

Preliminary Investigations of Inbreeding and Outcrossing in Larch

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Introduction

Selfpollination as the most efficient method of inbreeding has been carried out for a considerable period of time in the genus *Larix*, especially in the two species, European and Japanese larch, at the Arboretum in Harsholm. The possibility of controlled and forced selfpollination was first demonstrated by C. SYRACH-LARSEN in 1934 on a hybrid larch (*Larix gmelini* X *leptolepis*) and later on in 1938 and 1941 on Japanese and European larch, respectively. Since then many offsprings of the S_1 and S_2 -generations have been raised when flowers became available.

Originally when the selfpollination started the purpose was to evaluate the selected trees by studying the variation within families and the eventual emergence of recessive, deleterious genes (20).

These studies however have been somewhat neglected, but as soon as the first flowers appeared on individuals of the selfed families it was suggested by C. SYRACH-LARSEN (21) to cross inbreds of European and Japanese larch, respectively. Although very promising results from the hybrid, *Larix eurolepis*, were already available, an additional heterosis effect from the outcrossing was anticipated.

A few interspecific outcrossings were made in 1949 and 1951 of which only remain three trees of the $P \times S_1$ combination (S. 1760). In 1954, when larch flowered abundantly, a number of single crosses were carried out between individual of the S_1 generations of European and Japanese larch respectively, resulting in several hundred seedlings per cross. The families were planted out in equal sized plots without replications but with two plots of European larch from a selected seed source (Rundforbi) for comparison. The hybrids in the observation trial (F-no. 72) showed a luxuriant and uniform growth, but it was not possible to estimate how much of the heterosis effect was due to the species crossing or how much to the inbreeding/outcrossing effect. In comparison with the standard of European larch the hybrids grew from 118–132% better according to height measurements in 1961. This figure did not exceed similar comparisons in the same part of the country between hybrids of the $P \times P$ level and the same standard (11). An inbreeding/outcrossing effect was indicated by the relatively small standard deviation of the heights. For 6 of the 8 progenies this was below 10% of their respective mean heights, and the highest was almost 13%. These figures are slightly below standard deviations registered in hybrids of the $P \times P$ level.

We were thus still faced with the problem of how effective the method of inbreeding and outcrossing might be, especially when "ordinary" hybridization seemed so promising in itself. Of primary interest for our first investigations was therefore to test the effectiveness of the method as soon as more S_1 and S_2 families reached the reproductive state and to gain experiences in some of the problems related to inbreeding viz. inbreeding depression, the importance of selection of foundation trees and of inbred families and individuals. Although the heterosis effect deriving from the

interspecific crossing might obscure the effect of the inbreeding/outcrossing most of the testing has been done with hybrids. A very practical reason is that foresters are more inclined to accept hybrid larch for field trials in their forests than the pure species. It is thought justifiable also because the variation between hybrids seems to be of nearly the same magnitude as for European and Japanese larch respectively (KEIDING, 1962).

The effect of inbreeding in various aspects has been studied by many workers viz. ORR-EWING (1957–65), RIGHTER (1960), LANGNER (1961), SQUILLACE and KRAUS (1963–64), DIECKERT (1964), and FOWLER (1964–65). Outcrossing following inbreeding however have only been described for *Pseudotsuga menziesii* (ORR-EWING, 1965) and for *Larix* (KEIDING, 1962). Thus studies of inbreeding depression have hitherto been subject to far more investigations than outcrossing which may have its simple explanation in that most of the inbred material has not started to flower yet. Of course importance of inbreeding in other aspects viz. genetic gain by selection (*Pinus resinosa*, FOWLER, 1964–65), composition of seed orchards etc. has played a part too.

Inbred lines of the S_1 and in a few instances of the S_2 generations have been both measured and scored for various quantitative characters at Harsholm, but the results have not been very conclusive partly due to insufficient experimental lay-outs. Therefore the availability of flowers has to a large extent been determining for the selection of material for the outcrossings presented here.

Material

In the years of 1958 and 1960, when flowering in larch was abundant, a number of crossing series were carried out. The material connected with inbreeding and outcrossing resulted in six field trials of which the oldest, planted out in 1961, have been measured 3 times and scored for crookedness once. The youngest which were planted in the field in 1963 were measured in the autumn of 1966.

All the experiments serve the purpose of evaluating the effect of inbreeding and outcrossing, but three of them have the additional aim of investigating the effect of selection within inbred lines. The three latter are numbered F-no. 73, 89 and 92. For the sake of convenience the six experiments are treated in two groups termed A and B, the former containing F-no. 73, 76, 77 and 94 and the latter F-no. 73, 89 and 92.

Of the six experiments three are so-called small scale trials for early assessment (F-no.'s 76, 77 and 94) and two are almost identical, but placed in different parts of the country (F-no. 89 and 92).

The material and the experimental lay-outs for the six experiments are briefly described in the following.

Group A

Experiment F-no. 73: 2 clones of *Larix decidua* act as females for 7 individuals of *L. leptolepis*. One of the Eu-

Table 1. — Crossing scheme for F-no. 73.

P × P		P × S ₁	
♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch
V. 44	S. 127 ⁶	V. 44	S. 818 ⁵
V. 44	S. 127 ⁵⁰	V. 44	S. 1500 ⁴
		V. 44	S. 1500 ⁵
		V. 44	S. 1500 ⁶
		V. 44	S. 1500 ⁷
710/37	S. 127 ⁶	710/37	S. 818 ⁵
710/37	S. 127 ⁵⁰	710/37	S. 1500 ⁴
		710/37	S. 1500 ⁵
		710/37	S. 1500 ⁶
		710/37	S. 1500 ⁷
Eur. larch	Eur. larch	Eur. larch	Eur. larch
V. 44	V. 418	V. 44	S. 815 ⁵

The males of the S₁-generation derive from the males of the P-generation by selfing thus:

S. 818 = S. 127⁶, selfed

S. 1500 = S. 127⁵⁰, selfed

S. 815 = V. 418, selfed

ropean clones is in addition pollinated by 2 individuals of *L. decidua* making a total of 16 progenies. The trial is situated on the island of Bornholm.

The principle of the crossing series may be seen in the table 1.

The index after the family number indicate individual trees within that family. The two Japanese males of the P-generation are sibs and thus the inbred Jap. males are also related partly in the degree of cousins (S. 818 and S. 1500) and partly as sibs (S. 1500 nos. 4–7). The European larches of the P-generation are selected, unrelated plus trees.

The experimental lay-out is a 4 × 4 balanced lattice square with 5 squares which may also be considered as a randomized block design with 5 replications. The trees are planted at 2 × 2 m in plots of 20 trees (4 × 5) in an area, where the underlying rock causes a somewhat discontinuous variation in the depth of soil and thereby the conditions for growth. Year of planting: 1961 with 1/1 seedlings.

Experiment F-no. 76 and 77 at Hørsholm:

The principle of the crossing series is stated in table 2. In these two experiments a few more crosses at the P × P level and one of the S₁ × S₁ were included.

Again the inbreds derived from the males (females) of the P-generation by selfing. The following new inbred families appeared in F-no. 77:

S. 1194 = S. 127², selfed.

S. 648 = V. 44, selfed.

The experimental lay-out of F-no. 76 is a 5 × 5 latin square with quadratic plots of 4 trees and a planting dis-

Table 2. — Crossing scheme for F-no. 76 and 77.

P × P		P × S ₁		S ₁ × S ₁	
♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch
V. 418	S. 127 ⁵⁰	V. 418	S. 1500 ⁴	S. 815 ⁴	S. 1500 ⁷
		V. 418	S. 1500 ⁶		
		V. 418	S. 1500 ⁷		
V. 44	S. 127 ²	V. 44	S. 1194 ¹		
V. 418	S. 127 ²	V. 44	S. 1194 ²		
710/37	S. 127 ²	V. 44	S. 1194 ⁷		
		Jap. larch	Eur. larch		
		S. 127 ²	S. 648 ⁹		
		S. 127 ⁵⁰	S. 648 ⁹		

Table 3. — Crossing scheme for F-no. 94.

P × P		P × S ₁		S ₁ × S ₁	
♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch
V. 44	S. 127 ⁵⁰	V. 44	S. 1500 ⁷		
V. 44	S. 127 ⁶	V. 44	S. 818 ⁵	S. 1174 ²⁹	S. 818 ⁵
V. 44	S. 127 ²	V. 44	S. 1194 ¹		
V. 418	S. 127 ⁵⁰	V. 418	S. 1500 ⁷		
V. 418	S. 127 ⁶	V. 418	S. 818 ⁵		
V. 418	S. 127 ²	V. 418	S. 1194 ¹	S. 815 ⁵	S. 1194 ¹

tance of 1.25 × 1.25 m and of F-no. 77 a randomized block design with 5 replications. Plots contained 5 trees in a row. Planting distance the same as in F-no. 76. A removal of every alternate tree was carried out in March 1964 when the trees were beginning to compete.

Experiment F-no. 94, Flensburg plantation, Jutland:

This again is a small scale trial planted in the field in 1963 i.e. two years later than those previously described. Plants are from the controlled pollination in 1960.

The principle of the crossing series is given in table 3.

Again it should be noted that all individuals of the S₁ generation descend from the males or females employed in the P × P crossings by selfing. Each row in the table represents successive steps in the outcrossing. Thus the second and the sixth row have three steps, S. 1174 being V. 44, selfed, S. 818 = S. 127⁶, selfed, S. 815 = V. 418, selfed, and S. 1194 = S. 127², selfed.

The experimental lay-out is a 4 × 4 balanced lattice square or randomized block design with 5 replications. Each plot has 6 plants in 2 rows of 3, and the planting distance is 1.5 × 2.0 m.

Group B

Experiment F-nos. 89 and 92, Corselitze in Falster and Flensburg plantation:

The two experiments are almost identical in material and design. In the latter we had to include the offspring from a selected seed source of Japanese larch due to shortage of one of the combinations in the outcrossing programme. Plot size and planting distance are also slightly different, but the experimental design is the same.

The crossing programme is stated in table 4.

In principle 6 sibs of the inbred family, S. 1194, descending from S. 127² by selfing, are crossed onto the common tester V. 44. Beside testing the combining ability of the S₁ individuals they may all be compared with the crossing at the P × P level (V. 44 × S. 127²).

The experimental lay-out in both instances is a Youden square or a randomized block design with 4 replications. In F-no. 89 plots consist of 4 rows of 9 plants at a planting distance of 2.00 × 1.25 m, while in F-no. 92 the plots have 6 rows of 6 plants with a planting distance of 1.5 × 2.0 m.

Table 4. — Crossing scheme for F-nos. 89 and 92.

P × P		P × S ₁	
♀ Eur. larch	♂ Jap. larch	♀ Eur. larch	♂ Jap. larch
V. 44	S. 127 ²	V. 44	S. 1194 ¹
		V. 44	S. 1194 ²
		V. 44	S. 1194 ⁴
		V. 44	S. 1194 ^{6*}
		V. 44	S. 1194 ⁷
		V. 44	S. 1194 ⁹

*) Present in F-no. 89 only.

These differences are due to special considerations for the planting practice at the two forest districts.

Testing of individuals within an inbred line is also possible in F-no. 73. This experiment has already been described.

Method

The effect of inbreeding and outcrossing as well as the importance of selection have in the first instance been judged by the relative vigour of the offsprings. Height measurements of all trees in all the experiments have been carried out from one to several times. In one of the older experiments from 1961, F-no. 73, assessment of stem crookedness has begun and in F-no. 76 and 77 diameter at breast height has been measured. Recording of survival percentage and calculation of standard deviation have been made and contribute to a general evaluation of the method. The observations have been subject to analysis of variance. In the following however it is mainly the results of the height measurements that will be presented and discussed.

Results

Group A

In the experiments where measurements have been taken more than once, the rank of the different families in respect of height vary but little from one measurement to another. The small fluctuations observed may be ascribed to chance, as they are all well within the confidence limits. Therefore only the last measurements from 1966 are presented here.

The variation which has been registered, in some cases as significant differences between offsprings, seems to a greater extent to have its origin in the heritable variation between individuals than to be caused by the inbreeding and outcrossing. From table 5 it appears that two third of the $P \times S_1$ crosses show a slight improvement in height growth, while one third is slightly below. In the case of the

few $S_1 \times S_1$ crosses they are either below or equal to the corresponding $P \times P$ crosses.

The only example of outcrossing between individuals of European larch is found in F-no. 73 (S. 3910 and S. 3911. See table 5). The relative position of the two levels of outcrossing is the same as for the hybrids. The vigour of these two families however is significantly less than most of the hybrids. Using the cross, S. 3910 between the two non-inbred European larches, V. 44 and V. 418, as comparison the superiority in height growth of the hybrids ranges from 104–117%. Thus the heterosis effect deriving from the inter-specific crossing is much more pronounced than that coming from the inbreeding/outcrossing.

Further in this experiment there seems to be an example of specific combining ability. When the two European larches, V. 44 and 710/37, are pollinated with the Japanese males in turn, each pair of half-sibs differ only little in respect of vigour, and the two females seem to be equal in breeding value, except in one instance i. e. when S. 127⁶ is the pollinator. The family S. 3948 (710/37 \times S. 127⁶) has a significantly smaller mean height than family S. 3891 (V. 44 \times S. 127⁶). As the specific combining ability goes in a negative direction further use of this particular Japanese larch is problematic.

In the experiment F-no. 94 we again find that a majority of the $P \times S_1$ crosses are slightly superior to the $P \times P$ crosses. The picture is very similar to that of F-no. 73 (see table 5), only that one outcrossing at the $P \times S_1$ level is significantly less vigorous than its corresponding $P \times P$ cross (S. 4333 and S. 4334).

In the analysis of variance significant differences in height growth have been found. These differences however are nearly always found between progenies, which are not related in the context of inbreeding and outcrossing. As already mentioned the influence of the individual parents seem to account for most of the variation at these initial stages of the method.

Of great interest for the development of inbreeding and outcrossing is the possible correlation between good per-

Table 5. — Relative vigour of outcrossings in experiments, F-no. 73, 76, 77 and 94.

Exp. no.	$P \times P$				$P \times S_1$					$S_1 \times S_1$				
	family no.	♀ Eur. larch	♂ Jap. larch	M_H 1966	family no.	♀ Eur. larch	♂ Jap. larch	M_H 1966	% of $P \times P$	family no.	♀ Eur. larch	♂ Jap. larch	M_H 1966	% of $P \times P$
73	S. 3891	V. 44	S. 127 ⁶	5.06	S. 3894	V. 44	S. 818 ⁵	4.88	96					
	S. 3948	710/37	S. 127 ⁶	4.48	S. 3954	710/37	S. 818 ⁵	4.79	107					
	S. 3893	V. 44	S. 127 ⁵⁰	4.89	S. 3906	V. 44	S. 1500 ⁴	4.97	102					
	S. 3893	V. 44	S. 127 ⁵⁰	4.89	S. 3907	V. 44	S. 1500 ⁵	4.83	99					
	S. 3893	V. 44	S. 127 ⁵⁰	4.89	S. 3908	V. 44	S. 1500 ⁶	4.97	102					
	S. 3893	V. 44	S. 127 ⁵⁰	4.89	S. 3909	V. 44	S. 1500 ⁷	5.06	103					
	S. 3949	710/37	S. 127 ⁵⁰	4.94	S. 3950	710/37	S. 1500 ⁴	5.06	102					
	S. 3949	710/37	S. 127 ⁵⁰	4.94	S. 3951	710/37	S. 1500 ⁵	4.97	101					
	S. 3949	710/37	S. 127 ⁵⁰	4.94	S. 3952	710/37	S. 1500 ⁶	5.07	103					
	S. 3949	710/37	S. 127 ⁵⁰	4.94	S. 3953	710/37	S. 1500 ⁷	4.99	101					
	S. 3910	V. 44	V. 418	4.32	S. 3911	710/37	S. 815 ⁵	4.44	103					
			(Eur. larch)				(Eur. larch)							
76	S. 3958	V. 418	S. 127 ⁵⁰	6.40	S. 3959	V. 418	S. 1500 ⁴	6.45	101	S. 3923	S. 815 ⁴	S. 1500 ⁷	6.11	95
	S. 3958	V. 418	S. 127 ⁵⁰	6.40	S. 3961	V. 418	S. 1500 ⁶	6.14	96					
	S. 3958	V. 418	S. 127 ⁵⁰	6.40	S. 3962	V. 418	S. 1500 ⁷	6.40	100					
77	S. 3892	V. 44	S. 127 ²	5.74	S. 3895	V. 44	S. 1194 ¹	5.82	101					
	S. 3892	V. 44	S. 127 ²	5.74	S. 3896	V. 44	S. 1194 ²	5.50	96					
	S. 3892	V. 44	S. 127 ²	5.74	S. 3900	V. 44	S. 1194 ⁷	5.42	94					
94	S. 4321	V. 44	S. 127 ⁵⁰	3.10	S. 4320	V. 44	S. 1500 ⁷	3.20	103					
	S. 4323	V. 44	S. 127 ⁶	2.98	S. 4322	V. 44	S. 818 ⁵	3.03	102	S. 4338	S. 1174 ²⁹	S. 818 ⁵	2.86	96
	S. 4330	V. 44	S. 127 ²	2.77	S. 4324	V. 44	S. 1194 ¹	2.86	103					
	S. 4332	V. 418	S. 127 ⁵⁰	2.95	S. 4331	V. 418	S. 1500 ⁷	3.03	103					
	S. 4334	V. 418	S. 127 ⁶	3.04	S. 4333	V. 418	S. 818 ⁵	2.90	95*					
	S. 4336	V. 418	S. 127 ²	2.95	S. 4335	V. 418	S. 1194 ¹	2.84	96	S. 4317	S. 1194 ¹	S. 815 ⁵	2.96	100
											(Jap. larch)	(Eur. larch)		

formers of the parental generation and the inbred lines deriving from them. The material has been studied for that purpose. In view of the small representation from each line and the possible variation between sibs, the results must be taken with some reservation. However, three individuals of the S_1 -generation, S. 1500⁷, S. 818⁵, and S. 1194¹ have the same order of merit in combination with two European larches (V. 44 and V. 418), as their corresponding foundation trees, S. 127⁵⁰, S. 127⁶, and S. 127², when these latter are crossed with V. 44.

Group B: Selection within inbred lines

With a number of inbred families available at the reproductive stage further inbreeding (selfing) became feasible, but as the number of trees we could manage to selfpollinate was limited, the problem arose after which criterions such trees should be selected. Would it be possible to judge from the phenotypic appearance viz. vigour and form, which trees had the highest potential for further inbreeding and outcrossing? According to experiences from other fields of plant breeding it seems very difficult to give general rules for selection on appearance alone (22). Selecting for vigour usually slows down the process as it tends to favour the heterozygous individuals and thus delay, eventually exclude, the attainment of the homozygous state, which is the basis for obtaining heterosis. On the other hand consequent selection of the weakest plants may also lead to difficulties and disappointment as the inbreeding may stop at too early a stage. The safest but also the most laborious method is to test as many trees as possible within the family. Realizing the difficulties in setting up criterions for selection with regard to vigour, we grafted six trees from a number of inbred lines. Three of the trees belonged to the vigorous part and three to the slowest growing part. The clones thus established have only begun to flower recently, and it has not yet been possible to start extensive investigations. With the increasing number of inbred families it will however take up far too much space to proceed with grafting on those lines, and selection will have to be a combination of phenotypic selection and testing in progeny trials.

Experiences from the breeding of corn show that specific combining ability between inbred lines, which is the ultimate aim of the method, very often is correlated with general combining ability in the early stages of inbreeding. It is important therefore to single out individuals of the S_1 -families for general combining ability before the inbreeding is continued, and this may be done by using one or a few common testers.

Three experiments, F-nos. 73, 89, and 92 are in progress in order to investigate the variation in respect of combining ability within families as a basis for selection. The first, F-no. 73, has already been mentioned in connection with a more general test of inbreeding and outcrossing. In all three experiments the same clone of European larch, V. 44, has been crossed with a number of Japanese sibs from two S_1 -families. Further the foundation trees of the two families are included in the crossing series as males. In F-no. 73 a second European clone, 710/37 has been used as common tester.

In table 6 the average heights of the outcrossings in 1966 are stated partly in absolute measure and partly as a percentage of the $P \times P$ crossings i. e. the hybrids between the European clones and the foundation trees.

Looking at the experiment, F-no. 73 first it is noticeable that the mean heights of the progenies having sibs of family S. 1500 as male parents deviate only a few percent from that of the standard ($P \times P$ cross). Consequently the variation between these progenies is small, and none of the differences are significant at the $P:0.05$. In view of the uniform appearance of the individuals from family S. 1500 it is probable that the genetic constitution of these individuals vary but little. As regard to further inbreeding the prospects for family S. 1500 are not very promising provided the four individuals employed are sufficiently representative.

In family S. 1194 the six individuals tested seem to be more variable in respect of combining ability than those of S. 1500. The mean heights of some of the outcrossing exceed that of the standard ($P \times P$) with up to 14% and in both experiments, F-no. 89 and 92, there are significant differences between offsprings and standard as well as between offsprings (marked with an asterisk in table 6). It

Table 6. — Individual variation of sibs in respect of height growth within two inbred families, S. 1500 and S. 1194.

Exp. no.	$P \times P$				$P \times S_1$				
	S-no.	♀	♂	M_H m	S-no.	♀	♂	M_H m	% of $P \times P$
73	S. 3893	V. 44	S. 127 ⁵⁰	4.89	S. 3906	V. 44	S. 1500 ⁴	4.97	102
					S. 3907	V. 44	S. 1500 ⁵	4.83	99
					S. 3908	V. 44	S. 1500 ⁶	4.97	102
					S. 3909	V. 44	S. 1500 ⁷	5.06	103
	S. 3949	710/37	S. 127 ⁵⁰	4.94	S. 3950	710/37	S. 1500 ⁴	5.06	102
					S. 3951	710/37	S. 1500 ⁵	4.97	101
					S. 3952	710/37	S. 1500 ⁶	5.07	103
					S. 3953	710/37	S. 1500 ⁷	4.99	101
89	S. 4330	V. 44	S. 127 ²	2.15	S. 4324	V. 44	S. 1194 ¹	2.23	104
					S. 4325	V. 44	S. 1194 ²	2.11	98
					S. 4326	V. 44	S. 1194 ⁴	2.29	107
					S. 4327	V. 44	S. 1194 ⁶	2.46	114 [*]
					S. 4328	V. 44	S. 1194 ⁷	2.13	99
					S. 4329	V. 44	S. 1194 ⁹	2.38	111
92	S. 4330	V. 44	S. 127 ²	2.63	S. 4324	V. 44	S. 1194 ¹	2.67	102
					S. 4325	V. 44	S. 1194 ²	2.53	96
					S. 4326	V. 44	S. 1194 ⁴	2.77	105 [*]
					S. 4328	V. 44	S. 1194 ⁷	2.82	107 [*]
					S. 4329	V. 44	S. 1194 ⁹	2.77	105 [*]

* Denotes that the difference is significant at $P:0.05$ in proportion to the $P \times P$ cross.

may be noted that the rank of the offsprings in respect of vigour is the same in the two experiments except for one cross, S. 4328, although the climate and soil conditions at the two localities are very different. Further confirmations of the results may be found in experiment, F-no. 77 (see table 2 and 5), which includes four of the same progenies. The rank of the three outcrossings and the standard is the same as for experiment, F-no. 89.

The standard hybrid, Eur. larch, V. 44 \times Jap. larch, S. 127², presents a considerable improvement compared with offsprings of European and Japanese larch respectively. It compares well with other hybrids too, both in respect of vigour and adaptability. Therefore the significantly better growth of some of the outcrossings even at this early stage of the inbreeding indicate the potentialities of the breeding method.

Discussion

The following factors have originally been decisive for the investigations of the inbreeding/outcrossing method:

1. The widely used and often successful method of inbreeding and outcrossing in plant breeding.
2. The presence of quite a large number of inbred families derived from selfpollination.
3. The fact that the *Larix* species in question are obligate cross pollinators but may be selffertilized.
4. The ease with which the *Larix* species are vegetatively propagated.

Although very good results were obtained of *Larix decidua* \times *L. leptolepis* crosses it was expected that the inbreeding of the two species respectively followed by outcrossing in the hybrid combination might further increase the vigour and fitness of the offsprings. The evidence of such an increase in our first trials (experiment, F-no. 72) did not show up clearly. As a matter of fact it was doubtful whether inbreeding/outcrossing produced more vigour than "ordinary" hybrids between non-inbred parents although there was an indication of an increase in fitness. It was soon realized that the odds probably were too high. The level of the standards (the interspecific crosses) was high, the development of inbred lines at its beginning (mainly first generation selfed) and adequate representation of families and individuals not yet available. We had to contemplate whether it would be feasible for the breeding of larch and other forest tree species to take up more extensive investigations on the same lines as plant breeding of other crops in view of the time and space required.

The first four trials (F-nos. 73, 76, 77 and 94) laid down to investigate the method as such by comparing outcrossing after inbreeding with crosses between the foundation trees gave very little response, which after all is not surprising. Investigating the intra-family variation a little more thoroughly as described under group B the results, however, became more promising (F-no. 89 and 92). Under the circumstances it was very encouraging that the level of fast growing hybrids could be raised significantly. Hybrid larch usually grow from 10–20% better than the pure species. An additional increment in vigour of up to 14% in one case and more commonly of 5–10% is thus a very substantial improvement. It is reasonable therefore to believe that a more systematic approach to the problem over a longer period will yield valuable results, which in respect of *Larix* should be fairly easy to reproduce on a large scale.

Without going too much in details a programme for inbreeding and outcrossing might include the following measures:

1. Selfpollination of many selected, non-related trees.
2. Comparison of the inbred families in proper designed trials with careful study of variation within families and of inbreeding effect. Selection for form.
3. Test of inbred individuals (and families) with common tester for general combining ability.
4. Vegetative propagation of the most promising individuals.
5. Selection of individuals from the best F₁ crosses of unrelated parents for the establishment of doublecross seed orchards.
6. Continued inbreeding and outcrossing. Repetition of programme.
7. Search for individuals, subsequent lines, with specific combining ability.

re. 1. — How many trees it is necessary to selfpollinate will amongst other things depend of the trees' reaction to inbreeding. Some trees will suffer from inbreeding depression to an extent that further inbreeding will soon exclude itself. Other trees suffer very little or not at all and variation within the selfed offsprings is of the same magnitude as in normal cross pollination. Again some trees will disclose undesirable characters with the uncovering of deleterious, recessive genes. Usually a relatively small number of the trees tested will produce inbred offsprings which both stand further inbreeding and possess sufficiently discontinuous variation to be of value in outcrossing. The latter especially, which may be a sign of dominance or directional dominance, seems to be of great importance for the success of the method. It may be necessary therefore to test many trees and families to find the most suitable.

Our experience in larch so far is that relatively many trees suffer but little from inbreeding in the first generation and even in the second, and that the individuals from the inbred families do not vary more than in normal crossings. One explanation might be the rather crude method of sowing and transplanting hitherto practised by which the weaker plants disappear. Another might be that additive genetic variation is much more common than dominance for the characters we are examining.

As diversity in genetic constitution of the inbreds helps to get a higher degree of heterozygosity of the outcrossing it is of importance that the foundation trees are not related.

Selfpollination may also support the evaluation of selected trees by uncovering recessive genes and thus justify a more extensive programme of controlled pollination and progeny testing.

re. 2. — As already explained variation within progenies is of great importance for the successful utilization of the method. Further, selection for characters connected with form may be carried out with advantage at this and later phases, while selection for vigour is more problematic as discussed earlier. In any case it is essential to have the material screened, supported by statistical treatment of data in order to reduce the continued inbreeding to the most promising individuals.

re. 3 and 4. — The problems are very much the same as for all progeny testing i.e. availability of flowers, frequency of flowering, procurement of pollen, isolation technique etc., and the possibilities of testing a large number of progenies. Of special features in connection with inbreeding and outcrossing are the common testers and the reduced fertility of the inbred material. The former is solved here at Hørsholm by grafting a few large clones in hedges with ample space on both sides. The latter may be overcome by using the inbreds as males for the initial testing. If further

inbreeding is desired the inbreds have to be vegetatively propagated in a number of 5–10 ramets per clone (larch).

It is almost essential for the screening of many individuals and families to rely on small scale trials of a relatively short duration (4–6 years). It will be noted that some of the experiments mentioned in this paper are of that kind. As far as our experiences go they are reasonably reliable.

re. 5. — In forest tree breeding in general mass production of improved material or supposed improved material starts on the basis of interim results or before actual testing of the material has taken place. If promising outcrossings have been found at the early steps of inbreeding it would be sensible to establish seed orchards with the inbred parents that produced these outcrossings. For the first generations of inbreds (from selfing) it would probably not present any great problems. One such seed orchard in larch, F-no. 75 at Basnaes in South Zealand, is already made and has produced the first seed crop.

The following generations of inbreds would probably become increasingly difficult to establish and maintain in a seed orchard. It is doubtful too that seed production would be good enough for commercial use. As with breeding of maize we might employ the principle of doublecrosses*) or 4-way crosses grafting selected F_1 -hybrids as seed parents. A few such clones of *Larix eurolepis* from the most vigorous outcrossings have already been grafted in Hørsholm although we still have to test the actual doublecrosses.

re. 6. — Theoretically the inbreeding should continue until a complete homozygous state is reached of all members of the line. At that point no further changes would take place, and the maximum benefit of the outcrossings might show up.

In forest trees nobody knows how many generations of inbreeding are necessary to reach inbreeding minimum. Attempts have been made to obtain the S_3 -generation in larch, but even at this relatively early stage of inbreeding we had great difficulties in producing a few seedlings.

However, less than the maximum gain from the inbreeding and outcrossing might give substantial improvements as indicated by $P \times S_1$ crosses of experiment F-no. 89 and 92. Each such step forward may be maintained and utilized and therefore the very long and perhaps doubtful prospect of reaching complete homozygosis need not prevent the employment of the method. At the same time it should be emphasized that without careful examination and selection inbreeding and outcrossing do not automatically pay off and that extensive progeny trials are unavoidable. This presumably exclude a number of tree species

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Summary

Two species of larch, European larch (*Larix decidua*) and Japanese larch (*Larix leptolepis*) and the hybrid between them (*Larix eurolepis*) have been used for the investigations.

*) If four unrelated parents A, B, C, and D are crossed in pairs thus: $A \times B$ and $C \times D$ the crossing of the F_1 -hybrids ($A \times B$) \times ($C \times D$) constitute a doublecross. The vigour of the doublecross is found to be equal to that of the F_1 -hybrid.

The background and reasons for taking up the method of inbreeding and outcrossing for closer examination are outlined. To investigate the potentialities of the method six field trials have been established and these are described. In all the experiments it is possible to compare the hybrids of the $P \times P$ level i.e. crossings between individuals of the parental generation, with outcrossings in the hybrid combination after one generation of inbreeding of the respective species. Further in three of the experiments the combining ability of a number of sibs within two inbred families has been evaluated.

The stage of inbreeding is in all the examples first generation of selfing (S_1) and the outcrossings are at the level: $P \times P$, $P \times S_1$, $S_1 \times S_1$.

Various problems in connection with inbreeding and outcrossing are discussed viz. the application of the method for forest trees, the importance of selection, the importance of variation, utilization of interim results and mass production. A programme for inbreeding and outcrossing is outlined and the various points discussed.

The preliminary results indicate that extensive and systematic progeny testing is needed for making the method more efficient than interspecific crossing, at least when it concerns larch. However, the investigations also show that it is possible to obtain a significant increase in vigour of the outcrossings even when they are compared with fast growing hybrids.

With the last mentioned improvements in mind and the possibilities of converting them relatively quickly for mass production it is concluded that further investigations and employment of the method is justified.

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Geographic Variation in European Black Pine

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Introduction

European black pine (*Pinus nigra* ARNOLD) is a widely distributed species in southern Europe. It has been planted extensively in other parts of the world and in many areas is one of the most important timber trees. In the United States it has been used mostly as an ornamental but it also has a good potential for timber production.

Provenance studies in other widespread species has shown a great amount of genetic variability associated with the geographic origin of the seed. European black pine appears to be no exception. During the past century taxonomists have described many varieties and forms. A few unreplicated European provenance studies have also showed striking differences among trees grown from seed collected in different parts of the range.

My study was undertaken as part of a long range project for the improvement of black pine planted in north central United States. The total project includes replicated provenance-test plantations in several states. My work was concentrated on one of the most complete of these, at the W. K. Kellogg Forest in southeastern Michigan. This planta-

jective was to provide the basic information necessary for improvement of the species by breeding.

Distribution of European Black Pine

My main source of information was a German researcher RÖHRIG's (1957) excellent work published in *Silvae Genetica*. Other sources included MACDONALD *et al.* (1957), SEXTON (1947) and FRITZ HALLER's (1951) World Forest Atlas, SALVADOR (1927) and BIEL (1944). Valuable unpublished distribution data were obtained from Prof. LUIS CEBALLOS of Ciudad University, Madrid; Dr. PIERRE BOUVAREL of National Institute of Agronomy Research, Nancy; Prof. RICCARDO MORANDINI of Silvicultural Experiment Station, Firenze; Prof. MAX SCHREIBER of Hochschule für Bodenkultur, Institut für Waldbau, Wien; and the Turkish Forest Service.

European black pine has a large natural range in central and southern Europe and possibly northern Africa. It has a 13-degree latitudinal range from 35° to 48° N. and a 48-degree longitudinal range from 6° W. in Spain to 42° E. in Turkey (Fig. 1). The African stands were not sampled in the present study.

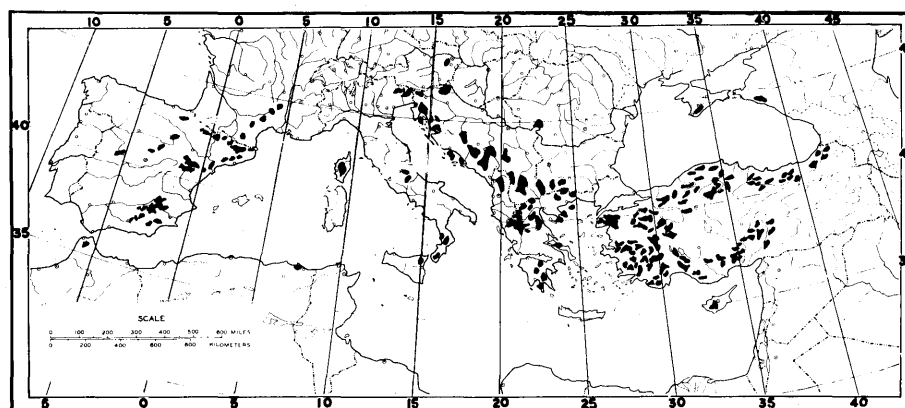


Fig. 1. — Natural distribution of European black pine.

tion furnishes information applicable to American conditions. In addition, being replicated, it offers solutions to some problems unsolved in the earlier work. My primary objective was to determine the genetic variability pattern in a variety of morphological, growth, and physiological characteristics, and to relate that pattern to features in the original habitat of the various seedlots. A secondary ob-

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Black pine is predominantly a mountain tree but can also be found at sea level along the shores of the Adriatic sea. In Spain, Corsica, and Italy black pine is limited to highland areas between elevations of 2,600 to 5,000 feet above sea level. On the French mainland it is found between elevations of 800 and 2,600 feet. In Austria and Yugoslavia, it is distributed between 1,000 and 3,000 feet; in southern Greece and Turkey it occurs up to 4,300 feet; in southern Taurus Mountains of Turkey it occurs higher than 6,000 feet.

The majority of the natural range is characterized by a Mediterranean type of climate — dry and hot summers with cool, moist winters. A few black pine stands are found at