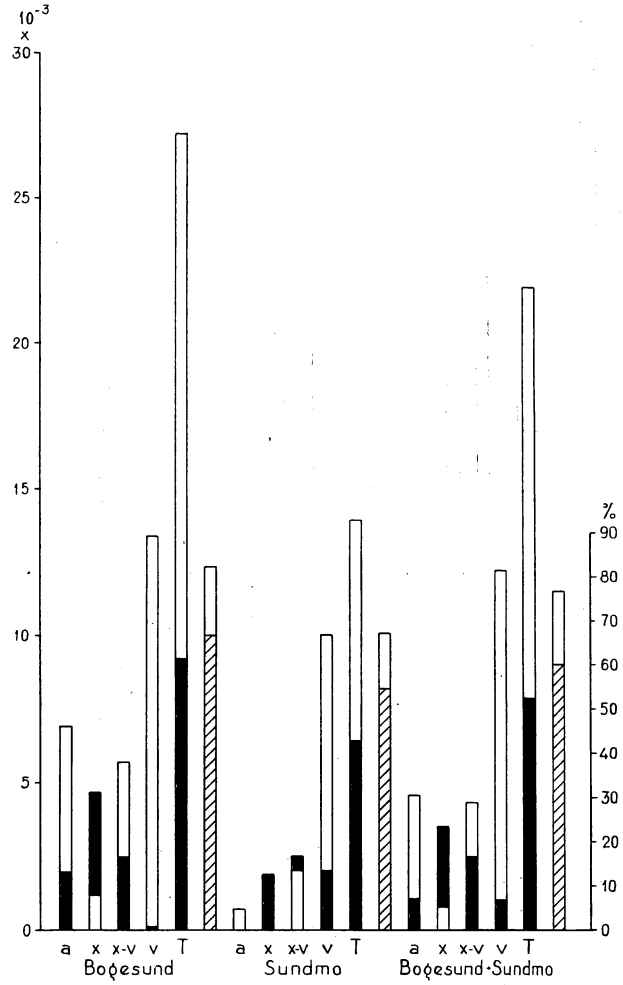

Fig. 6. Mutant rates of *albina* (a), of *xantha* (x), of *xanthoviridis* (x-v) and *viridis* (v) types and of the total (T) of these types in the progeny of 23 heterozygous trees in the greenhouse (▯), tested in 1953, and in the field (▮), tested in 1951 as well as the percentage of germinated seeds in the greenhouse (▯) and in the field (▨),

Mutantfrekvenser av *albina* (a), *xantha* (x), *xanthoviridis* (x-v) och *viridis* (v) typer och av deras summa (T) i avkomman av 23 heterozygota träd i växthusförsök (▯), år 1953, och i fältförsök, (▮) år 1951, samt fröets groningsprocent i växthus- (▯) och i fältförsök (▨).

phenomena should be mentioned. They may be either modifications in cases where seeds do not mature on account of insufficient temperature during the vegetation period, or they may be of genotypical nature (GUSTAFSSON and

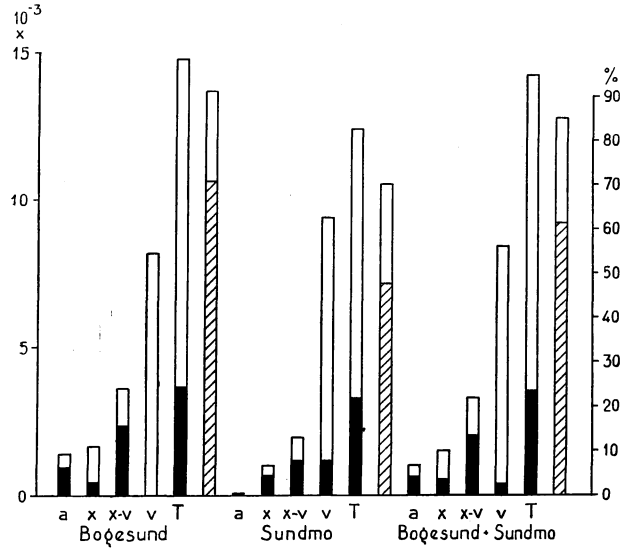

Fig. 7. Mutant rates of *albina* (a), *xantha* (x), *xanthoviridis* (x-v) and *viridis* (v) types and of their total (T) in the progeny of 28 heterozygous mother trees in the greenhouse (▯), tested in 1954 and in the field (▮), tested in 1951 as well as the percentage of germinated seeds in the greenhouse (▯) and in the field (▨),

Mutantfrekvenser av *albina* (a), *xantha* (x), *xanthoviridis* (x-v) och *viridis* (v) typer och av deras summa (T) i avkomman av 28 heterozygota träd i växthusförsök (▯), år 1954, och i fältförsök (▮), år 1951, samt fröets groningsprocent i växthus- (▯) och i fältförsök (▨).

Table 11. Relation between percentage of germinated seeds and mutant rates in the field trials of 1951 and in the greenhouse tests of 1953 & 1954.

Relation mellan procenttal grodda frön och mutantfrekvenser i fältförsök 1951 och växthusförsök 1953 & 1954.

	Percentage of germinated seeds		Mutant rates	
	In field trials	In green-house tests	In field trials	In green-house tests
1953. At Bogesund.....	66.2	82.2	9.1	27.2
At Sundmo.....	54.0	66.9	6.3	12.9
Σ	59.9	75.7	7.8	21.8
1954. At Bogesund.....	71.1	91.0	3.6	14.8
At Sundmo.....	47.7	69.9	3.3	12.3
Σ	61.2	85.0	3.5	14.2

SIMAK, 1953). It is the genotypical character of low germination energy that might be associated with chlorophyll deficient mutations.

The increase of mutant rates depending on the ageing of seeds on account of storage has been stated by many authors (GUSTAFSSON, 1936; STUBBE,

1952; KNAPP, 1941). In the present investigation on pine the significance of ageing in seeds was not elucidated.

The influence of light and temperature on the colour effect of the mutants is well known and has been described in literature. In dissimilar conditions one and the same mutation can reveal itself in different ways. In barley (GUSTAFSSON and NYBOM, 1950) *lutescens* mutation of *viridis* type grows yellow in field conditions, but in the greenhouse it is hardly discernible from normal individuals. This mutation is fully analogous to the corresponding mutation in pine. Likewise, the dissimilarity between the different mutation types in pine is more striking in field conditions than in the greenhouse. In this respect, too, pine displays analogy with barley (HOLM, 1954). On the other hand, the coloration of normal individuals in pine in field conditions is not so regular as in the greenhouse, which makes it more difficult to distinguish normal individuals from mutants. In the greenhouse, however, *viridis* type mutation, as mentioned above, supplies a continuous transition to normal individuals.

The results of the investigations in greenhouses and field experiments are compared in figures 6, 7 and 8. The results regarding the germination of seeds and mutant rates were interpreted previously. The distribution of mutations into types, in its turn, shows the faculty of the mutations to manifest themselves in a different manner depending on the environmental conditions. When interpreting this distribution *albinas* should be regarded apart from the other types, since *albinas* manifest themselves in a similar manner both in the greenhouse and in the field, but of course the mutation frequencies are higher in the greenhouse than in the field.

The destructive nature of the chlorophyll apparatus in *albina* mutations has been investigated in other plants. GUSTAFSSON (1946) characterizes the *albina* type in barley as being below the chromophoric threshold and (1942) states that the *albina* types in barley have a low number of minute and colourless plastids. v. EULER (1951) also explains that the primary cause of chlorophyll deficiency in *albinas* is the degeneration of plastids. KOSKI and SMITH (1951) investigating *Zea Mays White* seedling-3 come to the conclusion that "its chlorophyll deficiency is not due to a block of some step in the biosynthesis of chlorophyll, but rather to its inability to preserve chlorophyll when once formed".

Besides the above mentioned *albina* characteristics, one more peculiarity is discernible in pine, that is, a markedly expressed accumulation of anthocyanin at the base of the hypocotyl. The question as to whether the presence of anthocyanin in so great a concentration might be the cause of a particular physiological process characteristic for the *albina* mutations, has not been discussed in this paper. This peculiarity in the *albina* mutation of the pine

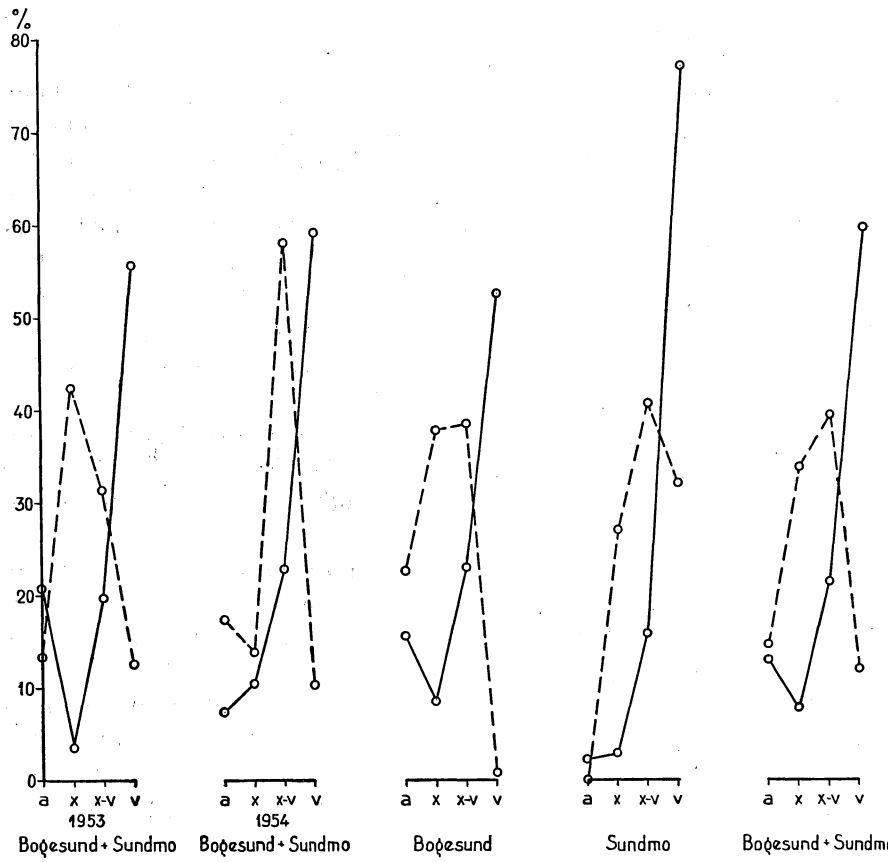

Fig. 8. Distribution of relative frequencies of mutants into the mutation types both in the greenhouse tests (—○—) and in the field trials (— —○— —). The same data and signs as used in figures 6 and 7.

Fördelning av relativa mutantfrekvenser i mutationstyper i växthus (—○—) och i fältförsök (— —○— —). Samma data och tecken som använts i fig. 6 och 7.

does not change, or rather remains almost unchanged both in the greenhouse and in the field.

The faculty of the other chlorophyll mutations to change their colour effect, depending on the environmental conditions, was previously mentioned. Doubtless the faculty of the mutation to manifest itself in a different manner is based on the anatomically physiological characteristics of the mutants. One of the most important of these characteristics is the shape and the coloration of the plastids, and the other, the biosynthesis of chlorophyll. Both can vary considerably in different mutations. The pigmentation of plastids varies from colourless in the case of lightest *xantha* types to subnormal or normal in *viridis* (GUSTAFSSON, 1942).

Taking into account what has been said above it is easier to understand

Table 12. Distribution of relative frequencies of mutants into types in the field trials of 1951 and in the greenhouse tests of 1953 & 1954.

Fördelningen av relativa mutantfrekvenser i olika mutationstyper i fältförsök 1951 och växthusförsök 1953 & 1954.

Year	In greenhouse					In field				
	Albina	*Xantha*	*Xantho-viridis*	*Virid-is*	Σ	*Albina*	*Xantha*	*Xantho-viridis*	*Virid-is*	Σ
				Bogesund trial						
1953	25.4	4.5	20.9	49.2	100.0	21.7	50.6	26.5	1.2	100.0
1954	9.2	11.2	24.5	55.1	100.0	25.0	11.3	63.7	—	100.0
Σ	15.8	8.5	23.0	52.7	100.0	22.8	37.8	38.6	0.8	100.0
				Sundmo trial						
1953	5.3	—	15.8	78.9	100.0	—	29.4	39.2	31.4	100.0
1954	—	8.0	16.0	76.0	100.0	—	20.0	45.7	34.3	100.0
Σ	2.3	4.5	15.9	77.3	100.0	—	27.0	40.9	32.1	100.0
				Bogesund & Sundmo trials						
Σ 1953 & 1954	12.9	7.7	21.5	57.9	100.0	14.6	34.0	39.4	12.0	100.0
				Bogesund & Sundmo trials						
1953	20.9	3.5	19.8	55.8	100.0	13.4	42.6	31.3	12.7	100.0
				Bogesund & Sundmo trials						
1954	7.3	10.6	22.8	59.3	100.0	17.4	13.9	58.3	10.4	100.0

the striking dissimilarity in the distribution of mutations into *xantha, xantho-viridis* and *viridis* types in the greenhouse and in the field respectively (fig. 8, table 12). Likewise, the differences in the climate of the Bogesund and Sundmo field trials also make themselves noticeable. *Xanthas* are found in the Sundmo field trial, but are completely missing in the greenhouse trial. At Bogesund *xanthas* are considerably represented in the field and slightly in the greenhouse. The same phenomenon as with *xanthas* in the Bogesund field trial has been observed with *xanthoviridis* at Sundmo, where *xantho-viridis* has a lower frequency in the greenhouse than in the field. On the other hand, when the results of the Bogesund field trial are compared with those of the greenhouse, *xanthoviridis* is found to dominate in the greenhouse. In the greenhouse the *viridis* type absorbs from the other types a considerable part of those mutations which in field conditions reveal themselves in another manner, as well as those which appear on account of a better germination of seeds. Thus, in the greenhouse, *viridis* type attains an enormous representation. On the other hand, the inverse distribution shown in fig. 6 does not occur in fig. 7, which can only be explained by low mutant rates. On account of low mutant rates in general and low germination percentage in field conditions, mutations in fig. 7 assume a random character. Nevertheless the

increase in the mutant rates of all types in the greenhouse is a regular and constant phenomenon, which in the *viridis* type assumes extensive proportions, similar to those discussed above.

Concluding the discussions of the present investigation where the correlation between chlorophyll mutations and environment has been dealt with, the analysis of their contents should be concentrated in two directions. In the first place it should be noted that in view of the germination conditions reaching their optimum in the greenhouse, the percentage of germinating seeds is highly accelerated in comparison with that in the field. The increased germination of seeds in its turn reveals new mutations which do not appear in field trials. Mutant rates accelerate with such velocity that their connection with the increase of germination percentage is by no means simple. The above-mentioned observations find their expression in table II. Secondly, in the greenhouse the regular temperature most favourable for the emergence of mutations, as well as the advantageous illumination, results in calling forth the distribution of mutation types which is different from that in the field. It should be mentioned, however, that *albinas* retain their constant attitude to environmental conditions. On the other hand, among other types *viridis* attains a striking majority. Further consequences of this phenomenon are contained in the discussion that follows.

5. Viability of Chlorophyll Mutations

Lethality is a well known phenomenon in chlorophyll mutations in homozygous state. The recessive lethal factor, however, remains concealed in heterozygous state and does not influence the viability of the individual. Even among mother trees endowed with the highest mutant rates there is not a single tree which deviates from other individuals in the same stand more than the usual variation of the phenotype permits. Their social state, and other characteristics, make these mother trees different from each other within one stand. Nevertheless, there is no cause to suppose that the explanation of these differences might be found in the recessive lethal factor. Since no genotype tests of these mother trees have been made, it is at present impossible to state whether the recessivity of the chlorophyll factors is complete or only partial. For the same reason also the heterozygous state of these trees cannot be questioned. In this connection it should also be mentioned that not all chlorophyll deficient factors are the cause of lethality in the state of homozygosity. Among the chlorophyll deficient factors there are some which, occurring in a double dose, reduce the viability of the homozygous individuals only partially or do not impair it at all. The viability of homozygous chlorophyll mutants is variable to such an extent that at the extreme points of this variation we find either absolute lethality or, on the other hand, that viability can reach the level of normal individuals. If, and under what environmental

conditions, this viability might attain the expression of superviability is too early to judge. However, taking into account what has been published in this connection, it is not out of the question (GUSTAFSSON, 1951, 1954).

The data on the viability of the chlorophyll mutations are based on the observations in the Bogesund experimental field from 1951 to 1952 and to 1954 respectively. Of the total number of mutants 83.3 %, that is, 1,139 mutant individuals, were found to be dead, which makes 0.1125 % of the total number of plants in the experiment; 33 mutants had turned green and could not possibly be distinguished from the normal; these make 2.4 % or 0.0032 % respectively; 196 mutants were alive which makes 14.3 % or 0.0194 % respectively. The above figures show that the viability of chlorophyll mutants is very low and that, under favourable conditions in the nursery, only one mutant appeared from among approximately 3,000 two-year specimens, and even then the degree of its chlorophyll deficiency was low. In the surviving mutants the above mentioned figures remained almost constant all through the following two years. Thus the number of chlorophyll mutants which survived at the end of a four-year investigation was 175, that is, 0.0173 % of all the plants in the experiment.

The viability of chlorophyll mutation types finds its expression in fig. 9, where the relative frequencies are shown separately for each stage of development; that is, for seedlings, first year as well as two-year plant mutants. The statistical basis of relative frequencies at seedling stage were 1,041 mutants; for first year plants, 195 mutants, and for two-year plants 232 mutants. The numerical basis of the two last groups might seem rather inconsiderable, but it should, however, be kept in mind that these seemingly insignificant figures are the result of investigations of more than one million plants and that the tendencies revealed by the relative frequencies are clearly pronounced.

The seedling mutants of all types are lethal. Among the first year plant mutants two individuals of the *albina* type and some of the *xantha* type survived on account of their green cotyledons. The *albinas* perished in their second year, but six individuals of the *xantha* type were still alive. In the course of the following three years two of them emerged as chimeras having the characteristics of a *xantha* as well as those of a normal individual. The rest of the survivors show a transition from *xanthas* to *xanthoviridis* type in different degrees. Nine *xanthoviridis* individuals found at seedling stage must be considered as an exception to the absolute lethality of this type. It is interesting to note that one of them turns *viridis* and in the following years completely loses its chlorophyll deficient traits. Five other individuals manifest themselves even in the following years as *xanthoviridis*. This fact conspicuously shows that single individual mutants can serve as an example of exceptional cases among lethal mutants of the same type. It cannot be denied that a more detailed classification of mutants into sub-types as found in GUSTAFSSON's

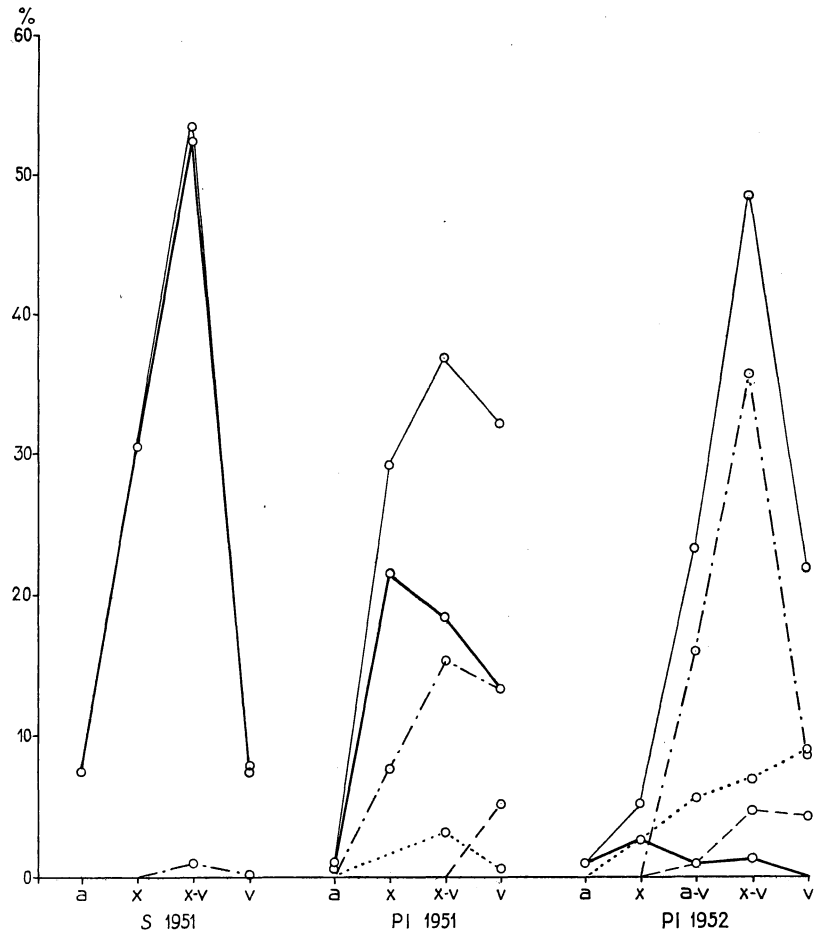

Fig. 9. Viability reaction of different mutation types at seedling stage (S 1951) and at plant stage in both first (Pl 1951) and second years (Pl 1952) in the field trial at Bogesund. Relative frequencies showing the distribution of mutants into mutation types (—○—) and into different viability groups: mutants which perished (—○—), mutants which had lost their chlorophyll deficient traits and turned green (— —○— —), mutants which survived after having emerged as new mutants either at seedling stage or at one of the plant stages (— . —○— . —), mutants which survived after having appeared at one or both of the previous stages (. . . ○ . . .).

Vitalitetseffekten hos olika mutationstyper i groddplantsstadiet (S 1951) och i plantstadiet under första (Pl 1951) och andra året (Pl 1952) i fältförsök vid Bogesund. Relativa frekvenser som visar fördelningen av mutanter i mutationstyper (—○—) och i olika vitalitetsgrupper: mutanter som dött (—○—), mutanter som upphört att visa sina klorofylldefekter (— —○— —), överlevande efter att ha uppträtt som nya mutanter antingen på groddplantstadiet eller på något av plantstadierna (— . —○— . —), överlevande mutanter efter att ha uppträtt på något av tidigare stadierna (. . .○ . . .).

(1940) scheme for barley would considerably clear up the matter. Nevertheless, there will always remain individual cases which as such correspond to the polygenic variability characterising the genetics of chlorophyll mutations.

In contrast to the absolute lethality of seedling mutants in the field trials,

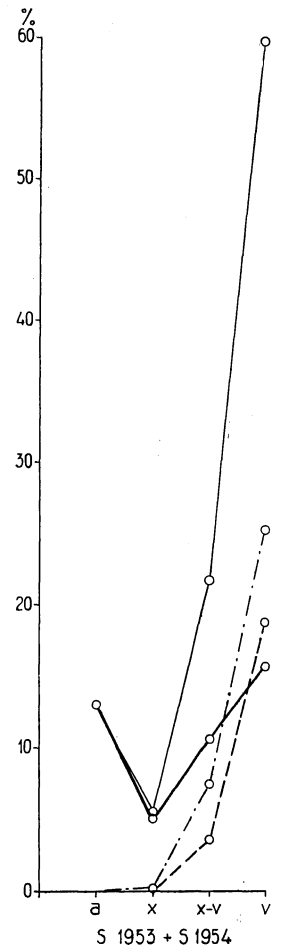

Fig. 10. Viability reaction of different mutation types in the greenhouse tests. The same signs as used in fig. 9.
Vitalitetseffekten hos olika mutationstyper i växthusförsök. Samma tecken som använts i fig. 9.

stands the partial lethality, i.e., semi-lethality of *viridis* seedling mutants in the greenhouse (fig. 10). It should be mentioned that these are the same mutations as were discussed previously (figs. 6, 7 and 8). Once again the correlation existing between environmental factors and the viability of mutations comes to light in the example above. No comments are really necessary in this case, since this phenomenon is well known in mutations of other plants (GUSTAFSSON, 1951).

Opposed to lethal mutants we find the surviving mutants as well as those which turn green (fig. 9). The latter appear already in the *viridis* type of first year plant mutants and are then more numerously represented in the second year in *viridis*, in *xanthoviridis* as well as in a lesser degree in *alboviridis* type. (The latter type has been included only in the second year plant mutants;

in other graphs it appears together with *xanthoviridis*). Those mutants which have the capacity to turn green are a most interesting phenomenon in the chlorophyll mutations of pine. Actually, this faculty by no means implies that the chlorophyll deficient factor in question ceases to reveal itself once and for all. There are many cases where a second year plant mutant turns completely green and can hardly be distinguished from normal individuals. However, in the following year, or even a year later it can appear again. On the other hand, the "surviving" mutants can be considered only as a divergent case of the mutants "endowed with the faculty to turn green". The only difference between the two groups of mutants is that the "surviving" mutants retain the chlorophyll deficient traits of the phenotype. Attention should be drawn to the two components of this group: firstly, mutants appearing and surviving during the second year and secondly mutants which had appeared in the first year, had survived and were still surviving in the second year. They appear at different ages of the plants and probably have a different genetic background. However, in the course of further development, during the following two years, no tangible differences between the two surviving mutations have been observed.

The very essence of the viability of chlorophyll mutations finds its expression in fig. 9 as follows. At seedling stage, in field trials, all types of mutations are lethal. On the other hand, in the first year plant mutants only *albinas* and *xanthas* are lethal, while other types are semi-lethal. Of the second-year plant mutants *albinas* and *xanthas* are still lethal, but the death-rate in other types is scarce. Actually it does not occur more frequently than in normal plants. The faculty of the plants to turn green and survive, as opposed to lethality, obtains here a most characteristic representation.

In connection with what has been said above, the second year mutants and partly also the mutants of the first year, on account of their surviving, acquire a certain significance and can possibly add to the hereditary variation of pine in nature. It is those mutations in which the chlorophyll deficient traits are only slightly expressed, probably corresponding to lesser mutated changes in the genotype of the individual, which are decreed to survive. The question arises if and how frequently can such mutants be found in natural pine stands. The above mentioned figures showed that out of 3,000 normal two-year plants in nurseries one chlorophyll deficient mutant can be expected. Assuming that the viability and competitive ability of these mutants in the years following is equal to that of normal individuals, and that *circa* one per cent of them reach the age of fifty years, when a stand of one hectar contains on the average 600 trees, a general estimate might be made that one homozygous chlorophyll deficient individual might be found on an area of five hectars under favourable environmental conditions. Without doubt this assumption is of a general nature and, under favourable outward conditions,

we might expect a more frequent occurrence of such trees in the stand depending on the chance concentration of chlorophyll mutations in the locality.

The question whether these chlorophyll mutations also have a bearing on the process of evolution belongs to one of the most cardinal problems on mutations, that is, the question of the importance and weight of minor mutations as such. In the case of chlorophyll deficient mutants in pine, only observations requiring years and even generations, might give a satisfactory answer as to their viability, their competitive ability in stands among other trees, as well as to their adaptive value in different environmental conditions.

At the same time it will fall within the sphere of further experiments to give answer to the question regarding the selectionary value of chlorophyll mutations in pine and their significance in breeding work.

III. Chlorophyll Mutations in Stands

Pine stands furnish a characteristic example of populations consisting of sexual and cross-fertilising organisms. The common fate of the individuals and of the population is revealed here in several aspects. Each of such stands is at the same time a social unit where the individuals are affected not only by external environment but also by internal environment of phytocenosis. Depending on the age of the trees, their ramification, density and other characteristics of phytocenosis, the internal climate of this unit can influence the biology of nutrition and reproduction of individuals in different ways. On account of this, individual trees do not reach maturity simultaneously, and neither does their periodical flowering and fruiting occur in the same year. The number of ♂ and ♀ flowers in the same individual and their relative proportion can vary from year to year, and it can also vary from individual to individual. It should also be mentioned that ♂ and ♀ flowers do not always blossom at the same time. Incidental changes in the meteorological conditions can deter the dispersal of pollen and the fertilisation of flowers. Thus external factors and the genotype of the individuals in the stand are harnessed together and render their reproduction as well as the reproduction of the whole stand rather intricate. As a reproduction unit every pine stand integrates in itself the hereditary material of all individuals, and the relationship established between them, preserved in the germ plasm of the stand, endures through many generations even after the present stand ceases to exist.

Naturally, the genotype of the stand does not remain unaltered. The composition of the stand, however, changes in connection with the fact that some trees perish and new trees are added to it, having reached their maturity. Unexpected and unaccountable changes can arise in the genotype of the stand caused by the pollen of the paternal constituent from other stands.

These changes in the germ plasm of the stands should also be associated with natural selection, migration and also with the possible origin of new mutations.

As all Mendelian populations, every forest stand contains great stores of potential variability. Among them we also find recessive chlorophyll deficient factors. This deleterious heredity reveals itself in the progeny of single individuals in the stand as homozygous mutants. This manifestation takes place only when the mutation is represented in the genotype of both gametes. Any individual mother tree can be one of the carriers of this heredity. The other carrier, however, can be any tree in the stand or any tree in other stands, which contributes to the common pollen pool, not excluding the mother tree itself. Many possibilities might be anticipated of which two extreme cases might be mentioned. Inbreeding in connection with complete or partial capacity of the mother tree for self-fertilisation can increase the mutant rate of the carrier. On the other hand, the mother tree, being at the same time a mutation carrier, can receive from the common pollen pool inheritance which is completely lacking in chlorophyll deficient factors. From the point of view of the inheritance of chlorophyll mutations the common pollen pool of the stand cannot remain permanent from year to year, since a changeable combination of trees take part in the flowering. The manifestation of chlorophyll mutations in the progeny of the stand thus grows complex and it is not at all astonishing that the mutant rates and also the proportion of mutation types fluctuate from year to year.

In connection with what has been said above, an absolutely reliable analysis of chlorophyll mutations in the stand would require a much greater investment of time and effort than used in the present investigation. The mode of procedure here has been simplified, but it cannot be denied that on account of the unknown components in the structure of the stand some uncertainty is attached to it. On the other hand, the method used gave the possibility to include a great number of stands as well as localities in the investigation (fig. 1). The grouping of these localities brings into the investigation also the principle of regionality.

As has been mentioned before, the investigation was carried out in the two experimental fields at Bogesund and Sundmo. The Bogesund field trial for the most part embraced stands from south and central Sweden, and the Sundmo trial was represented by those from north Sweden. From methodological point of view the division of the material into two parts serves at the same time as a test of the problem in the shape of two replications. Each stand, however, has for the most part been analysed only once; that is to say, seeds from the mother trees representing the stand were collected only once. Repeated collections of seeds in the course of two or three years successively had been carried out only in a few stands. Consequently, the investigation neither attempts to solve the dynamics of the inheritance of chlorophyll mutations in pine stands, nor the fluctuation of mutant rates year after year.

Fortunately a lucky coincidence was an advantage to the investigation. The yield of conifer seed in 1948 and 1949, when cones were collected, was more than ample, with a few exceptions (in 1948), in west and central Sweden. The pine crop was so abundant that only a few trees did not participate in the production of seed in the stands. The flowering of pine in 1947 and in 1948 had been as rich as was the yield of pine seeds in 1948 and 1949. Thus an assumption might be made that since almost all trees had been amply represented in the common pollen pool of the stand, chance played a lesser role than otherwise in the fertilisation process, on account of the abundance of the pollen.

1. Components of Chlorophyll Mutant Rates in Stands

Mutant rates in stands are the numerical expression of the concealed deleterious heredity which reveals itself in the progeny of individuals in the stands. At the same time it might be considered as a symbol expressing the soundness of the stand or its disposition to disease respectively.

From the statistical point of view the mutant rate value in the stand is a function of two components, that is to say, of the chlorophyll mutant frequencies in the progeny of individual mother trees, on the one hand, and of the percentage of mother trees which are heterozygous for chlorophyll mutations, on the other hand. In the absence of a complete analysis of the genotype of individuals these statistical values are the only known elements of the inheritance of chlorophyll mutations in stands.

The mutant rates of individual mother trees and the number of the mutated trees, that is, the percentage of the total number of trees in the stands are most variable. All kinds of combinations can be found here, for example, a great number of mutated trees with low mutant rates or vice versa. In some cases the result of these combinations may be the increase of mutant rates in the stand, but there are also reverse cases. In most cases, however, these combinations do not influence the extent of mutant rates in the stand at all. Nevertheless, the dissimilarity of such stands is quite obvious. Attempts have been made to express the dissimilar structure of these stands, that is, the heterogeneity which exists in the stands on account of the unequal participation of individual mother trees in the mutant rate value of the stands by computing its chi-square (χ^2) for each stand separately. For the computation of χ^2 an approximate formula was used,

$$\chi^2 = \frac{N}{n}\left(\frac{n_1^2}{N_1} + \frac{n^2_2}{N_2} + \cdots\cdots + \frac{n_k^2}{N_k}\right) - n,$$

where

$$N_1 + N_2 + \cdots\cdots N_k = N$$

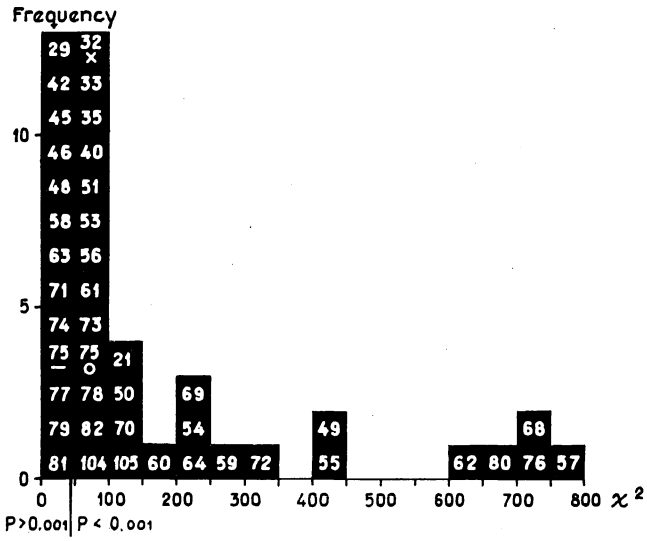

Fig. 11. Distribution of χ^2 values in 43 stands (designated by numbers) obtained from mutant rates in the progeny of individual heterozygous trees, in the field trial at Bogesund; (x) seeds obtained in 1948, (—) in 1949, (o) in 1950.

Fördelning av χ^2 värden för 43 bestånd utmärkta med nummer, vilkas mutantfrekvenser har erhållits från avkomman av enskilda heterozygota träd i fältförsök vid Bogesund; frön erhållna 1948 (x), 1949 (—) och 1950 (O).

denote the number of plants, that is, the number of germinated seeds for each mother tree separately and the total of the whole stand, and where

$$n_1 + n_2 + \cdots\cdots + n_k = n$$

denote the number of mutants for each tree separately and for the whole stand. The approximative computing of χ^2 is used because of $\left(\frac{n_i}{N_i}\right)$ being negligible.

The values of the chi-square characterising the structure, that is, the heterogeneity in stands, display a remarkably large dispersion. This dispersion is shown in the frequency distribution of χ^2 in fig. 11 representing stands in the Bogesund field trial, and in fig. 12 representing the Sundmo field trial. Class intervals of fifty chi-square values have been chosen. The statistical basis of the histogram (fig. 11) of the Bogesund field trial is 43 stands, and of that at Sundmo (fig. 12) 41 stands.

From the statistical point of view these histograms distinctly show a typical frequency distribution of chi-square values, and no essential divergencies are to be found when comparing the two histograms. There are few stands in which the size of χ^2 is large, but the fact that they consistently appear in both histograms proves that stands of such character are no excep-

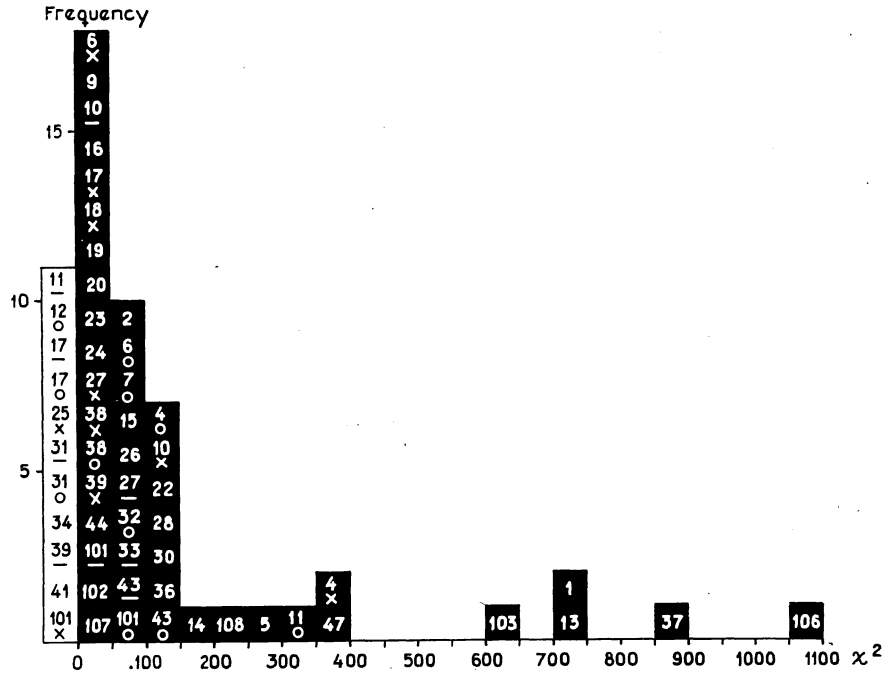

Fig. 12. Distribution of χ^2 values in 41 stands (designated by numbers) obtained from mutant rates in the progeny of individual heterozygous trees in the field trial at Sundmo. The same signs as used in fig. 11. Eleven stands were placed outside the graph on account of insufficient statistical basis.

Fördelning av χ^2 värden för 41 bestånd, utmärkta med nummer, vilkas mutantfrekvenser har erhållits från avkomman av enskilda heterozygota träd i fältförsök vid Sundmo. Samma tecken som använts i fig. 11. Elva bestånd har placerats utanför koordinatsystemet beroende på otillräckligt statistiskt material.

tions in nature. Here a certain parallelism with the contents of figs. 2 and 3 might be discerned, where large size mutant rates of individual mother trees stand in the same relation to the concentrations of low mutant rates as is the case in figs. 11 and 12. Actually it is not only a kind of resemblance but rather the repetition of the cases of mother trees endowed with high mutant frequencies.

From the biological point of view chi-square frequency distributions are significative, since they conspicuously show the mutual relationship existing between mutant rates of individual mother trees in each stand separately and the grouping of the stands depending on the numerical expression of their relationship.

The second component of chlorophyll mutations in stands, that is, the percentage of the mutated trees, finds expression in fig. 13 and table 13 in the distribution of relative frequencies for each of the two field trials separately. The frequency distribution, graphically expressed by means of polygons,

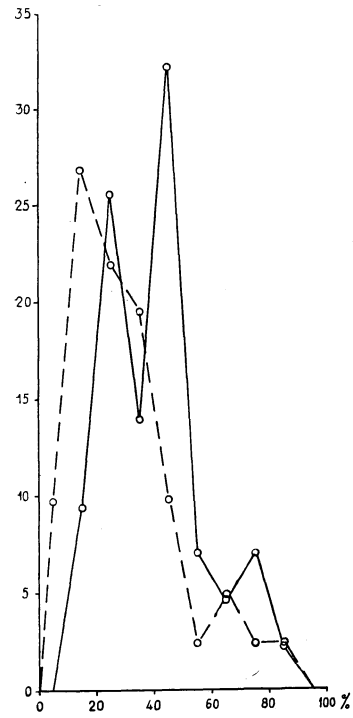

Fig. 13. Distribution of relative frequencies of stands, classified on the basis of the percentage of the mutated trees in stands (——) in the field trials at Bogesund and (— — — —) at Sundmo (class interval = 10 %).

Relativa frekvenser av bestånd fördelade efter procenttal muterade träd i bestånden (——) i fältförsöket vid Bogesund och (— —) vid Sundmo (klassbredd = 10 %).

shows a tendency to correspond to the normal frequency distribution but has a positive skewness. Seen from a biological aspect, cases with an extremely high percentage of mutated trees in the stands do not occur too often. Nevertheless they are not random occurrences, but rather a consistent natural phenomenon. It is difficult to say whether the cases where the percentage of mutated trees is high should be associated with certain environmental factors. From table 13 it may be seen that most of the stands containing a high percentage of mutated trees are located in districts with considerably low altitudes , that is, in districts with favourable climatic conditions. In advantageous geographic environment, however, which corresponds to the above mentioned cases of high percentage of mutated trees, a higher germinating capacity of the seeds is a common occurrence. The better the germinating capacity of seeds, the more the concealed inheritance of chlorophyll mutations is revealed and, consequently, the greater also the percentage of the mutated trees in the stand.

Another phenomenon of completely identical nature is to be seen in fig. 13, where the dissimilarity between the two polygons is most obvious. Most of the stands represented in the Sundmo field trial, originating from localities

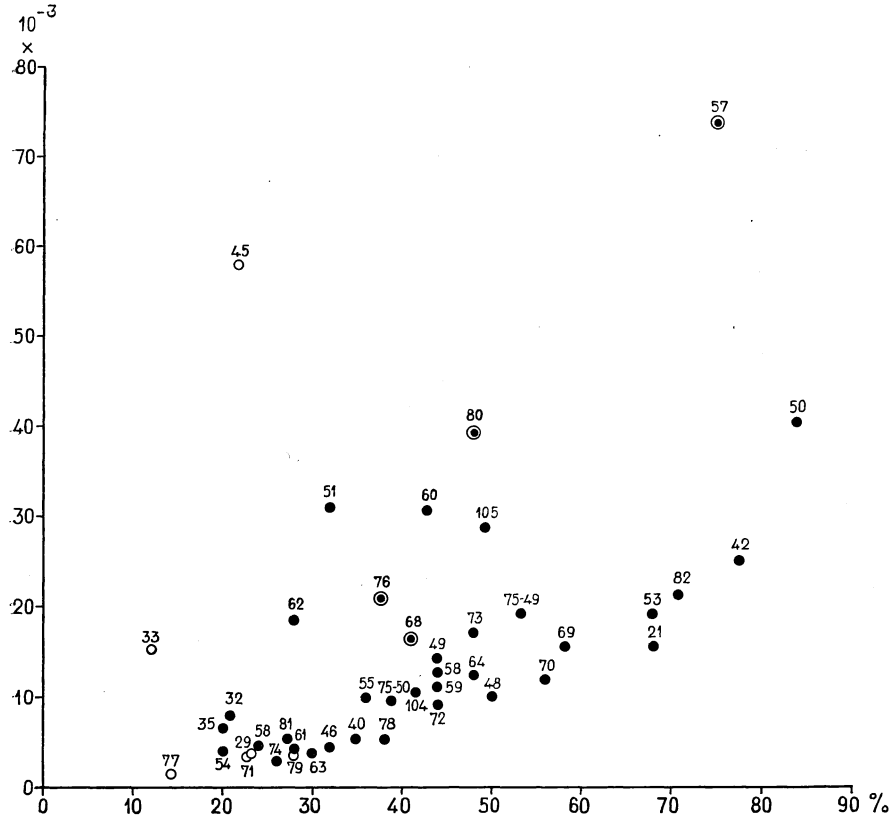

Fig. 14. Relation between the percentage of heterozygous mother trees in stands, designated by numbers (horizontal scale), and mutant rates in stands (vertical scale) in the field trial at Bogesund; o stands with insufficient statistical base, ◉ stands with only one heterozygous mother tree whose high mutant rate deviates considerably from that of other trees in the stand.

Relationen mellan procenttalet muterade heterozygota moderträd i bestånd (horisontala skalan) och mutantfrekvenser i bestånd (vertikala skalan) i fältförsök vid Bogesund. ○ bestånd med otillräckligt statistiskt material. ◉ moderträd vars höga mutantfrekvens avsevärt skiljer sig från andra träds.

in the north of Sweden, consistently show a lower percentage of mutated trees than do the stands in the Bogesund field trial, which might be explained by the low germinating capacity of the seeds from localities having unfavourable climatic environment as well as by inferior germination, the geographic environment at Sundmo being more unfavourable than at Bogesund. Nevertheless, it would be rather difficult to provide decisive evidence for the assumption that it is only the dissimilar germination of seeds and the divergencies in the Bogesund and Sundmo trials that are responsible for the high percentage of the mutated trees.

The correlation between the percentage of the mutated mother trees and

mutant rates in stands is graphically shown in fig. 14. The contents of this figure are the most important in this paper and require consideration of both a statistical and a biological nature. From the statistical point of view this correlation is of a complicated nature, since the values of both variables are widely dispersed. Viewed biologically this problem touches the sphere of mutation dynamics in stands, as well as their reproductive mechanism. When considering the contents of fig. 14, regarding the Bogesund field trial, reference should be made to fig. 12 which contains details of the former.

Fig. 15 supplements the statistical analysis of the correlation existing between the mutant rates in the stands and the percentage of the mutated trees. The contents of figures 14 and 15 differ only in regard to the values expressed by the vertical axis. By means of the coefficient of relative variation ($100 \times \varepsilon_m$) the total of the deviation of the mutant probability values of mother trees in stands is here shown in its relation to the mutant probability of the whole stand $\left(\frac{\delta_p}{p}\right)$.

ε_m values are calculated by use of the formula

$$\varepsilon_m{}^2 = \frac{\chi^2 - k + 1}{n}$$

where k denotes the number of mother trees in stands and n the total number of mutants in the stand.

The object with fig. 15 has been to control the correlation existing between the mutant rates in the stands and the percentage of the mutated trees. The dispersion of ($100 \times \varepsilon_m$) values in fig. 15 is very wide. However, as might have been expected, these values are higher in stands with low mutant rates (compare figs. 14 and 15). If we exclude four stands whose ε_m value is irrational on account of the negligible number of mutants, we see that the grouping of all other stands in fig. 15 distinctly shows a decrease of $100 \times \varepsilon_m$ values along the horizontal axis with the increase of the percentage of the mutated trees. The decrease of the values which are widely dispersed might be expressed by a straight line. The highest values ($100 \times \varepsilon_m$) are found in stands where several mother trees attain prominence by their high mutant rates. However, the most important thing in the contents of fig. 15 is the fact that no essential discrepancies are found when compared with fig. 14 as regards the conclusions which might be drawn from the increase of the percentage of the mutated trees.

The position of stands in fig. 14 is first of all dependent on the mutant rates of the stands and on the percentage of the mutated trees. As a further consequence of this dependence might be mentioned the grouping of most of the stands in such a way as to show a tendency to a positive linear correla-

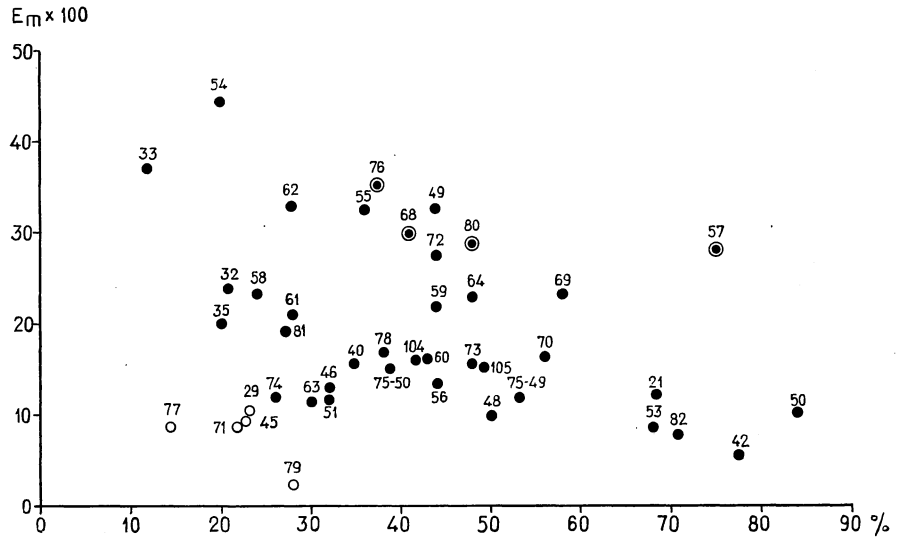

Fig. 15. Relation between the percentage of heterozygous mother trees in stands, designated by numbers (horizontal scale), and $100 \times \varepsilon m$ in stands (vertical scale). This figure is a supplement to fig. 14.

Relationen mellan procenttalet muterade heterozygota moderträd i bestånd (horisontala skalan) och $100 \times \varepsilon_m$ i bestånd (vertikala skalan). Denna figur utgör tillägg till fig. 14.

tion between the two variables. This means that the mutant rates in the stands increase simultaneously with the increase of the percentage of the mutated trees, and this correlation is expressed by a straight line. Some cases, however, deviate from this general tendency. Some of these deviations find a visible explanation in fig. 14. Such are cases where deviations are caused by an insufficient statistical basis, low number of plants, and cases where there is only one mother tree with a strikingly divergent mutant rate. The remaining deviations from the main grouping of stands with a linear correlation tendency in fig. 14 are rather similar to the above mentioned cases. Thus, for instance, the deviation in stand No. 51 is determined by two mother trees with high mutant rates, in stands Nos 105 and 60 by three such trees, while only stand No. 50 contains five such mother trees. From what has been said above, we might infer that the correlation between mutant rates in stands and the percentage of the mutated trees is also influenced by a third variable, that is, the mutant rates of individual mother trees. Low values of mutant rates do not offer a possibility to express these three variables in a statistically suitable manner.

With the exception of a few unreliable cases on account of insufficient statistical evidence (for instance stands 45 and 33) all other deviations in fig. 14 are associated with one or more mother trees endowed with high

mutant rates in each stand. Attention should also be drawn to the specific character of these trees, previously shown in figures 2, 4 and 12. However, viewed statistically, the stands containing them are only in minority. All other stands in fig. 14 have a grouping where the tendency to linear correlation between the mutation rates of stands and the percentage of the mutated trees is expressed in such a manner as does not induce one to look for another correlation or another tendency.

This phenomenon might seem rather paradoxical, since actually another grouping of stands could be expected than the one found in fig. 14. Viewed biologically, changes arise in the genotype of the stands with the increase of the percentage of the mutated trees, and this, in its turn, should result in a much more rapid increase of the mutant rates than in the graphical expression in fig. 14. It should, however, be kept in mind that the mutated mother trees contain chlorophyll deficient factors of both carriers, since they participate in the reproduction process of the stand both with ♀ flowers as well as with ♂ flowers. The greater the number of the mutated trees in the stand the greater is the possibility for the gametes of both carriers, having recessive traits of chlorophyll mutations, to meet. Even though we assume that the mutable changes in the genotype of a great number of mutated trees affect only a small number of genes, with the increase in the percentage of the mutated trees a rapid increase in the mutant rates might be expected. However, with the increase of the percentage of the mutated trees only a slow rise of mutant rates can be observed along the length of the horizontal axis of the graph.

The explanation of this contradiction, in the nature of things, lies deep, and it might be found in the paternal constituent of the reproduction system. Two alternatives might be suggested. The first possibility is that the ♀ flowers of the mutated trees do not receive the pollen pool of its own stand, that is to say, the paternal inheritance rich in mutable genes is not transmitted to them. The second possibility is that the ♀ flowers receive the pollen pool of its own stand as well as the recessive chlorophyll deficient factors contained in the stand, but on account of the poor quality of the pollen grains, or other causes, the recessive chlorophyll mutations are excluded from the fertilisation process of ♀ flowers.

The first alternative can hardly be fully realised. The following conjectural cases might be imagined. There might be years when no ♂ flowers are produced; when ♂ flowers suffer from frost, and when ♂ flowers come into blossom later than ♀ flowers, that is, after the latter had already been fertilised by pollen originating in other stands in which the recessive chlorophyll deficient traits are found on a smaller scale. On account of the divergent reproductive biology of the individuals in the stands, it can hardly be assumed that any of the

above mentioned cases can be fully realised. But, nevertheless, even a partial realisation of these conjectures would inevitably cause the emergence of mutants on a much larger scale, which would result in higher mutant rates, particularly in cases where more than eighty per cent of the trees are mutated.

The second alternative explaining the low mutant rates in the stands discussed above by the low quality of pollen containing chlorophyll deficient factors, or possibly having low pollen fertility, is more plausible. It is a well-known fact that pollen fertility of individual pine trees varies considerably (ANDERSSON, 1954; PLYM FORSHELL, 1953), and that, among other factors, also chromosomal disturbances cause reduced pollen fertility. Nevertheless, it is not known how the recessive chlorophyll mutation factor behaves in the male haploid gamete, that is, in the pollen grain which is the carrier of paternal heredity. It is quite possible that in the haploid gametes the mutation factor acts in the same way as it does in the diploid homozygous mutants, causing lethality, semi-lethality, or, at least, reducing viability (GUSTAFSSON, 1938; STADLER, 1951; MCCLINTOCK, 1951). Haploid plants which occur in rye and timothy populations furnish a good example of what has been said above. MÜNTZING (1946) writes that recessive destructive genes, which are a most usual occurrence in the cross-fertilizing populations, frequently reduce the viability of haploid individuals.

In any case, we might assume the existence of a barrier which prevents a relatively large part of pollen grains containing chlorophyll deficient traits taking part in the fertilization of ♀ flowers. This explanation, even if it is conjectural, agrees well with the statistical basis in fig. 14, as well as with biological considerations. Should the explanation prove to be correct, it might be of importance not only in this particular case, but would refer to the whole complex problem of the inheritance of recessive chlorophyll mutations in pine. However, in the absence of exact evidence, it remains only a hypothesis. A question, nevertheless, remains unanswered. What prevents recessive chlorophyll mutations from emerging in the stands and what interferes with a greater accumulation of the recessive chlorophyll deficient factors in pine stands, as is now the case?

2. Localities and Chlorophyll Mutant Rates in Stands

Stands completely void of chlorophyll mutations occur very seldom. At least a few mutants invariably emerge; even if they do not appear in the course of the first year, they may reveal their chlorophyll deficient traits in the second and third year. In the Bogesund field trial, representing the progeny of individual trees from 43 stands, there was not a single stand which did not produce mutants, but out of 14 stands whose progeny originated from mixed stands four did not produce mutants.

Table 13. Distribution of localities into percentage classes of mutated trees in stands.
Fördelning av orter i procenttalklasser muterade träd i bestånd.

Class intervals of the percentage of mutated trees	Number and locality in	
	the Bogesund trial	the Sundmo trial
0—10	—	34—Örnsköldsvik; 41—Särfors, Gryttjen; 4—Norway, Målselv: 39—Vålådalen; 44—Funäsdalen; 47—Färila
10—20	33—Gålgoberget; 35—Bispfors; Stadsforsen; 54—Kratte Masugn; 77—Kosta	6—Kitkiöjoki; 9—Norway, Bodö, 11—Gällivare; 14—Spikseleå; 16—Malmesjaure; 36—Bispfors; 38—Vallbo; 43—Idre; 101—Arjeplog; 103—Långvind
20—30	29—Hoting; 32—Lycksele, Storberget; 45—Grängesåsvallen; 58—Norway, Sånes; 61—Sandhamn; 62—St. Malm; 63—Lindfors; 71—Vimmerby; 74—Eckersholm; 79—Brömsebro; 81—Växtorp, Hallandsåsen	4—Norway, Målselv; 7—Kauppinen; 10—Ohtanajärvi; 13—Norway, Mo i Rana; 15—Telejokk; 19—Kalix; 20—Älvsbyn; 32—Lycksele, Storberget; 37—Hallen
30—40	40—Brämön; 46—Långvind; 51—Särna; Hundfjället; 55—Grangärde; 76—Kalmar; 78—Kinnared	2—Norway, Aronäs; 5—Norway, Tranöy; 10—Ohtanajärvi; 23—Maláträsk; 30—Vilhelmina; 106—Harrsjö; 107—Bispfors, Torresjölandet; 108—Galtström
40—50	48—Kilafors; 49—Orsa; 56—Norway, Vinje; 59—Bogesund; 60—Strömsholm; 64—Årjäng; 68—Lidköping; 72—Visby; 73—Särö; 75—Viebäck; 80—Färingtofta; 104—Böda; 105—Särö	33—Gålgoberget; 38—Vallbo; 43—Idre; 102—Långvattnet
50—60	69—Billingen; 70—Västervik; 75—Viebäck	28—Vindeln
60—70	21—Byske; 53—Älvkarleby	22—Jörn, Backen; 27—Vännäs
70—80	42—Sveg; 57—Norway, Bergen; 82—Vittskövle	27—Vännäs
80—90	50—Bunkris	26—Robertsfors

The lowest mutant rate in the Bogesund field trial is 0.10 per thousand (stand No. 111), and the highest 7.32 per thousand (stand No. 57). In the Sundmo trial the highest mutant rate is 8.67 per thousand (stand No. 11). The above figures show that the mutant rates fluctuate considerably and that the highest rate is almost one hundred times larger than the lowest. This

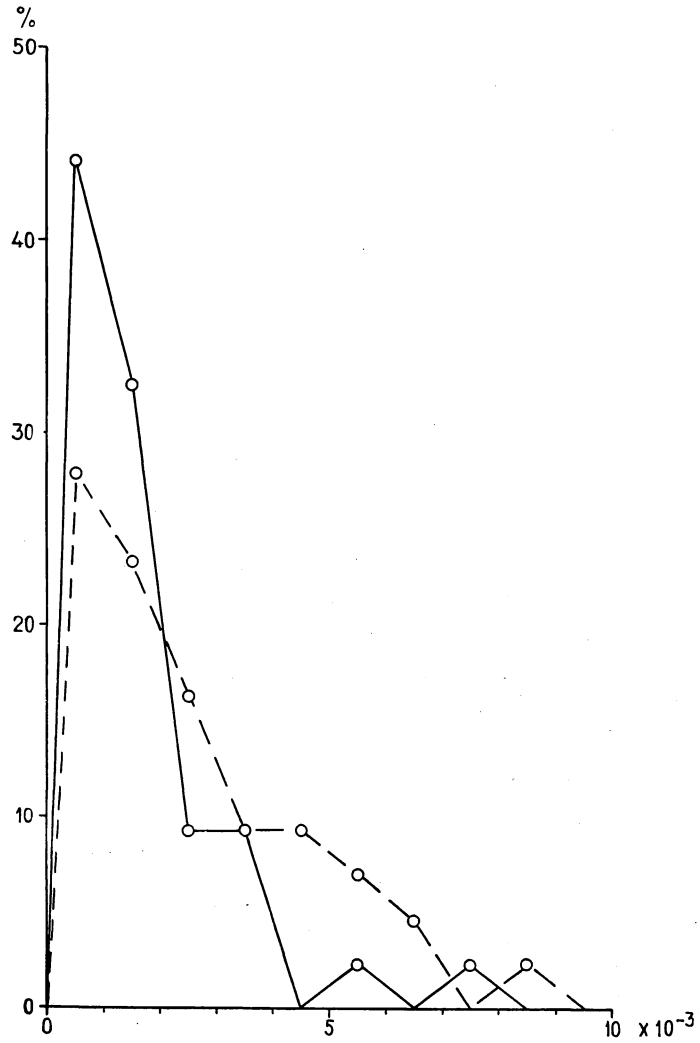

Fig. 16. Distribution of relative frequencies of stands, classified on the basis of mutant rates in stands (——) in the field trial at Bogesund and (— — — —) at Sundmo (class interval = one 10^{-3}).
Relativa frekvenser av bestånd fördelade efter mutantfrekvenser i bestånd (klassbredd = en 10^{-3}).

variation is shown in table 14 and it is also expressed by means of relative frequencies in fig. 16.

The graphical expression of the distribution of relative mutant frequencies in the stands corresponds to the truncated logarithmico-normal distribution. There are no essential divergencies in the distribution of stands in the Bogesund and Sundmo trials. Consequently, an assumption might be made that

the results of these trials typify chlorophyll mutant rates in pine stands at least as regards Scandinavia. Naturally, the statistical basis of the trials permits the making of only an approximate evaluation. However, from the contents of fig. 16 it might be seen that in 50 %—75 % of the cases of all pine stands from nought to two (0—2) per thousand chlorophyll mutants can be expected; in 20 %—40 % of the cases the number of chlorophyll mutants can vary from two to six (2—6) per thousand and in five per cent of the cases it can be higher than six per thousand. The existence of completely sound stands can scarcely be expected. It is hardly possible to span the store of all concealed chlorophyll mutations. Chance may always call forth the emergence of one or more mutated individuals in the stand.

The answer to the question as to whether mutant rates in stands are dependent on geographical environment is found in table 14.

The distribution of localities into classes in the table does not provide any evidence for the assumption that the mutant rates in stands are dependent on geographical environment. Most divergent localities occur in the same classes. Without doubt there are regions where mutant rates in stands seem to be higher than usual but there are also regions with a tendency to the contrary. Thus, for instance, in south-east Sweden (stands Nos. 70, 71, 77, 79 and 104) as well as in east Sweden (stands Nos. 34, 40, 41 and 110) mutant rates in the stands are low. On the other hand, in the north of Sweden, in the County of Västerbotten, as well as along the west coast of Scandinavia they are comparatively high. However, it would be too rash to draw conclusions from the above examples before answering the question as to on what scale these mutant rates fluctuate in smaller regions as well as how much they fluctuate from year to year.

Even when comparing most deviating cases in regard to geographical environment, for instance, mountainous and northern regions with those lying in the south or along the coast, it is hardly possible to find any pronounced divergencies in mutant rates. If differences are sometimes to be found, as in stands Nos. 45 and 33, they usually have a negligible statistical basis, resulting from low germinating capacity of seeds originating in mountainous and northern regions.

The mother trees in part C of the frequency polygons found in figs. 2 and 3 furnish a most valuable addition to the observations regarding the dependence of mutant rates in the stands upon geographical environment. In these trees the range of recessive mutations is so wide that even the low germinating capacity of the seeds cannot hinder the appearance of mutants on an extensive scale. In these considerations, trees having high mutant rates play a more important part than other trees, since their mutant frequencies, viewed statistically, are significant values. Trees having high mutant rates have

Table 14. Distribution of localities into mutant rate classes.
Fördelning av orter i mutantfrekvensklasser

Class intervals of mutant rates per thousand in stands	Number and locality in	
	the Bogesund trial	the Sundmo trial
0—1	29—Hoting; 32—Lycksele, Storberget; 35—Bispfors; 40—Brämön; 46—Långvind; 48—Kilafors; 54—Kratte Masugn; 55—Grangärde; 58—Norway, Sånes; 61—Sandhamn; 63—Lindfors; 71—Vimmerby; 72—Visby; 74—Eckersholm; 75—Viebäck; 77—Kosta; 78—Kinnared; 79—Brömsebro; 81—Våxtorp, Hallandsåsen	34—Örnsköldsvik; 41—Särfors, Gryttjen; 1—Norway, Gargialia; 6—Kitkiöjoki; 13—Norway, Mo i Rana; 14—Spikseleå; 19—Kalix; 20—Älvsbyn; 36—Bispfors; 38—Vallbo; 43—Idre; 101—Arjeplog
1—2	21—Byske; 33—Gålgoberget; 49—Orsa, Högståsen; 53—Älvkarleby; 56—Norge, Vinje; 59—Bogesund; 62—St. Malm; 64—Årjäng: 68—Lidköping; 69—Billingen; 70—Västervik; 73—Särö; 75—Viebäck; 104—Böda	2—Norway, Aronäs; 9—Norway, Bodö; 10—Ohtanajärvi; 15—Telejokk; 16—Malmesjaure; 22—Jörn, Backen; 27—Vännäs; 32—Lycksele, Storberget; 47—Färila; 108—Galtström
2—3	42—Sveg; 76—Kalmar; 82—Vittskövle; 105—Särö	10—Ohtanajärvi; 26—Robertsfors; 27—Vännäs; 39—Vålådalen; 101—Arjeplog; 103—Långvind; 107—Bispfors, Torresjölandet
3—4	50—Bunkris; 51—Särna, Hundfjället; 60—Strömsholm; 80—Färingtofta	4—Norway, Målselv; 28—Vindeln; 33—Gålgoberget; 38—Vallbo
4—5		23—Malåträsk; 30—Vilhelmina; 37—Hallen; 43—Idre
5—6	45—Idre, Grängesåsvallen	5—Norway, Tranöy; 44—Funäsdalen; 102—Långvattnet
6—7		7—Kiruna, Kauppinen; 106—Harrsjö
7—8	57—Norway, Bergen	
8—9		11—Gällivare, Linalompolo

obviously originated from most divergent localities, that is, from different stands. We find localities with alpine and sub-alpine climate (for instance stand 4—Altafjord, Northern Norway, 5—Tranöy, Norway, 11—Gällivare, 101—Arjeplog, 102—Långvattnet, 106—Harrsjö) in contrast to localities

having temperate climatic environment (for example stand 103—Långvind and stand 47—Färila). Localities with submaritime climate (80—Färingtofta, 105—Särö, 76—Kalmar and 57—Bergen, Norway) stand in contrast to subalpine localities (50—Bunkris and 51—Särna, Hundfjället) and the alpine stand (45—Städjan). The above observations are a sufficient proof that neither the mutant rates in the stands nor those of the individual trees are dependent on definite geographic environment.

By stating that neither chlorophyll mutant rates in pine stands nor those of individual mother trees show any dependence on the geographic environment we stress the fact that recessive chlorophyll mutations are a common occurrence in the genotype of pine stands. In this respect pine stands are similar to other populations being pronouncedly cross-fertilizing as well as containing large stores of concealed deleterious genetic factors. These factors do not lower the viability of the individuals in a heterozygous state, whereas they reduce the viability of the homozygous individuals (PLOUGH, 1941; DUBININ, 1946; DOBZHANSKY, 1949, 1951; STEBBINS, 1951; GUSTAFSSON, 1951, 1954).

How the recessive chlorophyll mutations preserved in the genotype of the pine stands during the course of many generations reveal themselves is a rather intricate problem. In this connection two points discussed above are the most important. Mutant rates in stands are in the first place restricted by a barrier which prevents chlorophyll mutation factors of ♀ and ♂ flowers to meet and emerge as homozygous mutants, as illustrated by fig. 14. This barrier might be the reduced viability of haploid gametes endowed with mutation factors. It is difficult to say whether this refers both to male and female gametes. Nevertheless, the reduced viability of the male gametes or even their complete lethality seem to be of decisive importance. The second point requiring attention is the dependence of mutant rates on the germinating capacity of the seeds, which is proved by the greenhouse tests (figs. 6 and 7). Mutant rate values sink when the germinating capacity of seeds is low. The two points mentioned above are naturally dependent on environmental conditions, whose divergencies in different localities are more than obvious. However, as seen from table 14 the result of the impact of all environmental conditions influencing mutant rates does not show that a single environmental factor has attained prominence as compared with the others.

In the present publication, however, the data regarding the extensive variation of chlorophyll mutant rate values in pine stands are so ample that there is enough convincing evidence to maintain that this variation is a natural phenomenon, which, taking into account what has been said above, might be explained by the genetic drift. Whether this is the only explanation of the problem must, for the time being, be left unanswered.

3. Localities and Distribution of Mutation Types in Stands

This chapter embraces two problems, that is, the segregation of chlorophyll deficient factors into mutation types in stands, and the possible divergency of this segregation depending on the locality.

As seen from table 15, no stands had been found where chlorophyll mutations in the progeny of individual trees were represented only by a single type. This fact is most important, since it proves that the hereditary material of chlorophyll mutations in stands is by no means of a simple but rather of a complex character. The distribution of stands containing either two, three or

Table 15. Number of stands with different combinations of mutation types
Antal bestånd med olika kombinationer av mutationstyper

Mutation type	Stands with 4 mutation types	Stands with 3 mutation types	Stands with 2 mutation types	Σ
Albina	*Albina, xantha, xanthoviridis, viridis* 24 *(28.6 %)*	*Albina, xanthoviridis, viridis* 3 *(3.5 %)* *Albina, xantha, xanthoviridis* 2 *(2.4 %)*	—	29 ***(35.5 %)***
Xantha	*Xantha, albina, xanthoviridis, viridis* 24 *(28.6 %)*	*Xantha. xanthoviridis, viridis* 23 *(27,4 %)* *Xantha, albina, xanthoviridis* 2 *(2.4 %)*	*Xantha, xanthoviridis* 10 *(11.9 %)*	59 ***(70.2 %)***
Xanthoviridis	*Xanthoviridis, albina, xantha, viridis* 24 *(28.6 %)*	*Xanthoviridis, xantha, viridis* 23 *(27,4 %)* *Xanthoviridis, albina, viridis* 3 *(3.5 %)* *Xanthoviridis, albina, xantha* 2 *(2.4 %)*	*Xanthaviridis, viridis* 22 *(26.2 %)* *Xanthoviridis, xantha* 10 *(11.9 %)*	84 ***(100.0 %)***
Viridis	*Viridis, albina, xantha, xanthoviridis* 24 *(28.6 %)*	*Viridis, xantha, xanthoviridis* 23 *(27.4 %)* *Viridis, albina, xanthoviridis* 3 *(3.5 %)*	*Viridis, xanthoviridis* 22 *(26.2 %)*	72 ***(85.7 %)***
Σ	24 ***(28.6 %)***	28 ***(33.3 %)***	32 ***(38.1 %)***	84 ***(100.0 %)***

all four mutation types has the following sequence: 38 %, 33 % and 29 %. Of all mutation types the *xanthoviridis* type emerges most frequently. It manifests itself in all 84 stands (= 100 %). Mutation types show the following sequence depending upon the number of stands (expressed in %) in which they occur: *xanthoviridis* (100 %), *viridis* (86 %), *xantha* (70 %) and *albina* (36 %). The combination of mutation types which occurs most frequently is *xanthoviridis*, *viridis* and *xantha* and all three together are found in 55 % of stands. *Xantha* and *xanthoviridis* together are found in 75 % of stands. Also in this table the *albina* type occurs less frequently, similar to the case regarding the segregation of mutations into types in the progeny of individual trees (tables 4, 5 and 6).

In order to find the answer to the question whether and to what extent the distribution of chlorophyll mutations in stands varies in different regions, all 84 localities were divided into three groups or regions (table 16), depending on the number of days with the normal mean $t° \geqq + 6°$ C (LANGLET, 1936). Region 1 embraces northern Scandinavia and mountain districts, but region 3 the southern part of the country and coastal districts. Region 2 lies between the two regions mentioned above.

When comparing the three regions the following divergencies in the distribution of mutation types may be noted. The relative values of *albina* are low in region 1 and 2. The highest *xantha* values are found in region 3 (26 %), they fall in region 2 (23 %) and drop very low in region 1 (10 %). The relative frequencies of *xanthoviridis* are, as a matter of fact, almost the same in all regions. On the other hand, the *viridis* type displays a reverse tendency when compared with that of the *xantha* type. The highest frequency of the *viridis* type is found in region 1 (29 %), diminishes in region 2 (18 %) and drops even lower in region 3 (9 %). As seen from the foregoing, the distribution of mutation types in the three regions differs considerably.

When trying to find the cause of the divergency in the distribution of mutation types, three possibilities should be mentioned. Each of them, independent of others, might be influential in determining the result. These possibilities are: chance, the impact of environment and divergencies of a genuine genotypical nature.

It is hardly possible to disregard chance and the impact of environment, since the data of the investigation were obtained in two field trials with different climatic conditions. Dissimilar germination conditions influence the distribution of mutation types (figs. 6, 7 and 8). However, the fact that the investigation was carried out in two field trials is only a lesser part of the environmental influences. The short duration of the vegetation period in region 1, in contrast to region 3, plays a more important role and is the cause of the low germination of pine seeds called forth by modificative influences.

Table 16. Distribution of mutants into mutation types in three regions.

Fördelning av mutanter på mutationstyper i tre regioner

Σ days with normal average t° ≧ + 6° C	Σ Stands	Σ Seedlings	Σ Plants	*Albina* Seedlings	*Albina* Plants	*Xantha* Seedlings	*Xantha* Plants	*Xanthoviridis* Seedlings	*Xanthoviridis* Plants	*Viridis* Seedlings	*Viridis* Plants
					Region 1						
90—120	27	428 *(70.2 %)*	182 *(29.8 %)* Σ = 610	10	20 Σ = 30 *(4.9 %)*	25	33 Σ = 58 *(9.5 %)*	234	109 Σ = 343 *(56.2 %)*	159	20 Σ = 179 *(29.4 %)*
					Region 2						
120—160	35	1005 *(72.3 %)*	386 *(27.7 %)* Σ = 1 391	23	36 Σ = 59 *(4.2 %)*	220	100 Σ = 320 *(23.0 %)*	556	208 Σ = 764 *(54.9 %)*	206	42 Σ = 248 *(17.8 %)*
					Region 3						
160—200	22	531 *(78.2 %)*	150 *(22.0 %)* Σ = 681	55	1 Σ = 56 *(8.2)*	152	23 Σ = 175 *(25.7 %)*	281	104 Σ = 385 *(56.6 %)*	43	22 Σ = 65 *(9.5 %)*

The decrease in the germinating capacity of the seeds, in its turn, brings about deviations in the distribution of mutation types. Attention should also be drawn to the low number of seedling mutants in comparison with that of plant mutants in the *albina* and *xantha* types in region 1, which should be associated with the decrease in the germinating capacity of the homozygous seeds, previously discussed (figs. 6 and 7). However much care might be given to evaluating the connection between the distribution of types and the regions, part of the divergency in the distribution of types should be ascribed to genotypical causes. The contents of fig. 8 favourably support this inference. Here, in the greenhouse trials high values of the *viridis* type mutants originating from seeds obtained in northern districts are confirmed.

The variation of the distribution of relative frequencies of mutation types in different localities is very wide. A similar phenomenon regarding the variation of mutant rates in stands was noted in the preceding chapter. This variation should be considered as a natural phenomenon which could be explained by the genetic drift. However, the distribution of the relative frequencies of mutation types in different regions varies considerably and it would be difficult to account for this as being a matter of chance or as caused by the genetic drift. The excess of the relative frequencies of the *viridis* type at the expense of the *xantha* type in region 1, that is, in northern Sweden and in mountain districts, has already been mentioned and it would be most important to elucidate this problem.

This fact, in its turn, induces one to look for a tendency to evolutionary processes created by geographic environment. In order to test the inference that this fact is due to genotypical causes it is necessary to carry out further investigations and draw a more definite line of demarcation between the genotype and the variation of faculties influenced by environmental conditions.

Summary

The present publication deals with spontaneous chlorophyll mutations in pine and their numerical values in the progeny of individual trees in natural stands.

Seeds for the investigation were obtained from 77 localities in Sweden, from 11 in Norway, eight in Germany and one in Holland (fig. 1). Seeds were sown in the nurseries of the experimental fields at Bogesund and Sundmo. The investigations were carried out from 1951 to 1952 and 1954 respectively. In the Bogesund experimental field the material used for the investigations comprised 1,012,000 plants originating from 1,016 individual

trees in 43 stands, and in the Sundmo experimental field it was made up of 757,000 plants originating from 1,015 individual trees from 43 stands. At Bogesund the number of plants from mixed seed samples originating from 15 stands was 87,000, and at Sundmo 98,000 from 18 stands. In 1953 replications were made in the greenhouse in Stockholm using 23 seed samples of individual trees, which gave 6,400 seedlings and plants. The replications of 1954 gave 8,700 seedlings and plants from 28 seed samples of individual trees.

The classification of mutants (table 1) was based upon the system of chlorophyll mutations elaborated by GUSTAFSSON (1940) when experimenting with barley. The phenotypical traits of the seedling and plant mutants such as divergencies in coloration of cotyledons, hypocotyl, primary needles, and needles gave the possibility to set apart 4 and 5 mutation types respectively, namely: *albina, xantha, xanthoviridis, viridis* and *alboviridis*. Such a simplified scheme of classification fully corresponded to the extensive scope of the investigation material. A characteristic feature in pine seedling mutants is their divergent hypocotyl pigmentation. The pigmentation of anthocyanin was particularly striking in the basal part of the *albina* hypocotyl.

The interaction of anthocyanin and chlorophyll in the seedling mutants and the function of anthocyanin in pine in general will be discussed in a later publication.

The sum total of all kinds of mutants in the experimental field at Bogesund was 1,368. The average mutant rate was 1.351 per thousand, which makes one mutant per 740 plants. In the Sundmo experiment the total of mutants was 1,317 and the average mutant rate 1.740 per thousand, that is, one mutant per 575 plants. In the Bogesund experimental field the progeny of 60 per cent of the trees did not produce mutations of any kind, and in the Sundmo field 70 per cent. At Bogesund the highest mutant rate of 410 mutated trees was 8.3 per cent and at Sundmo 25 per cent from 295 trees.

High mutant rates of individual trees is an interesting but by no means surprising phenomenon. It illustrates the capacity of heterozygotes to preserve concealed, recessive and destructive traits in their genotype. The manifestation of mutations by such high rates should be explained, at least in most of the individual trees, by inbreeding, which is the result of the self-fertilizing faculty of these trees. The variation of mutant rates, shown in figs. 2 and 3 by means of frequency polygons, corresponds to the truncated logarithmico-normal distribution. Frequency polygons are divided into parts A, B and C. Each of these parts not only shows the divergent dispersion of the mutants, but also differs from the others by the distribution of mutation types.

In the progeny of individual trees seedling and plant mutants were revealed in most different variations. On the other hand, the average data in both experiments were almost similar (table 2). In approximately 70 % of the

trees only seedling mutants emerge in the progeny. 20 % of the trees gave only plant mutants and 10 % of the trees produced both seedling and plant mutants. Only three per cent of the mutated trees consistently produced both seedling mutants and plant mutants of the first and second year. The repeated manifestation of mutations in the progeny of the same tree through all the stages of development shows the consistency of the process. The mutual relationship of seedling and plant mutants is clearly revealed in those individuals which at the seedling stage can hardly be suspected to be mutants, whereas as plants they reveal their chlorophyll deficient character. The genetic background for the divergencies between seedling and plant mutants might be found in their polygenic variability. The number of mutated genes, the mechanism of gene action in the development of individuals as well as environmental influences might explain the wide variation in seedling and plant mutants.

The numerical relationship between seedling and plant mutants in both experiments was quite similar (table 2). In the Bogesund experimental field there were 76 % seedling mutants and 24 % plant mutants. The respective figures at Sundmo were 70 % and 30 %. Both mutant groups combined gave the following distribution into mutation types expressed in %:

	Albina	*Xantha*	*Xanthoviridis*	*Viridis*
At Bogesund.....	6	30	52	12
At Sundmo.....	5	13	56	26

Low percentage of the mutants of the *xantha* type and high percentage of the *viridis* type at Sundmo is a phenomenon which was also ascertained by the subsequent greenhouse tests. It is more probable that this divergency is due to the retarded germination of seeds in the Sundmo experiment on account of climatic conditions, as well as to the low germinating capacity of seeds originating from mountainous and northern localities.

Low *albina* frequencies and high *xanthoviridis* frequencies are a characteristic feature in pine, and in this respect chlorophyll mutations in pine greatly differ from those in barley, but are similar to those in the pea species.

The differences in the distribution of relative frequencies of mutation types in graphs A, B and C (figs. 4 and 5, table 4) provide decisive evidence for the dependence of this distribution on the mutant rates of the trees. The distribution of mutants into types expressed in per cent in part C of the frequency polygons is as follows:

	Albina	*Xantha*	*Xanthoviridis*	*Viridis*
At Bogesund.....	10	48	33	9
At Sundmo.....	10	25	57	8

The difference between this distribution and the one mentioned before, as well as between graphs A and B is obvious. The cause of such a deviation of the distribution of mutation types in trees having high mutant rates might be found in the increased number of mutated genes.

The distribution of trees with one, two, three and four mutation types in their progeny (tables 5 and 6) is as follows:

At Bogesund	73.7 %	21.2 %	4.9 %	0.2 %
At Sundmo	61.0 %	33.9 %	3.4 %	1.7 %

It appears from the table that most mutations emerge as one single type.

The distribution of mutation cases in the progeny of individual trees containing one, two, three and four mutaion types shows the following sequence of values (tables 5 and 6):

At Bogesund.....	56 %	32 %	11 %	1 %
At Sundmo.....	42 %	46 %	7 %	5 %

The distribution of all mutations into types is as follows:

	Albina	*Xantha*	*Xanthoviridis*	*Viridis*
At Bogesund.....	3 %	24 %	55 %	18 %
At Sundmo	6 %	9 %	49 %	36 %

It differs from the values of relative frequencies which show the distribution of mutants into types (table 4). The divergency between the two series of values might be explained by the varying average number (M) of individual mutants per mutation (tables 7 and 8) in different mutation types.

The sequence of mutation types showing the average number of mutants per one mutation is as follows:

	Albina	*Xantha*	*Xanthoviridis*	*Viridis*	M
At Bogesund.....	5.3	2.9	2.5	1.6	2.5
At Sundmo	2.6	4.5	3.5	2.2	3.1

This sequence of mutation types is similar to "the hypothetical sequence in the genotypical size of the different types" (GUSTAFSSON, 1936) which was obtained in the progeny of X-rayed barley seeds.

An analogous case is the distribution of mutations with one, two, three and >3 individual mutants which is as follows (tables 7 and 8):

At Bogesund.....	57 %	20 %	8 %	15 %
At Sundmo	46 %	22 %	12 %	21 %

If the inferences obtained in the experiments with barley could be applied to spontaneous chlorophyll mutations in pine, the origin of most mutations

of the *viridis*, *xanthoviridis* and of the *xantha* type should be associated with the greater structural changes in the genotype than is the case in the mutations of the *albina* type. On the other hand, low rates of the *albina* type and the remarkably high rates of the *xanthoviridis* and *viridis* types in pine stand in striking contrast to the corresponding values in barley. This contradiction is left unexplained, but one of the conjectural explanations of this phenomenon could be zygotic sterility or at least a certain decrease in the germinating capacity of homozygotes in pine, varying in different mutation types.

The results of trials both in nurseries and in the greenhouse show that the difference between the field and greenhouse mutant rates is quite significant ($P < 0.001$) for those mother trees whose mutant rates are relatively low (table 10, fig. 7), and that it also exists, but with less evidence ($P = 0.05$), in trees containing high mutant rates (table 9, fig. 6).

The increase of the mutant rates in the greenhouse might be explained by better germination conditions of the seeds than in the field trials (table 11). One may surmise that just the seeds endowed with chlorophyll deficient factors have an impaired germinating capacity. There is no direct evidence to prove this assumption but there are points which supply favourable support. For instance, dwarf chlorophyll mutants were rare in field trials but in the greenhouse they occurred frequently.

The influence of light and temperature on the colour effect of the mutants calls forth the manifestation of most divergent mutants (figs. 6 and 7) in the field trial as compared to the greenhouse. The distribution of relative frequencies in mutation types in the greenhouse (fig. 8) is inverse in relation to that obtained in the field trial. The average percentage of the distribution of mutation types in the greenhouse and in the field trials is as follows:

	Albina	*Xantha*	*Xanthoviridis*	*Viridis*
Field trials.....	15	34	39	12
Greenhouse replication....	13	8	21	58

The values of the relative frequencies of the *albina* type do not change in the greenhouse, since the pigmentation of this mutation type is not influenced by the climatic environment. Low relative frequencies in the *xantha* type and high frequencies of the *viridis* type are a common occurrence. In the greenhouse the *viridis* type absorbs from the other types a considerable part of those mutations which in field conditions reveal themselves in another manner, as well as those which appear on account of better germination. Thus, in the greenhouse, the *viridis* type attains an enormous representation being 53 % at Bogesund and 77 % at Sundmo.

In the course of two years in the Bogesund field trial from 1,368 mutants 83 % perished, 3 % turned green and it was impossible to set them apart

from the normal individuals, 14 % or 196 mutants survived, preserving their chlorophyll deficient traits. The survivors made 0.02 % of the total number of germinated seeds, that is, one mutant emerged from among 3,000 two-year specimens. Chlorophyll deficient traits of the surviving mutants are negligible. In field trials all types of seedling mutants were lethal (fig. 9). In the greenhouse the *viridis* type mutants were semi-lethal, but those of other types lethal. The first year mutants of the *albina* and *xantha* type were lethal, but the other types semi-lethal. In the plant mutants of the second year *albinas* and *xanthas* were still lethal but the death-rate in other types was scarce and actually it did not occur more frequently than in normal plants. The faculty of plants to turn green is opposed to lethality. Some of the *viridis* plant mutants turned green already in the course of the first year, but they were more often found in the plant mutants of the *viridis*, *xanthoviridis* and *alboviridis* type in the second year.

On account of their surviving the second year plant mutants and partly also the plant mutants of the first year acquire a certain significance and can possibly add to the hereditary variation in pine in nature. It was those mutations in which the chlorophyll deficient traits were only slightly represented which were decreed to survive. Assuming that the viability and competitive ability of the mutants in the years following is equal to that of normal individuals a general estimate might be made that one homozygous chlorophyll deficient individual might be found on an area of five hectars in natural stands.

As a reproduction unit every pine stand integrates in itself the hereditary material of all individuals. The genotype of the individuals in the stand and external factors are harnessed together. Mutant rate value in the stand, viewed statistically, is a function of two components, that is to say, of chlorophyll mutant rates of individual mother trees, on the one hand, and the percentage of mother trees in which these chlorophyll mutations manifest themselves, on the other hand.

The mutual relationship existing between mutant rates of individual mother trees in each stand separately is expressed by means of chi-square and the grouping of the stands depending on the numerical expression of their relationship is found in figs. 11 and 12. The histograms in these figures show a typical frequency distribution of chi-square values.

The percentage of the mutated trees in stands finds its expression in fig. 13 and table 11. The frequency distribution of these values shows a tendency to correspond to the normal distribution with positive skewness. Extremely high percentage of mutated trees (the highest of which is 88 %) in the stands does not occur too often, but is, nevertheless a natural phenomenon. Most of the stands containing a high percentage of mutated trees are located in districts with favourable climatic conditions. The extremely high percentage

of mutated trees thus should be associated with a better germinating capacity of the seeds originating from localities with beneficial geographic environment. The circumstance that the stands originating from localities in the north of Sweden (experiments at Sundmo) consistently showed a lower percentage of mutated trees than did the stands originating from localities in south and central Sweden (experiments at Bogesund) should be explained by the low germinating capacity of the seeds from localities having unfavourable climatic environment.

The correlation between the percentage of the mutated mother trees and mutant rates in stands is both of a statistical and biological nature, and is expressed in figs. 14 and 15. The mutant rates in stands slowly increase simultaneously with the increase of the percentage of mutated trees. The tendency to linear correlation between the mutant rates of stands and the percentage of the mutated trees is expressed in such a manner as does not induce one to look for another correlation or another tendency. This phenomenon might seem rather paradoxical, since actually with the increase in the percentage of the mutated trees a rapid increase of the mutant rates might be expected. The explanation of this contradiction might be found in the paternal constituent of the reproduction system, that is, in the low quality of pollen containing chlorophyll deficient factors. Thus, due to the elimination of male gametes, mutant rates in stands are restricted.

In the Bogesund trial mutant rates in stands vary from 0.10 to 7.32 per thousand and in the Sundmo trial from 0 to 8.67 per thousand. The frequency distribution (fig. 16, table 14) corresponds to the truncated logarithmico-normal distribution. An assumption might be made that the results of these trials typify chlorophyll mutant rates in pine stands, at least as regards Scandinavia. We might expect that in general in 50 %—75 % of all pine stands mutant rate would be from 0 to 2 per thousand; in 20 %—40 % of stands from 2 to 6 per thousand and in 5 % of all stands there would be the possibility to find > 6 per thousand mutants.

The distribution of localities into classes (table 14) does not provide any evidence for the surmise that the mutant rates in stands or those of the individual trees are dependent on definite geographical environment.

On account of the ample scope of the material used in the investigation there is enough convincing evidence to maintain that the extensive variation of chlorophyll mutation rates in pine stands is a natural phenomenon, which might be explained by the genetic drift.

The distribution of stands containing either two, three or all four mutation types in the progeny of individual trees is as follows: 38 %, 33 % and 29 % (table 15). No stands had been found containing only one single type. Mutation types show the following sequence depending upon the percentage of

stands in which they occur: *xanthoviridis* (100 %), *viridis* (86 %), *xantha* (70 %) and *albina* (36 %).

The variation of the distribution of relative frequencies of mutation types in different localities is very wide. When dividing all 84 stands in 3 regions (region 1 embraces northern Scandinavia and mountain districts, region 3 the southern part of the country and region 2 lies between region 1 and region 3) the following divergencies in the distribution of mutation types in these regions may be noted (table 16). The relative frequencies of *xantho-viridis* are almost the same in all regions. Values of *albina* are low in region 1 and 2. The highest *xantha* values are found in region 3 (26 %), they fall in region 2 (23 %) and drop very low in region 1 (10 %). The *viridis* type displays a reverse tendency when compared with that of the *xantha* type. The highest frequency of the *viridis* type is found in region 1 (29 %), diminishes in region 2 (18 %) and drops lower in region 3 (9 %). Chance, the impact of environment and differences of a genuine genotypical nature should be mentioned when trying to find the cause of the divergency in the distribution of mutation types. However much care might be given to evaluating all influences caused by the environment (figs. 6, 7 and 8), part of the divergency in the distribution of types should be ascribed to genotypical causes. This inference induces one to look for a tendency of evolutionary processes created by geographic environment. However, further investigations are necessary to set apart the genotypic factors from the variation of faculties influenced by environmental conditions.

Acknowledgments

I owe a debt of gratitude to Professor ÅKE GUSTAFSSON for the possibility afforded to me to work at the present publication and for all generous support and valuable suggestions received.

My sincere thanks are also due to Fil. lic. BERTIL MATÉRN for his assistance in the statistical analysis of the investigation material.

I also wish to thank my wife, fil. kand. ALEKSANDRA EICHE, for her help and for translating this publication into English.

Further I have to thank Mrs. KERSTIN LINDAHL for the drafting of graphs.

My collaborators and co-workers have been unsparing in their help with the routine work of the investigation and I take the opportunity of expressing my grateful appreciation of their assistance and patience.

Last but not least I wish to express my indebtedness to Mr. JAMES A. WILLIAMS for his aid in connection with the reading of the manuscript and the proofs.

Literature Cited

ANDERSSON, E., 1954. Några data om pollenvariationen och pollenfertiliteten hos gran och tall. Some data concerning the pollen variation and pollen fertility of spruce and pine. — Svensk papperstidn. 7 & 18.

DEMEREC, M., 1935. Behaviour of chlorophyll in inheritance. — Cold Spring Harbor Symp. Quant. Biol. 3.

DOBZHANSKY, TH., 1939. Experimental studies on genetics of freeliving populations of *Drosophila*. — Biol. Reviews 14.

—— 1951. Genetics and the origin of species. New York.

DUBININ, N. P., 1946. On lethal mutations in natural populations. Genetics 31.

EULER, H. v., 1951. Chlorophylldefekte Mutanten. — Festschr. z. Feier d. 200—jähr. Best. d. Akad. d. Wissensch. in Göttingen.

GUSTAFSSON, Å., 1938. Studies on the genetic basis of chlorophyll formation and the mechanism of induced mutating. — Hereditas 24.

—— 1940. The mutation system of the chlorophyll apparatus. — Lunds Univ. Årsskr. N. F. Avd. 2. 36.

—— 1942. The plastid development in various types of chlorophyll mutations. — Hereditas 28.

—— 1946. The origin of albina and xantha mutations in barley. — Acta Radiol. 27.

—— 1951. Mutations, environment and evolution. — Cold Spring Harbor Symp. Quant. Biol. 16.

—— 1952. Statens skogsforskningsinstitut 1902—1952. — Medd. Stat. Skogsforskn.-inst. 42.

—— 1954. Mutations, viability, and population structure. — Acta agr. scand. 4.

GUSTAFSSON, Å., & NYBOM, N., & WETTSTEIN, U. v., 1950. Chlorophyll factors and heterosis in barley. — Hereditas 36.

GUSTAFSSON, Å., & NYBOM, N., 1950. The viability reaction of some induced and spontaneous mutations in barley. — Hereditas 36.

HOLM, G., 1954. Chlorophyll mutations in barley. — Acta agr. scand. 4.

KLINCKSIECK, P., & VALETTE, TH., 1908. Code des Couleurs. — Paris.

KNAPP, E., 1941. Die Mutation. — Handb. d. Pflanzenz. Bd. 1.

KOSKI, V. M., & SMITH, J. H. C., 1951. Chlorophyll formation in a mutant, white seedling. — 3. Arch. Biochem. 29. New York.

LANGLET, O., 1936. Studier över tallens fysiologiska variabilitet och dess samband med klimatet. — Medd. Stat. Skogsforskn.inst. 29.

LINDSTRÖM, E. W., 1925. Genetic factors for yellow pigment in maize and their linkage relations. — Genetics 10.

MCCLINTOCK, BARBARA, 1951. Chromosome organization and genic expression. — Cold Spring Harb. Symp. Quant. Biol. 16. New York.

MÜNTZING, A., 1946. Sterility in rye populations. — Hereditas 32.

NILSSON-EHLE, H., 1922. Über freie Kombination und Koppelung verschiedener Chlorophyllerbeinheiten bei Gerste. — Hereditas 3.

PLOUGH, H. M., 1941. Spontaneous mutations in Drosophila. — Cold Spring Harb. Symp. Quant. Biol. 9. New York.

PLYM FORSHELL, C., 1953. Kottens och fröets utbildning efter själv- och korsbefruktning hos tall. (*Pinus silvestris* L.). — Medd. Stat. Skogsforskn.inst. 43.

SIMAK, M., & GUSTAFSSON, Å., 1954. Fröbeskaffenheten hos moderträd och ympar av tall. — Medd. Stat. Skogsforskn.inst. 44.

STADLER, L. J., 1932. On the genetic nature of induced mutations in plants. — Proc. 6th Int. Congr. Genet., 1.

—— 1951. Spontaneous mutation in maize. — Cold Spring Harb. Symp. Quant. Biol. 16.

STEBBINS, G. L., JR., 1950. Variation and evolution in plants. — London.

STUBBE, H., 1952. Über einige theoretische und praktische Fragen der Mutationsforschung. — Abh. Sächs. Akad. Wiss. Leipzig. Bd. 47.

Sammanfattning

Föreliggande publikation behandlar tallens klorofyllmutationer och deras frekvenser hos avkomman från enskilda träd i naturliga bestånd.

Frön till försöken insamlades från 77 växtplatser i Sverige, 11 i Norge, 8 i Tyskland och 1 i Holland (fig. 1). Fröna såddes i plantskolor i Bogesund och Sundmo. Försöken pågick under tiden 1951—1954. Försöket i Bogesund omfattade 1 012 000 plantor, som härstammade från 1 016 enskilda träd i 43 bestånd. I Sundmo var motsvarande siffror 757 000 plantor från 1 015 enskilda träd i 43 bestånd. I Bogesund var antalet plantor, uppkomna ur blandade fröprov, 87 000 från 15 bestånd och i Sundmo 98 000 från 18 bestånd. År 1953 gjordes kontrollförsök i växthus i Stockholm med 23 fröprov från enskilda träd av vilka erhölls 6 400 groddplantor. Kontrollförsöken 1954 resulterade i 8 700 groddplantor från 28 fröprov av enskilda träd.

För klassificering av klorofyllmutanterna användes det system, som utarbetats av GUSTAFSSON i försök med korn. Sådana fenotypiska kännetecken hos groddplant- och plantmutanter som avvikelser i färg hos hjärtbladen, stjälken, primära barren samt barren möjliggjorde särskiljande av 4 resp. 5 mutationstyper, nämligen *albina, xantha, xanthoviridis, viridis* och *alboviridis*. En sådan förenklad klassificering var lämplig med hänsyn till försöksmaterialets stora omfattning. Ett karakteristiskt drag hos tallens groddplantmutanter är deras skiftande stjälkpigmentering. Antocyanpigmenteringen var särskilt iögonfallende vid rothalsen av albinastjälken (tabell 1). Dessa färgnyanser hos groddplantmutanterna samt antocyanets funktion hos tallen i allmänhet kommer att behandlas i ett senare meddelande.

Den totala summan av alla mutanter i försöksfältet i Bogesund var 1 368. Mutantfrekvensen var 1,351 ‰, vilket är en mutant på 740 plantor. I Sundmoförsöket uppgick antalet mutanter till 1 317 och mutantfrekvensen till 1,740 ‰, dvs. en mutant på 575 plantor. I Bogesundförsöket hade 60 % av moderträden inga mutanter i avkomman och i Sundmoförsöket var motsvarande värde 70 %. I Bogesund var hos 410 muterade träd den högsta mutantfrekvensen per träd 8,3 % och i Sundmo hos 295 träd var motsvarande värde 25 %.

Höga mutantfrekvenser hos en del enskilda träd är ett intressant men inte på något vis förvånande fenomen. Det illustrerar heterozygoternas förmåga att dölja recessiva och destruktiva faktorer i sin genotyp. Så hög mutantfrekvens kan förklaras, åtminstone hos de flesta enskilda träd, med inavel och är i så fall resultatet av förmågan till självbefruktning hos dessa träd. Mutantfrekvensens variation, illustrerad av frekvenspolygonerna i fig. 2 och 3, motsvarar den stympade logaritmiskt normala frekvensfördelningen. Frekvenspolygonerna är indelade i A-, B- och C-delar. Var och en av dessa delar visar inte endast mutanternas olikartade spridning utan också den olika fördelningen av mutationstyperna.

I avkomman från enskilda träd erhölls groddplant- och plantmutanter med mycket skiftande fördelning. Däremot var de genomsnittliga värdena i båda försöken nästan lika (tabell 2). Hos ca 70 % av träden förekom endast groddplantmutanter i avkomman. 20 % av träden gav endast upphov till plantmutanter och 10 % av träden till både groddplant- och plantmutanter. Endast 3 % av de muterade träden gav konsekvent upphov till både groddplantmutanter och mutanter bland ett- och tvååriga plantor. Den genetiska bakgrunden till olikheterna mellan groddplant- och plantmutanter orsakas av deras polygeniska variation, dvs. antalet

muterade gener i plantorna, deras samverkan under de enskilda plantornas utveckling såväl som skiftande miljöpåverkan under olika utvecklingsstadier.

Det numeriska förhållandet mellan groddplant- och plantmutanter var lika i båda försöken (tabell 2). I Bogesunds försöksfält fanns 76 % groddplantmutanter och 24 % plantmutanter. Motsvarande värden i Sundmo var 70 % och 30 %. Tillsammans gav båda mutantgrupperna följande fördelning på olika mutationstyper, uttryckt i %:

	albina	*xantha*	*xanthoviridis*	*viridis*
I Bogesund	6	30	52	12
I Sundmo	5	13	56	26

Det låga procenttalet mutanter av *xanthatypen* och det höga procenttalet av *viridistypen* i Sundmo är en företeelse, som också tydligt visade sig i senare växthusförsök. Det är troligt att denna olikhet mellan Bogesunds- och Sundmoförsöken i viss mån beror på hämmad groning hos fröna i Sundmoförsöket på grund av svårare klimatbetingelser, samt också på den låga groningsförmågan hos frön, som härstammar från fjälltrakter och nordligt belägna orter, men givetvis kan det även finnas andra orsaker.

Låga *albinafrekvenser* och höga *xanthoviridisfrekvenser* är ett karakteristiskt drag hos tallen, som i detta avseende i hög grad skiljer sig från kornet, men liknar ärterna.

Olikheterna i fördelningen av mutationstypernas relativa frekvenser i polygondelarna A, B och C (fig. 4 och 5, tabell 4) utgör ett säkert bevis för fördelningens beroende av mutantfrekvenserna hos träden. Mutanternas fördelning på olika typer uttryckta i % i del C av frekvenspolygonen är följande:

	albina	*xantha*	*xanthoviridis*	*viridis*
I Bogesund	10	48	33	9
I Sundmo	10	25	57	8

Olikheten mellan denna fördelning och den genomsnittliga, såväl som mellan polygondelarna A och B är uppenbar.

Fördelningen av träd med en, två, tre eller fyra mutationstyper i sin avkomma (tabellerna 5 och 6) är följande:

I Bogesund	73,7 %	21,2 %	4,9 %	0,2 %
I Sundmo	61,0 %	33,9 %	3,4 %	1,7 %

Det framgår av tabellen att de flesta mutationerna uppträder som endast en typ i enskilda träds avkommor.

Fördelningen av mutationsfall med en, två, tre eller fyra mutationstyper samtidigt i enskilda träds avkommor (tabellerna 5 och 6) är följande:

I Bogesund	56 %	32 %	11 %	1 %
I Sundmo	42 %	46 %	7 %	5 %

Den genomsnittliga fördelningen av mutationer (således icke mutanter) är följande:

	albina	*xantha*	*xanthoviridis*	*viridis*
I Bogesund	3 %	24 %	55 %	18 %
I Sundmo	6 %	9 %	49 %	36 %

Dessa värden skiljer sig från dem man erhåller för mutanternas fördelning i olika typer (tabell 4). Skillnaden mellan de två serievärdena kan förklaras med variationen av medeltalet (M) för enskilda mutanter per mutation (tabellerna 7 och 8) hos olika mutationstyper.

Antalet individ per avkomma och mutationstyp är genomsnittligen följande:

	albina	*xantha*	*xanthoviridis*	*viridis*	M
I Bogesund	5,3	2,9	2,5	1,6	2,5
I Sundmo	2,6	4,5	3,5	2,2	3,1

De här erhållna resultaten överensstämmer med »the hypothetical sequence in the genotypical size of the different types» (GUSTAFSSON, 1938), som erhölls i avkomman av röntgenbestrålat korn.

Ett analogt fall är fördelningen av mutationer med en, två, tre och > 3 mutanter per avkomma enligt följande (tabellerna 7 och 8):

I Bogesund	57 %	20 %	8 %	15 %
I Sundmo	46 %	22 %	12 %	21 %

Om de vid kornförsöken erhållna resultaten även kan tillämpas vid klorofyllmutationer hos tallen skulle ursprunget till de flesta mutationerna av *viridis*- och *xanthatyperna* kunna sättas i samband med grövre strukturella förändringar inom genotypen, vilket inte är fallet hos mutationer av *albinatypen*. Å andra sidan står de låga frekvenserna av *albinatypen* och de anmärkningsvärt höga frekvenserna av *xanthoviridis*- och *viridistyperna* hos tallen i skarp kontrast till motsvarande värden hos korn. Denna motsägelse är svårtolkad, men en av de antagliga förklaringarna till företeelsen kan vara zygotisk sterilitet eller åtminstone en viss minskning av groningsförmågan hos de muterade recessiverna, vilken varierar i olika mutationstyper.

Olikheten mellan mutantfrekvenserna i fält- och i växthusförsöken är starkt signifikativ ($P < 0{,}001$) för de moderträd, vilkas mutantfrekvenser är relativt låga (tabell 10, fig. 7) och den existerar också, men med mindre påtaglighet ($P = 0{,}05$) hos träd, som har höga mutantfrekvenser (tabell 9, fig. 6).

Ökningen av mutantfrekvenserna i växthusförsöken kan förklaras med frönas bättre groningsförhållanden därstädes (tabell 11). Förmodligen har de frön, som är behäftade med klorofylldefekter, en nedsatt groningsförmåga. Det finns inget bestämt stöd för detta antagande, men vissa tecken tyder härpå. Dvärgklorofyllmutanterna var t. ex. sällsynta i fältförsöken men i växthusförsöken förekom de allmänt.

Inverkan av ljus och temperatur på mutanternas pigmentbildning åstadkommer en annan fördelning av mutanterna på typer (fig. 6 och 7) i fältförsöken jämfört med växthusförsöken. Fördelningen av mutationstypernas relativa frekvenser i växthusförsöken (fig. 8) är omvänd till fältförsökens. Mutanternas genomsnittliga fördelning på mutationstyper i % är följande:

	albina	*xantha*	*xanthoviridis*	*viridis*
Fältförsök	15	34	39	12
Växthusförsök	13	8	21	58

De relativa frekvenserna av *albinatypen* ändras inte i växthusförsöken emedan pigmentering hos *albinas* inte påverkas av klimatförhållandena. De låga relativa

frekvenserna av *xanthatypen* och de höga frekvenserna av *viridistypen* är en allmän företeelse. I växthusförsöket upptar *viridistypen* en stor del av de mutationer, som under fältförhållanden uppträder i annan form samt även de, som framträder tack vare frönas bättre groning. I växthusförsöken uppgår *viridistypen* till 53 % i avkomman av moderträd, som ingår i Bogesunds fältförsök, och till 77 % i Sundmo.

Av de 1 368 mutanterna i Bogesunds fältförsök dog 83 % under loppet av två år, 3 % antog grön färg och var omöjliga att skilja från de normala plantorna, 14 % eller 196 mutanter överlevde och behöll sina bristfälliga klorofyllegenskaper. De överlevande utgjorde 0,02 % av det totala antalet grodda frön, dvs. en mutant förekom bland 3 000 tvååriga plantor. Hos de överlevande mutanterna var klorofylldefekterna föga framträdande. I fältförsöken var alla slag av groddplantmutanter letala (fig. 9). I växthusförsöken var *viridistypens* groddplantmutanter semiletala, de andra letala. Första årets *albina-* och *xanthaplantmutanter* var letala i fältförsöken, de andra mutanttyperna semiletala. Av andra årets plantmutanter var *albina-* och *xanthatyperna* fortfarande letala, men däremot var dödlighetsfrekvensen låg hos de andra typerna eller ungefär densamma som hos normala plantor. Plantor med förmågan att antaga grön färg är den extrema motsatsen till plantor, som saknar denna förmåga, dvs. är letala. En del av *viridis*-plantmutanterna antog normal grön färg redan under första året, men oftare blev *viridis-*, *xanthoviridis-* och *alboviridistypernas* plantmutanter gröna först andra året.

Tack vare förmågan att överleva får andra och delvis första årets plantmutanter en signifikativ betydelse, som antagligen bidrar till tallens ärftliga variation i naturen. Endast mutanter med svaga klorofylldefekter överlevde. Under förutsättning att mutanternas livsstyrka och konkurrensduglighet under de följande åren är densamma som de normala plantornas, kan man allmänt räkna med att det förekommer en tall med homozygota klorofylldefekter på en yta av fem ha i naturliga bestånd.

Vidare försök kommer att besvara frågan om den selektiva betydelsen av tallens klorofyllmutationer i förädlingsarbetet.

Såsom fortplantningsenhet förenar varje tallbestånd i sig alla sina träds arvsmassa. Mutantfrekvensvärdet i beståndet är ur statistisk synpunkt en funktion av två komponenter, dvs. enskilda moderträds klorofyllmutantfrekvenser å ena sidan och procenttalet moderträd, hos vilka klorofyllmutationer framträder, å andra sidan.

Sambandet, som existerar mellan enskilda moderträds mutantfrekvenser i varje bestånd för sig kan uttryckas medelst chi-kvadratvärden och beståndens gruppering med hänsyn till dessa värdens storlek finner man i fig. 11 och 12. Dessa figurers histogram visar en för chi-kvadratvärden typisk frekvensfördelning.

Procenttalet muterade träd i bestånden framgår av fig. 13 och tabell 13. Dessa värdens frekvensfördelning visar en viss likhet med normalfördelningen men har en positiv snedhet. Extremt höga procenttal muterade träd (av vilka det högsta är 88 %) i bestånden är mindre vanliga. De flesta av dessa bestånd är belägna i områden med relativt gynnsammare klimatförhållanden. Detta skulle kunna sättas i samband med bättre groningsförmåga hos frön, som härstammar från orter med fördelaktig geografisk miljö.

Omständigheten att bestånden från orter i norra Sverige (försöken i Sundmo) konsekvent visade ett lägre procenttal muterade träd än bestånden från orter i södra och mellersta Sverige (försöken i Bogesund) skulle likaså kunna förklaras

med den låga groningsförmågan hos frön från orter med ogynnsam klimatmiljö samt också med frönas bättre groningsförhållanden i Bogesunds fältförsök.

Sambandet mellan procenttalet muterade moderträd och mutantfrekvensen i avkomman från samtliga träd i bestånd är både av statistisk och biologisk natur och finns uttryckt i fig. 14 och 15. Beståndens mutantfrekvenser ökar långsamt med ökat procenttal muterade träd. Detta samband liknar en lineär korrelation. Beståndens gruppering i fig. 14 tyder inte på något annat samband. Detta kan förefalla ganska paradoxalt, eftersom man i verkligheten kan vänta sig en snabb ökning av mutantfrekvenser med ökat procenttal muterade träd. Förklaringen till denna motsägelse kan troligen sökas hos den hanliga delen av reproduktionssystemet, dvs. i den låga grobarheten av de pollenkorn, som är behäftade med klorofylldefektfaktorer. Beståndens mutantfrekvenser kan begränsas genom de hanliga gameternas elimination.

I Bogesundsförsöket varierar beståndens mutantfrekvenser från 0,10 till 7,32 ‰ och i Sundmoförsöket från 0 till 8,67 ‰. Frekvensfördelningen motsvarar den stympade logaritmiskt normala fördelningen. Antagandet att dessa försöksresultat exemplifierar tallbestånds klorofyllmutantfrekvenser kan göras åtminstone angående Skandinavien. Man kan vänta sig att hos 50—75 % av alla tallbestånd mutantfrekvensen i allmänhet bör variera från 0 till 2 ‰, hos 20—40 % av bestånden från 2 till 6 ‰ och hos 5 % av alla bestånd kan man finna > 6 ‰ klorofyllmutanter.

Beståndens fördelning på mutantfrekvensklasser (tabell 14) tyder inte på att tallbeståndens eller de enskilda trädens mutantfrekvenser skulle sammanhänga med en bestånd geografisk miljö.

Med hänsyn till det i försöket använda materialets omfattning finns det tillräckligt med övertygande bevis för påståendet, att klorofyllmutationsfrekvensernas stora variation i tallbestånden är en naturlig företeelse, som kan förklaras med »genetic drift».

Fördelningen av bestånd, som innehåller två, tre eller alla fyra mutationstyperna i enskilda träds avkomma är följande: 38 %, 33 % och 29 % (tabell 15). Inga bestånd med endast en mutationstyp har påträffats. Mutationstyperna uppvisar följande värden, beroende på procenttalet bestånd i vilka de förekommer: *xanthoviridis* 100 %, *viridis* 86 %, *xantha* 70 % och *albina* 36 %.

Mutationstypernas relativa frekvenser varierar betydligt på olika orter. Om man indelar alla 84 bestånden i 3 regioner (region 1 omfattar norra Skandinavien och fjälltrakterna, region 3 den södra delen av landet och region 2 området däremellan) kan man iaktta följande skillnader i mutationstypernas fördelning (tabell 16). *Xanthoviridistypens* relativa frekvenser är nästan desamma i alla regioner. *Albinatypens* värden är låga i region 1 och 2. De högsta *xanthavärdena* förekommer i region 3 (26 %), sjunker i region 2 (23 %) och har ett mycket lågt värde i region 1 (10 %). *Viridistypen* visar en tendens motsatt *xanthatypens*. Dess högsta frekvens förekommer i region 1 (29 %), minskar i region 2 (18 %) och har ett ännu lägre värde i region 3 (9 %). Slumpen, inverkan av miljön och skillnader av rent genotypisk art bör nämnas, när man söker orsakerna till olikheterna i mutationstypernas fördelning. Fastän miljöinflytandet här spelar en väsentlig roll, bör dock en del av olikheterna tillskrivas den genotypiska variationen, dvs. det pågår en evolutionsprocess påverkad av den skiftande geografiska miljön.

Ytterligare undersökningar är nödvändiga för att helt klarlägga de genotypiska faktorernas andel i den fenotypiska variationen hos klorofyllmutanterna.