the samples from Klosterheden and Ulborg are polymorphic (*Table 2*). A similar pattern is seen at the *Ugpp* locus, where the sample from FP623 is polymorphic but where the other 2 samples are almost fixed for allele 2. At the other 2 loci 2 alleles are found with relative high frequencies in all 3 populations. The highest differentiation measured with WRIGHT's F_{ST}-statistic according to WEIR and COCKERHAM (1984) was observed at the *Ugpp* locus with a value of 0.12. The values at the other three loci are somewhat smaller, being in the range of 0.016 to 0.027. An average across the 4 loci was 0.029. Despite the low values at 3 of the loci, the 3 populations are significantly differentiated at all loci. *G*-tests for homogeneity (SOKAL and ROHLF, 1981) on the allele numbers are significant at the 0.1% level. The populations are all differentiated from each other: a pairwise comparison of the three populations where the test statistics are summed across the loci does also reveal significant differences among them with significance levels smaller than 0.01%.

The increased level of variation found in the present study in comparison to our previous published result (SIEGISMUND et al., 1996) suggests that the import of noble fir to Denmark has not been accompanied with a severe bottleneck that has eroded the genetic variation. As noted by SIEGISMUND et al. (1996) noble fir has a level of variation at quantitative characters that is comparable to what is found in other conifer species.

The present study indicates that the outcrossing rate differs between the 2 investigated stands being lower in Klosterheden ($t_m = 0.90$) than in Ulborg ($t_m = 0.98$). However, the difference was not found to be significant on a 5% level. Both stands are uniform and fairly dense. The difference could be due to the more windy conditions in Ulborg compared to Klosterheden. SIEGISMUND et al. (1996) estimated $t_m = 1.00$ in the Viborg seed orchard, which they found puzzling considering the low level of embryonic lethal genes reported by SORENSEN et al. (1976). A similar finding has been reported for *Picea omorika* by KUITTINEN and SAVOLAINEN (1991). *Picea omorika* is self-fertile but is highly outcrossing, which is explained by the separation of male and female flowers and by a protogynous flowering.

The present study shows that outcrossing rates significantly lower than 1.00 can be found. Noble fir therefore appears to resemble other coniferous species by reproducing according to a mixed mating system, although still with a low level of selfing (see e.g. references in MUONA, 1990).

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References

CLAYTON, J. W. and TRETIAK, D. N.: Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. **29**: 1169–1172 (1972). — Danish Land Development Service: Registration report for FP 623. Hedeselskabet, Krogårdsvej 6, DK-8882 Fårvang. Unpublished. [in Danish] (1979). — GUO, S. W. and THOMPSON, E. A.: Performing the exact test for HARDY-WEINBERG proportions for mutiple tests. Biometrics **48**: 361–372 (1992). — HARRIS, H. and HOPKINSON, D. A.: Handbook of Enzyme Electrophoresis in Human Genetics. North Holland, Amsterdam (1976). — HOLM, S.: A simple sequentially rejective multiple test procedure. Scand. J. Stat. **6**: 65–70 (1979). — KUITTINEN, K. and SAVOLAINEN, O.: *Picea omorika* is a self-fertile but outcrossing conifer. Heredity **68**: 183–187 (1991). — LANGE, J.: Introduction History of Cultivated Plants in Denmark. DSR Forlag, Copenhagen. [in Danish, English abstract] (1994). — LARSEN, J. B.: Danish forest tree species, races, seed supply and choice of provenances. Dansk Skovforenings Tidsskrift **68**: 1–100 [in Danish] (1983). — MANCHENKO, G. P.: Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Boca Raton, USA (1994). — MUONA, O.: Population genetics in forest tree improvement. In: Plant Population Genetics, Breeding, and Genetic Resources. (Edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER and B. S. WEIR). pp. 282–298. Sinauer Associates Inc., Massachusetts (1990). — RICE, W. R.: Analyzing tables of statistical tests. Evolution **43**: 223–225 (1989). — RITLAND, K.: A series of FORTRAN computer programs for estimating plant mating systems. J. Heredity **81**: 235–237 (1990). — RITLAND, K. and JAIN, S.: A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity **47**: 35–52 (1981). — SIEGISMUND, H. R., KJÆR, E. D. and NIELSEN, U. B.: Mating system estimates and effective population numbers for an isolated noble fir (*Abies procera*) clonal seed orchard in Denmark. Can. J. For. Res. **26**: 1135–1141 (1996). — SOKAL, R. R. and ROHLF, F. J.: Biometry. 2nd edition. W. H. Freeman and Company, San Francisco (1981). — SORENSEN, F. C., FRANKLIN, J. F. and WOOLLARD, R.: Self-pollination effects on seed and seedling traits in noble fir. Forest Science **22**: 155–159 (1976). — SORENSEN, C. F. and MILES, R. S.: Inbreeding depression in height, height growth, and survival of douglas-fir, ponderosa pine, and noble fir to 10 years of age. Forest Science **28**: 283–292 (1982). — WEIR, B. S. and COCKERHAM, C. C.: Estimating *F*-statistics for the analysis of population structure. Evolution **48**: 1358–1370 (1984). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. In: Isozymes in Plant Biology. (Edited by D. E. SOLTIS and P. S. SOLTIS). pp. 5–45. Chapman and Hall, London (1989).

Mode of Fertilization and its Individual Variation in *Larix gmelinii* var. *japonica*

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Summary

Fertilization mode, that is random, selective cross- or selective self-fertilization after mixed pollination (pollination with equal mixture of outcross and self pollen) was investigated for 6 clones of *Larix gmelinii* var. *japonica*. The seed fertilities expressed as proportions of filled seeds and germinatible seeds for the numbers of full size-seeds per cone after mixed pollination were examined, and these were compared with those resulting from complete outcross- and self-pollination. Five clones showed significant inbreeding depression in seed fertility. For 4 clones, fertilities of seeds from mixed pollination were similar to those resulting from cross-pollination, suggesting selective cross-fertilization. Selective cross-fertilization is possibly one of factors which causes a high outcrossing rate in coniferous tree species. For 1 clone, the fertility of seeds from mixed pollination was similar to that resulting from self-pollination in 1993, suggesting selective self-fertilization. This clone, however, showed random fertilization in 1995, that is to say, fertility of seeds after mixed pollination was between seed fertility from cross-pollination and that from self-pollination. This was not significantly different from the expected fertility from random fertilization between cross and self pollen. The limitation of outcross pollen caused by a decrease of effective population size was considered as a possible factor which allows the existence of some selfers in coniferous tree species.

Key words: fertilization mode, self-fertilization, cross-fertilization, random fertilization, *Larix gmelinii* var. *japonica*.

FDC: 165.41; 181.52; 232.318; 174.7 *Larix gmelinii*; (520).

Introduction

Selfing rate and degree of inbreeding depression affect individual fitness in self-compatible plant species. It is regarded as a mating strategy for a parental plant, which determines what percent of ovules are to be self-fertilized or cross-fertilized. At the species level, most of the species are classified into a high selfing rate group or a low selfing rate group, although some animal pollinated species show intermediate selfing rate and there is great variation among populations (LANDE and SCHEMSKE, 1985; AIDE, 1986; BARRETT and ECKERT, 1990).

Generally, coniferous tree species show strong inbreeding depression (SORENSEN, 1969; FRANKLIN, 1970, 1972; SORENSEN and MILES, 1982; FOWLER and PARK, 1983; WILCOX, 1983; PARK and FOWLER, 1984; CHARLESWORTH and CHARLESWORTH, 1987) and high outcrossing rate (FOWLER, 1965; OHBA et al., 1971; FARRIS and MITTON, 1984; CHARLESWORTH and CHARLESWORTH, 1987; BARRETT and ECKERT, 1990), which maintain high genetic diversity (HAMRICK, 1979). In coniferous tree species, which lack self-incompatibility systems such as those in angiosperms (BATEMAN, 1952), a high outcrossing rate has been attributed to the strong inbreeding depression at the early stages of embryo development (SORENSEN, 1969, 1982; PARK and FOWLER, 1984; KNOWLES et al., 1987; KANAZASHI et al., 1990; HUSBAND and SCHEMSKE, 1996). Cytological examinations have shown that self-fertilization occurs normally, but then self-fertilized embryos fail to develop in some coniferous tree species (ORR-EWING, 1957; HAGMAN and MIKKOLA, 1963; MERGEN et al., 1965). On the basis of these observations, the decrease of seed fertility after self-pollination in conifers has been attributed to the post-zygotic abortion of self-fertilized embryos resulting from homozygosity of recessive lethal and deleterious genes. The embryonic genetic load has been estimated in some coniferous tree species (SORENSEN, 1969; FRANKLIN, 1972; FOWLER and PARK, 1983; PARK and FOWLER, 1984; BISHIR and NAMKOONG, 1987; KANAZASHI et al., 1990).

Fertilization mode, that is random or selective fertilization between outcross and self pollen, is another mechanism which affects outcrossing rate. In this paper, I use the term "selective fertilization" as discriminative fertilization between outcross and self pollen at the stage after pollination prior to fertilization. It is possible that cross-fertilization occurs more frequently than self-fertilization (selective cross-fertilization) after simultaneous pollination with both outcross and self pollen, and this might be one of the factors which results in a high outcrossing rate in conifers. Selective cross-fertilization with mixture of outcross and self pollen is known in some angiosperms species as cryptic self-incompatibility (BOWMAN, 1987; CASPER et al., 1988; WELLER and ORNDUFF, 1989; CRUZAN and BARRETT, 1993). It would be important in many coniferous tree species because self-pollination can happen frequently on account of monoecy and homogamy. In *Cryptomeria japonica*, selective cross-fertilization with both of outcross and self pollen was observed for 1 clone (OHBA et al., 1972). In *Pinus densiflora,* the relationship between the proportion of self-pollination and that of selfed filled seeds was well explained by archegonial polyembryony and zygotic lethal alleles under the assumption of the random fertilization between self and outcross pollen (KANAZASHI et al., 1990). Information about fertilization mode, however, is not complete at present.

On the other hand, outcrossing rate varies between individuals. IWASA (1990) pointed out the importance of selfing rate at the individual level, besides population average. He suggested the possibility that selfing rate varies according to the amount of available resources of each individual plant. Although information about individual variation in outcrossing rate in tree species is scanty, some studies have reported individuals that apparently have a relatively high selfing rate (PERRY and KNOWLES, 1990; SORENSEN, 1994). In *Pinus monticola* and *Pinus elliottii,* some individuals showed selective cross-fertilization while others showed selective self-fertilization, with an equal mixture of self and outcross pollen (BARNES et al., 1962; KRAUS and SQUILLACE, 1964). It is unknown whether these selfers are maintained in the population or not. More information about individual outcrossing rate or fertilization mode is needed.

The purpose of this study was to clarify the fertilization mode after mixed pollination (pollination with equal mixture of self and outcross pollen) in *Larix gmelinii* var. *japonica,* and to determine whether individual differences of fertilization mode exist or not.

Materials and Methods

Larix gmelinii var. *japonica* occurs in Eastern Siberia, Sakhalin and Kuril Islands. It does not occur naturally in Hokkaido, northern Japan where it has been introduced artificially from Sakhalin and Kuril Islands. The material used in this study were collected from these artificial forests. Thirty individuals were chosen and grafted at Hokkaido Forest Research Institute, Bibai (43° 15' N, 141° 50' E), Hokkaido, northern Japan in 1973. Six clones among them were used for this study. These 6 clones were chosen on the basis of practical matters of pollination experiments, that is, a quantity of male and female strobili enough for artificial pollination, and synchronicity of development of strobili between clones in pairs for outcross-pollination.

Pollination experiments were conducted in 1993, 1994, and 1995. The most of the clones produced many strobili and seeds in 1992 and 1995, but produced few female strobili, except for a few clones in 1993. Pollen shedding is coincident with receptivity of female strobili within the same clone, although there is a time lag in flowering of about 1 week between clones. The investigated clones and treatment at each year are shown in *table 1*. Pollen for pollination experiments was collected from mid to late April, then dried and processed. For mixed pollination, an equal weight of self and outcross pollen were blended. Female strobili were isolated with paper bags from mid to late April in each year. Pollination with sufficient amount of pollen was made twice during late April, at the most receptive stage of the female strobili. The paper bags were removed in mid May. Cones from the controlled pollinations were collected in mid August, together with about 10 open pollinated cones from each clone. In *Larix gmelinii*

Table 1. – Summary of years, clones, treatments and results of this study.

Pollination year	Mother tree	No. of ramet used	treatment	outcross pollen donor	No.of cone harvested	Average per cone No.of full-size seeds	Average per cone No.of filled seeds	Average per cone No.of germinatible seeds
1993	V545	3	CROSS	V544	8	33.8	25.3	23.5
			MIX	V544	5	36.0	16.5	15.0
			SELF		6	36.3	15.3	13.8
	V544	3	CROSS	V545	8	37.1	10.5	6.8
			MIX	V545	7	35.1	9.3	6.3
			SELF		5	41.2	1.4	1.2
1994	Toyooka641	2	CROSS	V545	11	26.2	11.9	10.6
			MIX	V545	11	28.8	13.5	12.6
			SELF		7	31.0	1.7	1.3
1995	Kabaoka194	1	CROSS	Sapporp1	15	35.3	12.0	11.4
			MIX	Sapporo1	28	30.1	8.3	8.0
			SELF		10	30.2	2.2	2.0
	Sapporo1	3	CROSS	Kabaoka194	11	28.5	4.0	2.0
			MIX	Kabaoka194	25	33.6	7.8	4.4
			SELF		22	29.5	5.5	2.6
	Toyooka121	3	CROSS	V545	18	45.9	26.7	25.7
			MIX	V545	17	43.9	26.1	25.4
			SELF		25	38.8	16.6	15.2
	V545	3	CROSS	Toyooka121	32	34.9	23.8	21.4
			MIX	Toyooka121	47	34.6	18.8	14.6
			SELF		35	33.5	9.2	7.7

var. *japonica,* both empty seeds as well as filled seeds develop into full size-seeds. Fertilities of seeds, (the proportion of filled seeds and germinatible seeds for the numbers of full size-seed) were examined for each cone from each treatment. Seeds were stored for one month at 4°C. Then they were sown on agar, and germination was recorded after 14 days at 25°C. Ungerminated seeds were dissected to determine the proportion of filled seeds. All proportions were arcsine-transformed before analysis to achieve normality.

Fertility of seeds after complete cross-pollination (Fc) and self-pollination (Fs) can be expressed as follows, taking archegonial polyembryony into consideration (Sorensen, 1982).

$$[1] \qquad Fc = 1 - (1 - a)^n$$

$$[2] \qquad Fs = 1 - (1 - b)^n$$

where a and b are probability of a egg cell fertilized and developing into a viable embryo or a germinatible seed after cross- and self-pollination respectively, and n is the number of embryos per ovule. If the probability of fertilization by self pollen is equal to that by cross pollen, if fertilization is random between self and outcross pollen, the fertility of seeds after mixed pollination, P can be expected as follows:

$$[3] \qquad P = 1 - \{1 - (a + b)/2\}^n$$

Parameters a and b can be estimated from Fc and Fs using equations [1] and [2]. Parameter n is unknown for *Larix gmelinii* var. *japonica*. In *Larix leptolepis*, which occur naturally in central Japan, the number of archegonia per ovule ranged from 0 to 6, and most ovules contained 3 or 4 archegonia (Kaji, 1974). In this paper, it is assumed that n takes an integer from 1 to 6.

If there are significant differences among Fc, Fs and P, it is possible to determine the fertilization mode as follows. Fertility of seeds from mixed pollination (Fm) was compared with Fc, Fs and P, calculated by equation [3] changing n from 1 to 6. Fertilization mode was regarded as random when there were no significant differences between Fm and P, or nonrandom in the other case. Furthermore, in the case of nonrandom, the fertilization mode was classified as selective cross-fertilization if there were no significant differences between Fm and Fc, and it was classified as selective self-fertilization if there were no significant differences between Fm and Fs.

Results

The proportions of filled seeds and germinatible seeds of each pollination for all clones were shown in *table 1* and *figure 1*. The results were not altered in analysis by using either the proportion of filled seeds or germinatible seeds, except for clone V545 in 1995. Fs was significantly lower than Fc for all clones except for Sapporo 1 (Tukey's HSD test, $P < 0.05$). Fs was significantly different among clones (one-way ANOVA, $P < 0.001$). In clone V545, differences of seed fertility between 1993 and 1995 were insignificant for Fc and Fs respectively.

In clone Sapporo 1, there was no significant difference between Fc and Fs (Tukey's HSD test, $P < 0.252$, 0.492 for proportion of filled seeds and germinatible seeds, respectively). Therefore, fertilization mode is not clear for this clone. It is probable that timing of controlled pollination was not appropriate for this clone, because Fc is much lower than seed fertility from open pollination.

Selective cross-fertilization mode was observed for 4 clones, V544, Toyooka 641, Kabaoka 194, and Toyooka 121. Fm was significantly different from P with $n = 1$ to 6 (*t*-test, $P < 0.05$), and not significantly different from Fc for those clones. Seed fertility of open pollination was not significantly different from Fc and Fm, except for clone Toyooka 121.

In clone V545, selective self-fertilization mode was observed in 1993. Fm was significantly different from Fc (Tukey's HSD test, $P < 0.05$) and P with $n = 1$ to 6 (*t* -test, $P < 0.05$) and not significantly different from Fs (Tukey's HSD test, $P < 0.655$, 0.766 for proportion of filled seeds and germinatible seeds, respectively) and seed fertility from open pollination ($P < 0.403$, 0.251). In 1995, fertilization mode of clone V545 was

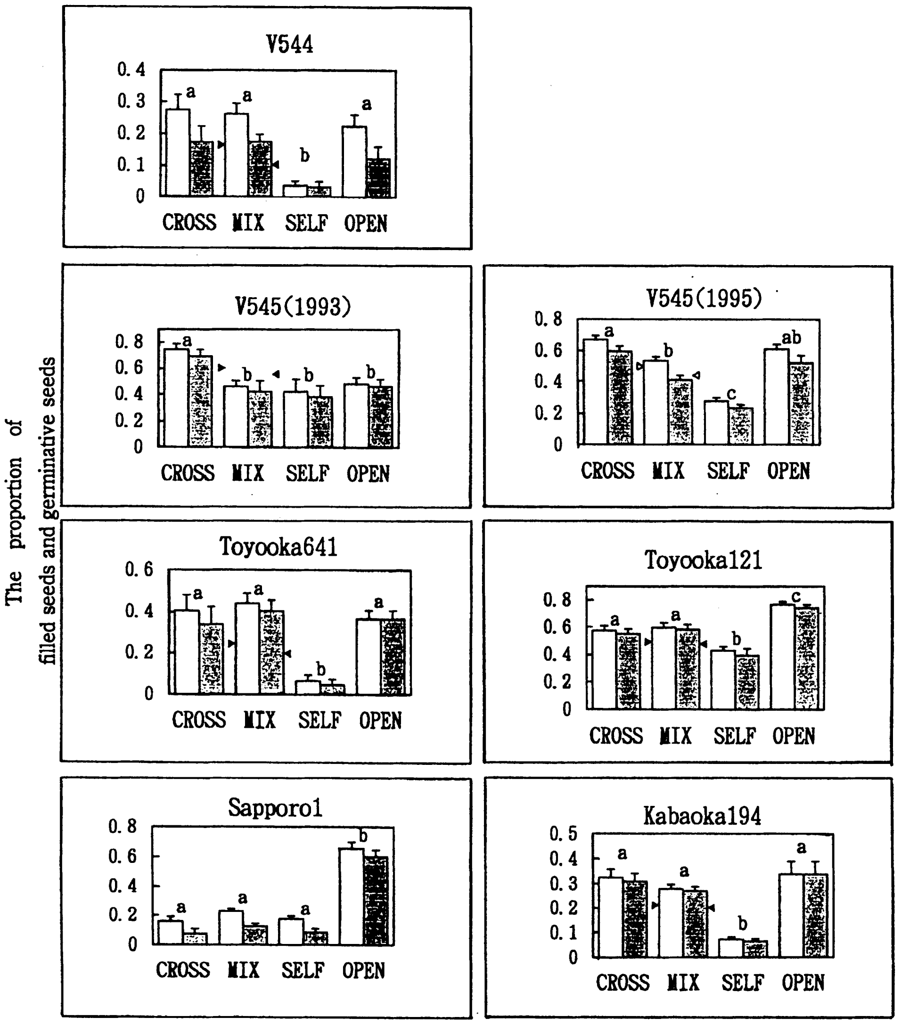

Fig. 1. – Mean proportions of filled seeds (unshaded bars) and germinatible seeds (shaded bars) after each pollination for 6 clones (± S.E.). Outcross pollen donors for CROSS and MIX are shown in *table 1.* Means not having the same letter are significantly different from each other (TUKEY's HSD test, $P < 0.05$). Significance of difference were not altered by using either the proportion of filled seeds or germinatible seeds. Triangles in MIX indicate expected fertilities from random fertilization between outcross and self pollen, P, which is calculated by equation [3] with $n = 3$. Solid triangles indicate significant difference between seed fertility of MIX and P when $n = 3$.

reexamined with different pollen donor from that in 1993. F_M was significantly different from that of F_C and F_S (TUKEY's HSD test, $P < 0.05$), and not significantly different from P when $n = 3$ to 6 (*t*-test, $P < 0.160$, 0.422 for proportion of filled seeds and germinatible seeds respectively, when $n = 3$). When $n = 1$ to 2, proportion of filled seeds of mixed pollination was significantly different from expected value from random fertilization (*t*-test, $P < 0.05$), while germination rate was not significantly different from expected one.

Discussion

The high outcrossing rate in conifers has been attributed to the strong inbreeding depression at an early stage of embryo development (SORENSEN, 1969, 1982; PARK and FOWLER, 1984; KNOWLES et al., 1987; KANAZASHI et al., 1990). Greater attention should be paid to the pre-zygotic mating system which plays an important role in determining outcrossing rate. Selective cross-fertilization was observed in 4 of the 6 clones of *Larix gmelinii* var. *japonica* in this study. Besides strong inbreeding depression and archegonial polyembryony, selective cross-fertilization can be one of factors which maintains a high outcrossing rate in conifers.

The possible causes of selective cross-fertilization are low germination ability and/or low pollen tube growth rate of self pollen, compared with those of cross pollen. At present, it is unclear which is the main cause. Although normal germination and pollen tube growth of self pollen has been observed directly in *Larix leptolepis* (KAJI, personal communication) and some other conifers (ORR-EWING, 1957; HAGMAN and MIKKOLA, 1963; MERGEN et al., 1965), it has not been detected in *Larix gmelinii* var. *japonica*. Further cytological observations are needed.

Under strong inbreeding depression, selective cross-fertilization is regarded as more adaptive than random fertilization and selective self-fertilization, especially where self-pollination occurs frequently. There is selection for increased outcrossing if inbreeding depression is greater than 0.5, assuming that the cost of outcrossing is constant at 0.5 (LANDE and SCHEMSKE, 1985; YAHARA, 1992). If low fertility of self-pollinated seeds are due to the abortion after fertilization, the survival rate of self-fertilized seeds before germination is less than a half of that of cross-fertilized seeds for clones V544, Toyooka 641, and Kabaoka 194. Thus, the degree of inbreeding depression throughout the all stages of life would be much greater (FRANKLIN, 1970; SORENSEN and MILES, 1982; FOWLER and Park, 1983; Wilcox, 1983) and it would be strong enough to favor selective cross-fertilization.

Interestingly, fertilization mode was not same for all clones surveyed. Selective self-fertilization and random fertilization were observed in clone V545. Although the proportion of filled seeds was significantly different from the expected value from random fertilization when n = 1 to 2 in 1995, random fertilization is more probable than nonrandom one, considering an insignificant difference in germination rate, and high frequency of ovules which contain 3 or 4 archegonia in *Larix leptolepis* (Kaji, 1974). Fertilization mode was regarded as random in *Pinus densiflora* (Kanazashi et al., 1990). Selective self-fertilization has been reported in *Pinus elliottii* (Kraus and Squillace, 1964) and *Pinus monticola* (Barnes et al., 1962), especially in complete self-fertile individuals (trees that normally set as many viable seeds when selfed as when outcrossed). These fertilization modes will increase the selfing rate of individuals under natural conditions. Some studies reported significant levels of selfing rate in other conifer species at the population level (Knowles et al., 1987; Perry and Knowles, 1990) or at the individual level (Perry and Knowles, 1990; Sorensen, 1994). Limitation of outcross pollen, caused by decrease of population size or density (Farris and Mitton, 1984; Knowles et al., 1987; Perry and Knowles, 1990) would lead to the obligatory high selfing rate. Selective self-fertilization and random fertilization might be retained under such conditions. There is no information about the natural population from which clone V545 originates. Therefore discussion about population size or density is impossible in this study.

Clone V545 tends to produce female strobili every year regularly compared with other clones (Sato, personal observation). Only V545 and other a few clones produced seeds in 1993, in contrast to the bulk of clones which produced many seeds in 1995. If the amount of male strobili produced is positively correlated with that of female strobili, available outcross pollen is insufficient for individuals which produce female strobili in a poor crop year, like clone V545. The limitation of outcross pollen might happen to specific individuals as a result of a decrease of effective population size such as in this clone.

In clone V545, fertilization mode in 1993 was different from that in 1995. One possible factor of this change is difference in pollen donor. A change of fertilization mode of one individual depending on the pollen donor was also observed in *Pinus monticola* (Barnes et al., 1962). Further pollination experiments with various pollen donors will be needed to test this hypothesis. Another possible hypothesis is that maternal plants change fertilization mode year to year, according to the amount of total resource level, or availability of cross pollen. Optimal allocation to outcrossed seeds, selfed seeds and reserve for the next year under the differential reproductive success through each resource investment was modeled by Iwasa (1990). According to his model, optimal selfing rate changes depending on the total resource level. Maternal plants should produce more selfed seeds as the total resource increases, if available outcross pollen is limited. Experimental manipulation of resource level would be needed to test this hypothesis. Availability of cross pollen affects reproductive success through outcrossed seeds. Increase of reproductive success through outcrossed seeds with an increase of resource investment reaches a plateau more quickly when available outcross pollen is severely limited. Consequently, optimal selfing rate increases when the amount of outcross pollen decreases. Pollination experiment in both a mast year and a poor crop year with same pollen donor will be useful to test this hypothesis.

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References

Aide, T. M.: The influence of wind and animal pollination on variation in outcrossing rates. Evolution **40**: 434–435 (1986). — Barnes, B. V., Bingham, R. T. and Squillace, A. E.: Selective fertilization in *Pinus monticola*. II. Results of additional tests. Silvae Genet. **11**: 103–111 (1962). — Barrett, S. C. H. and Eckert, C. G.: Variation and evolution of mating systems in seeds plants. *In*: Biological approaches and evolutionary trends in plants. *Edited by* S. Kawano. Academic Press, London. pp. 229–254 (1990). — Bateman, A. J.: Self-incompatibility systems in angiosperms. Heredity **6**: 285–310 (1952). — Bishir, J. and Namkoong, G.: Unsound seeds in conifers: Estimation of numbers of lethal alleles and of magnitudes of effects associated with the maternal parent. Silvae Genet. **36**: 180–185 (1987). — Bowman, R. N.: Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). Amer. J. Bot. **74**: 471–476 (1987). — Casper, B. B., Sayigh, L. S. and Lee, S. S.: Demonstration of cryptic incompatibility in distylous *Amsinckia douglasiana*. Evolution **42**: 248–253 (1988). — Charlesworth, D. and Charlesworth, B.: Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. **18**: 237–268 (1987). — Cruzan, M. B. and Barrett, S. C. H.: Contribution of cryptic incompatibility to the mating system of *Eichhornia paniculata* (Pontederiaceae). Evolution **47**: 925–934 (1993). — Farris, M. A. and Mitton, J. B.: Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. Evolution **38**: 1151–1154 (1984). — Fowler, D. P.: Effects of inbreeding in red pine, *Pinus resinosa*, Ait. III. Factors affecting natural selfing. Silvae Genet. **14**: 37–46 (1965). — Fowler, D. P. and Park, Y. S.: Population studies of white spruce. I. Effects of self-pollination. Can. J. For. Res. **13**: 1133–1138 (1983). — Franklin, E. C.: Survey of mutant forms and inbreeding depressions in species of the family Pinaceae. USDA For. Serv. Res. Pap. SE-61 (1970). — Franklin, E. C.: Genetic load in loblolly pine. Am. Nat. **106**: 262–265 (1972). — Hagman, M. and Mikkola, L.: Observations on cross-, self-, and inter-specific pollinations in *Pinus peuce* Griseb. Silvae Genet. **12**: 73–79 (1963). — Hamrick, J. L.: Genetic variation and longevity. *In*: Topics in plant population biology. *Edited by* O. T. Solbrig, S. Jain, G. B. Johnson and P. H. Raven. Columbia University Press, New York. pp. 84–113 (1979). — Husband, B. C. and Schemske, D. W.: Evolution of the magnitude and timing of inbreeding depression in plants. Evolution **50**: 54–70 (1996). — Iwasa, Y.: Evolution of the selfing rate and resource allocation models. Plant Species Biol. **5**: 19–30 (1990). — Kaji, K.: On the pollination and development of ovules, and on the sterility of seeds in Japanese larch (*Larix leptolepis* Gord.). Bull. Hokkaido For. Res. Inst. **12**: 1–12, (in Japanese with English summary) (1974). — Kanazashi, A., Kanazashi, T. and Yokoyama, T.: The relationship between the proportion of self-pollination and that of selfed filled seeds in consideration of polyembryony and zygotic lethals in *Pinus densiflora*. J. Jpn. For. Soc. **72**: 277–285 (1990). — Knowles, P., Furnier, G. R., Aleksiuk, M. A. and Perry, D. J.: Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. **65**: 1087–1091 (1987). — Kraus, J. F. and Squillace, A. E.: Selfing vs. outcrossing under artificial conditions in *Pinus elliottii* Engelm.. Silvae Genet. **13**: 72–76 (1964). — Lande, R. and Schemske, D. W.: The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution **39**: 41–52 (1985). — Mergen, F., Burley, J. and Furnival, G. M.: Embryo and seedling development in *Picea glauca* (Moench) Voss. after self-, cross-, and wind-pollination. Silvae Genet. **14**: 188–194 (1965). — Ohba, K.: Selective fertilization using pollen of Wogon-Sugi as a marker. Trans. 83th Jpn. For. Soc. 204–206 (in Japanese) (1972). — Ohba, K., Iwakawa, M., Okada, Y. and Murai, M.: Estimation of the degree of the natural self-fertilization by the frequencies of chlorophyllous variants in Japanese red pines, *Pinus densiflora* Sieb. et. Zucc., and the inheritance of the variants. J. Jpn. For. Soc. **53**: 327–333 (in Japanese with English summary) (1971). — Orr-Ewing, A. L.: A cytological study of the effects of self-pollination of *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genet. **6**: 179–185 (1957). — Park, Y. S. and Fowler, D. P.: Inbreeding in black spruce (*Picea mariana* (Mill.) B. S. P.): self-fertility, genetic load, and performance. Can. J. For. Res. **14**: 17–21 (1984). — Perry, D. J. and Knowles, P.: Evidence of high self-fertilization in natural populations of eastern white cedar (*Thuja occidentalis*). Can. J. Bot. **68**: 663–668 (1990). — Sorensen, F. C.: Embryonic genetic load in coastal Douglas-fir, *Pseudotsuga menziesii*. var. *menziesii*. Am. Nat. **103**: 389–398 (1969). — Sorensen, F. C.: The roles of polyembryony and embryo viability in the genetic system of conifers. Evolution **36**: 725–733 (1982). — SORENSEN, F. C.: Frequency of seedlings from natural self-fertilization in Pacific Northwest ponderosa pine (*Pinus ponderosa* DOUGL. ex LAWS). Silvae Genet. **43**: 100–108 (1994). — SORENSEN, F. C. and MILES, R. S.: Inbreeding depression in height, height growth, and survival of Douglas-fir, ponderosa pine, and noble fir to 10 years of age. For. Sci. **28**: 283–292 (1982). — WELLER, S. G. and ORNDUFF, R.: Incompatibility in *Amsinckia douglasiana* (Boraginaceae): distribution of callose plugs and pollen tubes following inter- and intra- morph crosses. Amer. J. Bot. **76**: 277–282 (1989). — WILCOX, M. D.: Inbreeding depression and genetic variances estimated from self- and cross-pollinated families of *Pinus radiata*. Silvae Genet. **32**: 89–96 (1983). — YAHARA, T.: Graphical analysis of mating system evolution in plants. Evolution **46**: 557–561 (1992).

Genetic Differentiation Among and Within Natural and Planted *Cupressus sempervirens* L. Eastern Mediterranean Populations[1]

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Abstract

The aim of the study was to extend our knowledge of the geographic patterns of genetic variation of *Cupressus sempervirens* L., and to try to relate the earliest plantations of *C. sempervirens* in Israel to its ultimate seed origins. Seeds of 22 populations, most of them of *C. sempervirens* var. *horizontalis*, were used for the analysis of the genetic diversity within and among East Mediterranean populations. Bulk seed lots from 13 populations, and single-tree seed collections, representing about 30 trees from each of the populations from Cyprus, Syria and Turkey, and from 267 trees from 20 Israeli plantations were available. Horizontal starch gel electrophoresis was used to resolve allele patterns in 22 loci encoding 13 enzyme systems. The mean number of alleles per locus was 1.7; the over all percentage of polymorphic loci was 41.7% (S.E. ± 1.1). The over all mean observed heterozygosity and the expected heterozygosity, *i.e.*, genetic diversity within populations – were 0.149 (S.E. ± 0.021) and 0.181 (S.E. ± 0.03), respectively; and the over all mean total genetic diversity among the natural populations was 0.192 (S.E. ± 0.032). The fixation indices, F_{it}, F_{is}, F_{st} and G_{st} for each polymorphic locus, over all the populations, are 0.180, 0.333, 0.187 and 0.049, respectively. The phylogenetic tree enabled us to define three main groups: a north-eastern Mediterranean group which includes the populations from central Mediterranean Turkey, Syria and Iran; a low-altitude east Aegean group which includes the populations of Kos, 2 Turkish population on the shores of the Aegean Sea together with populations on the island of Samos; a third group which includes populations from Crete, Cyprus, Rhodes, Jordan and plantations in Israel. The results imply that plantations of *Cupressus sempervirens* in Israel probably originated from seed material imported from Crete, Cyprus, Rhodes and Jordan.

Key words: Cupressus sempervirens, genetic diversity, populations structure, polymorphism, isoenzymes.

FDC: 165.3; 174.7 *Cupressus sempervirens;* (4-015).

Introduction

Natural relict stands of *Cupressus sempervirens* L. (Italian cypress or Mediterranean cypress) are growing in Iran, Syria, Jordan, Lebanon and Libya and on the Aegean islands, Kos, Samos and Rhodes, and there are larger forests of this species in Turkey, Cyprus and Crete (PAVARI, 1934; ZOHARY, 1973). This tree species grows under various Mediterranean climates (EMBERGER *et al.*, 1963), it grows from sea level up to 2000 m or more, and on a variety of bedrock formations and soils types, and therefore, in a variety of plant associations (ZOHARY, 1973). Natural relicts and forests of *C. sempervirens* are composed of var. *horizontalis* (MILL.) GORDON, which grows spreading branches. Single specimens of var. *pyramidalis* NYMAN (var. *stricta* AITON), the erect form which has a columnar or narrowly conical habit occur rarely (BOLOTIN, 1964). The erect form was most probably selected for ornamental and short rotation planting (MAKKONEN, 1968), and spread all around the Mediterranean in ancient times. Unfortunately, no paleobotanical evidence is known which could support the division of this species into the 2 most widespread varieties and help us to identify the exact range of distribution in the past.

Due to the importance of *C. sempervirens* L. for afforestation, and for ornamental and windbreak plantings all around the Mediterranean region and in other parts of the world, where this species has been introduced, there is considerable interest in the geographic patterns of genetic variation. However, so far, quantitative information on the gene pool of natural relict stands of *C. sempervirens* L. is available only for several Greek populations (PAPAGEORGIOU *et al.,* 1994, 1995); information is lacking on the gene pool of other geographic regions where this species grows naturally.

Contrary to a previously published opinion (LIPHSCHITZ and BIGER, 1989), natural occurrences of this species, that could be used as seed sources, are not known to have existed in Cis Jordan during recent centuries, although this area is included within the phytogeographical range of *C. sempervirens* L. (ZOHARY, 1973). Consequently, the seed source and/or the origin of trees that were planted in the last 2 centuries in this area is most likely foreign.

Therefore, the aims of the present study were twofold: (i) to extend our knowledge of the geographic patterns of genetic

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