1935–1938 (1969). — NAMKOONG, G., KANG, H. C. and BROUARD, J. S.: Tree Breeding: Principles and Strategies. Springer-Verlag, Berlin. 180 pp. (1988). — NIELSEN, U. B.: Genetisk variation i Sitkagran (*Picea sitchensis* (BONG.) CARR.) i højdevækst, stammeform og frosthærdighed – vurderet ud fra danske proveniens-, afkoms-, og klonforsøg. Forskningscentret for Skov & Landskab., Forskningsserien 9–94, 332 pp. (1994). — NYLINDER, P.: Wood quality and fibre product. In: Proc. IUFRO-5 Meet., Vol. 2, Republic of South Africa, pp. 832–844 (1973). — OLESEN, P. O.: The Interrelation Between Basic Density and Ring Width of Norway Spruce. Det Forstlige Forsøgsvæsen i Danmark **34**, 340–359 (1976). — PETTY, J. A., MACMILLAN, D. C. and STEWARD, C. M.: Variation of density and growth ring width in stems of Sitka and Norway spruce. Forestry **63**: 39–49 (1990). — ROBERTSON, A.: The sampling variance of the genetic correlation coefficient. Biometrics **15**: 469–485 (1959). — RYDHOLM, S. A.: Pulping Processes. Interscience Publishers, New York. 1269 pp. (1965). — SAS® Institute Inc.: SAS Procedures Guide, Version 6. SAS® Institute Inc., Cary, NC (1990a). — SAS® Institute Inc.: SAS/STAT User's Guide, Version 6, Vol. 1 and 2. SAS® Institute Inc., Cary, NC (1990b). — SAS® Institute Inc.: SAS/IML Software: Usage and Reference, Version 6. SAS® Institute Inc., Cary, NC (1990c). — SHAPIRO, S. S. and WILK, M. B.: An analysis of variance test for normality (complete samples). Biometrika **52**: 591–611 (1965). — SHELBOURNE, C. J. A. and LOW, C. B.: Multi-trait index selection and associated genetic gains of *Pinus radiata* progenies at five sites. N. Z. J. For. Sci. **10**: 307–324 (1980). — SNEDECOR, G. W. and COCHRAN, W. G.: Statistical Methods. The Iowa State University Press, Ames, U.S.A.. 593 pp. (1976). — VAN BUIJTENEN, J. P., EINSPAHR, D. W. and PECKHAM, J. R.: Micropulping loblolly pine grafts selected for extreme wood specific gravity. Silvae Genet. **17**: 15–19 (1968). — WELLENDORF, H.: Divers nucleus breeding: selection in different directions from one central multiple-trait selected breeding population. In: Evolution and Tree Breeding, CTIA/WFGA Conf., Victoria, BC., pp. 87 (1995). — WHITE, T. L. and HODGE, G. R.: Predicting Breeding Values with Applications in Forest Tree Improvement. Kluwer Academic Publishers, Dordrecht. 367 pp. (1989). — WILCOX, M. D. and SMITH, H. D.: Selection indices for wood quality in loblolly pine. In: Proc. 12th South. For. Tree Improve. Conf., Louisiana. pp. 322–342 (1973). — YANCHUK, A. D. and KISS, G. K.: Genetic variation in growth and wood specific gravity and its utility in the improvement of interior spruce in British Columbia. Silvae Genet. **42**: 141–148 (1993). — ZHANG, S. Y.: Wood density considerations in black spruce (*Picea mariana*) tree improvement program. In: Evolution and Tree Breeding, CTIA/WFGA Conf., Victoria, BC.. pp. 91 (1995). — ZOBEL, B. J. and VAN BUIJTENEN, J. P.: Wood Variation. Its Causes and Control. Springer-Verlag, Berlin. 363 pp. (1989).

Inheritance and Linkage Relationships of Allozymes, and Estimation of Outcrossing Rates in a Seed Orchard of *Cunninghamia konishii* HAY.

By CH.-T. WANG and T.-P. LIN[1])

Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei, Taiwan

(Received 16th July 1997)

Summary

Inheritance and linkage relationships of 11 polymorphic loci from 9 enzyme systems in *Cunninghamia konishii* were analyzed by horizontal starch gel electrophoresis using megagametophyte haploid tissues collected from Chuyunshan seed orchard, Taiwan. The outcrossing rate was also estimated based on multilocus and single-locus models. The observation of segregated female gametophytes of heterozygous trees revealed simple MENDELIAN inheritance for most of the allozyme loci. Linkage relationships were examined for 39 pairs of polymorphic allozyme loci. Three pairs with significant joint segregation were detected: *Mdh-1/6Pgd-2* with a recombination value (R) 0.098, *Fest-1/Fest-3* with R = 0.168, and Fest-2/Fest-3 with R = 0.038. Single-locus estimates (t_s) of outcrossing rate varied between 0.640 and 0.991 with an average of 0.847. Compared with the single-locus outcrossing rate estimates, the multilocus estimate (t_m = 0.902) indicated that a part of the inbreeding may be biparental.

[1]) Correspond: Dr. Tsan-Piao Lin, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei, Taiwan.
Contribution no. 98 of Taiwan Forestry Research Institute.
FAX: 886 (2) 2389-5531
E-mail: tpl@serv.tfri.gov.tw

Key words: *Cunninghamia konishii* HAY., isozyme, inheritance, linkage, outcrossing rate.

FDC: 165.3; 165.41; 232.311.3; 174.7 *Cunninghamia konishii* (529.1).

Introduction

In recent decades electrophoretic variants of enzymes have become very popular genetic markers in forest genetic research. Defined allozyme loci need to be confirmed before the population genetic structure can be studied. Testing of inheritance of allozyme polymorphisms can be performed by direct observation of segregating haploid gametophytes in coniferous species. Allozyme markers can also be used in the study of mating systems of natural populations of conifers. lt is known that significant inbreeding depression due to selfing can decrease the survival and growth of seedling progeny of many conifer species (SORENSEN and MILES, 1982). Knowledge of the mating system of a species is of practical significance when using open-pollinated seeds for reforestation (YEH *et al*., 1983).

Simple MENDELIAN inheritance of allozyme loci and linkage relationships have been described for *C. lanceolata* (MÜLLER-STARCK and LIU, 1988), and the genetic structure was also studied (MÜLLER-STARCK and LIU, 1989). *C. lanceolata* was introduced to Taiwan from the mainland China, and is cultivated widely at low elevations, especially on private forest land. *C. konishii* HAY. also known as *C. lanceolata* (LAMB.) HOOK. var. *konishii* (HAY.) FUJITA is endemic to Taiwan. *C. konishii* represents the country's valuable timber produced from old growth. It is usually found scattered in forests of *Chamaecyparis* spp. at an elevational range of 1,300 m to 2,800 m (LIU, 1966), and accompanied by *Taiwania cryptomerioides*, *Pseudotsuga wilsonii* and *Pinus* spp. *C. konishii* has been used in reforestation programs of national forest land over the past 20 years and composes man-made plantations of about 10,000 ha.

In this study, we report on the inheritance and linkage of 11 allozyme loci in 9 enzyme systems of *C. konishii* grown in a clonal seed orchard. The mating system is also characterized.

Materials and Methods

Seed collection

The seed orchard of *C. konishii* was established between 1968 and 1974, at Chuyunshan, Tungsyh (Taichung County) at an elevation of 700 m. It is composed of 25 grafted clones on 10 ha and has been producing abundant seeds since 1983. Clones originated from old growth forests of *C. konishii* in central Taiwan. In 1994, cones from 18 individual trees (families) were collected, and seeds were extracted and stored at –20 °C until analysis.

Seeds were soaked on moistened filter paper in petri dishes at a cyclically alternating temperature 15 °C and 25 °C (12 + 12 hours/cycle) growth chamber until the seeds started to germinate. Twenty female gametophytes from each of the 18 trees were first sampled to infer maternal genotypes and estimate the outcrossing rate with their corresponding diploid embryos. Then an additional 40 to 80 megagametophytes per tree with observed heterozygotes at 2 or more loci were examined and used for allozyme segregation analysis and recombination rate estimation.

Electrophoresis

Horizontal starch gel electrophoresis was used for separating isozymes: AAT, FEST, G6PD IDH, MDH, 6PGD PGI, PGM, and SKDH. Gametophytes and embryos were ground with extraction buffer according to the procedures described by FERET (1971). Electrophoresis and staining followed the procedure described by CHELIAK and PITEL (1984).

The zone specifying the slowest migrating bands was designated as 1, the next as 2, and so on. Within each zone, the slowest migrating bands were designated as a, the next as b, and so on.

Data analysis

Genotypes of mother trees were inferred from allozymes in their megagametophytes. The inheritance of allozyme polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the expected 1:1 ratio. Linkage analysis was examined as described by BAILEY (1961) and MATHER (1951). Segregation data of doubly heterozygous maternal trees were analyzed with the expected segregations by the chi-square test in contingency tables.

Four polymorphic loci, *Pgi-1*, *Skdh-1*, *Skdh-2*, and *6pgd-2*, resolved clearly in both gametophytes and embryos, were used to estimate mating parameters. Multilocus estimate of outcrossing rate (t_m) and single-locus estimations of outcrossing rate (t_s) were estimated using the maximum likelihood estimation procedure of the Generalized Multilocus Estimation Program of RITLAND (1990).

Results and Discussion

Inheritance of isozyme patterns

Nine of the 30 enzyme systems tested could be resolved clearly enough for inheritance studies. The 13 loci resolvable from the megagametophyte tissues of *C. konishii* were *Aat-2, Fest-1, Fest-2, Fest-3, G6pd-1, Idh-2, Mdh-1, 6Pgd-1, 6Pgd-2, Pgi-1, Pgm-2, Skdh-1* and *Skdh-2*. MENDELIAN inheritance was tested only for the 11 staining polymorphic allozyme loci. In summary, no significant deviation from the expected 1:1 segregation ratio was detected indicating that these allozyme loci had codominant alleles and simple MENDELIAN segregation. No segragation distortion was observed in allozymes of *C. Ianceolata* either (MÜLLER-STARCK and LIU, 1988), a species closely related to *C. konishii*. There was no heterogeneity in allozyme segregation in megagametophytes among trees.

AAT: There were three zones of AAT activity on the gel. Only *Aat-2* stained consistently. Three single-banded alleles were observed (This equaled *Got-B* of *C. lanceolata* (MÜLLER-STARCK and LIU, 1988)). Two of three possible combinations were detected but there was no tree with *b*, *c* genotype observed.

IDH: Two zones of activity were found for IDH, the faster migrating zone (*Idh-2*) was polymorphic with two alleles (*a* and *b*) in only one tree. The slower zone with poor activity could not be resolved clearly.

FEST: There were three zones of activity on gels stained for FEST. *Fest-1* and *Fest-2* both have two alleles, *Fest-3* has three alleles. FEST from embryo tissue could not be resolved clearly.

G6PD: One zone of activity (*G6pd-1*) was evident on gels stained G6PD. In this zone, three alleles were observed. Enzyme activity from embryo tissue could not be resolved clearly.

MDH: There were multiple bands of activity on gels stained for MDH. Only *Mdh-1* which could be distinguished was polymorphic with two alleles. Even though MDH from young leaf tissue could be resolved, no locus could be defined.

6PGD: Gels stained for 6PGD had two zones of activity. Four single-banded alleles were observed in the faster migrating zone (*6Pgd-2*, same as *6PGDH-A* in *C. lanceolata* (MÜLLER-STARCK and LUI, 1988)). *6Pgd-1* was monomorphic (same as *6PGDH-B*).

PGI: Two zones of PGI activity were observed. The slower migrating zone (*Pgi-1*) had two alleles (same as *PGI* in *C. lanceolata*).

PGM: Two zones of activity occurred on gels stained for PGM. The slower migrating zone (*Pgm-1*) was monomorphic with two bands for the young leaf tissue of *C. konishii* but no band for the megagametophytes. *Pgm-2* had three alleles in the young leaf tissue but only one band was observed in haploid tissue.

SKDH: Two zones of activity occurred on gels stained for SKDH. The slower migrating zone (*Skdh-1*) had two alleles (same as *SKDH-B*), the faster migrating zone (*Skdh-2*) had three alleles (same as *SKDH-A* in *C. lanceolata*). *Skdh-1b* and *Skdh-2a* were overlapping.

Different enzyme systems are employed by different authors because of their different eletrophoretical methods. In *C. lanceolata*, 5 enzyme systems with 10 loci were reported by MÜLLER-STARCK and LIU (1988) and recently more enzymes and loci were studied by YEH *et al.* (1994). However, among the common enzyme systems, a monomeric enzyme structure for SKDH and a dimeric structure for AAT, IDH, 6PGD, and PGI were observed in both *C. lanceolata* and *C. konishii*. The number of alleles within a locus in *C. konishii* was less than in *C. lanceolata*.

Linkage relationship

Of the 55 possible 2-locus combinations formed from 11 polymorphic loci, 40 pairs of allozyme loci were tested in at least one tree. Thirty-one of these 40 pairs were analyzed based on more than one tree. The number of trees employed for linkage analysis and results of statistical tests are presented in *table 1*. Two-locus segregation with non-random joint segregation for 6 combinations (including separate combinations for coupling and repulsion in *Mdh-1/6Ppg-2* and *Fest-2/Fest-3*) are presented in *table 2*. Heterogeneity among trees for 2-locus combinations has been reported in several conifer species. A major cause may be due to alleles being in different combinations that is, in coupling and repulsion in different trees (ADAMS and JOLY, 1980; ADAMS *et al.*, 1990; BEAULIEU and SIMON, 1994). If there were more than 2 alleles in the locus, the heterogeneity would be more apparent. We need, therefore, to transform the data with more than two alleles per locus or repulsion condition into an uniform type for analyzing pooled data. *Mdh-1/6Pgd-2*, *Fest-1/Fest-3*, and *Fest-2/Fest-3* pairs represented homogeneity joint segregation among families and appeared to be tightly linked. The chisquare tests did not reject homogeneity of recombination frequencies among trees for these three combinations (data not shown).

According to the observed recombination values for the significantly linked loci, two linkage groups can be constructed. *Mdh-1* and *6Pgd-2* were linked with a recombination value of 0.098. Similar linkage has also been reported in other conifers. The other group including *Fest-1*, *Fest-2*, and *Fest-3*. *Fest-1/Fest-3* and *Fest-2/Fest-3* was found to be closely linked with recombination values of 0.168 and 0.038, respectively. *Est-2/Est-3* linkage was also detected in Japanese red pine (NA'IEM, *et al.*, 1993) and Masson pine (HUANG *et al.*, 1994), in

Table 1. – Number of tree analyzed for linkage for each pair of allozyme loci (upper right half) and results of statistical tests for linkage (lower left half).

	Aat-	*Fest-*			*G6pd-*	*Mdh-*	*6Pgd-*	*Pgi-*	*Skdh-*		*Idh-*
	2	*1*	*2*	*3*	*1*	*1*	*2*	*1*	*1*	*2*	*2*
Aat-2		4	1	6	4	4	5	-	4	9	-
Fest-1	NS		-	3	-	1	1	-	5	5	-
2	NS	-		3	3	3	2	-	-	1	-
3	NS	*	*		7	6	5	1	4	9	-
G6pg-1	NS	-	NS	*		6	9	2	2	8	-
Mdh-1	NS	NS	NS	NS	NS		7	-	1	6	-
6Pgd-2	NS	NS	NS	NS	NS	*		2	3	9	-
Pgi-1	-	-	-	NS	*	-	NS		2	2	-
Skdh-1	NS	NS	-	NS	NS	NS	NS	NS		8	1
2	NS	NS	NS	*	NS	NS	NS	NS	NS		1
Idh-2	-	-	-	-	-	-	-	-	NS	NS	

–: 2 locus combination not tested.
*: Significant linkage at 5% level in at least 1 tree.
NS: Not significant.

Table 2. – Segregation in 2-locus combinations with significant deviation from random joint segregation, χ^2 values for heterogeneity among families (χ^2H), and deviation from 1:1:1:1 joint segregation (χ^2).

Combination	No. of	Sample	Segregation class				Heterogeneity		Deviation	
locus A/B	families	size	A_iB_i	A_iB_j	A_jB_i	A_jB_j	χ^2H (df)	p	χ^2	p
Fest-1 / Fest-3	3	232	98	18	21	95	3.44 (6)	0.753	102.29	< 0.01
Fest-2 / Fest-3	3	160	77	3	3	77	5.73 (6)	0.454	136.90	< 0.01
Mdh-1 / 6Pgd-2	7	615	273	30	30	282	20.22 (18)	0.321	398.37	< 0.01
Fest-3 / G6pd-1	1	84	26	12	18	28	-	-	7.16	< 0.01
Fest-3 / Skdh-2	1	85	27	17	16	25	-	-	4.24	< 0.05
G6pd-1 / Pgi-1	1	89	31	23	12	23	-	-	4.55	< 0.05

which *Est-2/Est-3* was linked completely, but *Est-1* was not included in that group. The exact order of the three *Fest* loci could not be determined because of the lack of a double heterozygous maternal tree for *Fest-1* and *Fest-2* in this study.

Other possible linkages were *Fest-3/G6pd-1* (R = 0.357), *Fest-3/Skdh-2* (R = 0.388), and *G6pd-1/Pgi-1* (R = 0.393) based on only 1 significant (P < 0.05) case of linkage. When we pooled the trees employed, the recombination values of *Fest-3/G6pd-1, Fest-3/Skdh-2*, and *G6pd-1/Pgi-1* were 0.427, 0.459, and 0.457, respectively, and no longer significant for random joint segregations. Significant linkage in one but not in all trees has also been reported for other conifer species (CEBUREK and WUEHLISCH, 1989; GEBUREK *et al.*, 1990; LEWANDOSKI *et al.*, 1992). The possible reason for different recombination values among trees is related to environmental effect or modification of chromosome structure, i.e., insertion, translocation, deletion, reversion, etc.

Three linkage relationships, *Pgi-1/6Pgd-2*, *Pgi-1/Skdh-2*, and *6Pgd-2/Skdh-2*, reported in *C. lanceolata* (MÜLLER-STARCK and LIU, 1988) were not detected in *C. konishii*, even though they were tested for. Linkage between *Aat* and *Pgi* loci in many conifer species including *C. lanceolata* has been reported, but we were not able to test for it due to lack of doubly heterozygous trees.

Mating system

Estimated allozyme frequencies of pollen and outcrossing rates with standard deviation are given in *table 3*. The single-locus estimates of outcrossing (t_s) were different among loci and ranged 0.640 to 0.991. Differences observed among single-locus estimates have also been reported for several conifer species. The large interlocus variation may be due to violation of the assumptions of the mixed-mating model (ENNOS and CLEGG, 1982; Brown *et al.*, 1984; EL-KASSABY *et al.*, 1987).

The outcrossing rate of multilocus estimation (t_m = 0.902) is greater than the mean of all single-locus estimates and similar to the conifer average (0.90) by allozymes in progeny arrays (see the compilation in SURLES *et al.*, 1990). In general, multilocus estimation will be more accurate than a single-locus model. Since the multilocus model is more powerful in distinguishing related mating (in addition to selfing, inbreeding due to other causes biases the single-locus estimates downward), the t_m estimator provides a better estimate than t_s (SHAW *et al.*, 1981; SHAW and ALLARD, 1982). Inbreeding other than selfing in stands can be inferred by comparing t_m and t_s estimates (SHAW and ALLARD, 1982). If we assume that t_m and t_s estimates are statistically independent (in fact, they are estimated with the same data), the difference between them would become significant in the case when inbreeding other than selfing occurs. Some proportion of related mating other than selfs occurs in the clonal seed orchard of *C. konishii* probably because related trees grow nearby.

In this study, the embryos were not analyzed further if the enzyme system was monomorphic within families, and this resulted in less family data for in *6Ppg-2* and *Pgi-1* loci (*Table 3*.). We assume that the individual genotype including heterozygotes and homozygotes of different loci would not affect the estimates of outcrossing rate. Comparing with t_s (0.991) of the *Skdh-1* locus based on 12 families, the additional estimate (t_s' = 0.984) in which 7 families having a homozygous maternal tree were omitted was not significantly different (data not shown). Thus, there is no evidence to reject the above assumption in this study.

The mating system parameters are not constant, but are affected by both environmental and genetic variation (MITTON, 1992). Also it has been found that outcrossing rates can be higher in seed orchards than in natural populations of the same species (MUONA and HARJU, 1989). Therefore, the outcrossing rate of the estimate based on a single population and a single year may not be applicable to other populations of *C. konishii*.

Table 3. – Estimates of the mating system parameters for 4 loci of the clonal seed orchard of *C. Konishii* in Chuyunshan, Tungsyh.

Locus and allele	No. of seeds/family	Allelic frequency of pollen	Outcrossing rate (t_s)
6Pgd-2	138/7		0.640 (0.059)
a		0.457 (0.047)	
b		0.231 (0.040)	
c		0.312 (0.046)	
Skdh-1	237/12		0.991 (0.028)
a		0.266 (0.032)	
b		0.734 (0.032)	
Skdh-2	237/12		0.865 (0.022)
a		0.080 (0.020)	
b		0.512 (0.037)	
c		0.408 (0.036)	
Pgi-1	38/2		0.893 (0.066)
a		0.115 (0.061)	
b		0.885 (0.061)	
Average			0.847
Multilocus (t_m)			0.902 (0.052)

Standard deviation is in parantheses.
Allele *d* was incorporated in *c* in *6Pgd-2* locus.

Acknowledgments

Financial support provided by the Council of Agriculture (86-AST-1.5-FOD-03) is greatly acknowledged.

Reference

ADAMS, W. T. and JOLY, R. J.: Linkage relationships among twelve allozyme loci in loblolly pine. J. Hered. **71**: 199–202 (1980). — ADAMS, W. T., NEALE, D. B., DOERKSEN, A. H. and SMITH, D. B.: Inheritance and linkage of isozyme variants from seed and vegetative bud tissues in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (MIRB.) FRANCO). Silvae Genet. **39**: 153–167 (1990). — BAILEY, N. T. J.: Introduction to mathematical theory of genetic linkage. Oxford University Press, London. 288 pp. (1961). — BEAULIEU, J. and SIMON, J.-P.: Inheritance and linkage relationships of allozymes in *Pinus strobus* L.. Silvae Genet. **43**: 253–261 (1994). — BROWN, A. H. D., BARRETT, S. C. H. and MORGAN, G. F.: Mating system estimation in forest trees: models, methods and meaning. *In*: Population Genetics In Forestry. GREGORIUS, H. R. (Ed). Springer-Verlag, Berlin. pp. 32–49 (1984). — CHELIAK, W. M. and PITEL, J. A.: Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National For. Institute, Can. For. Serv. Information Rep. PI-X-42. pp. 19–45 (1984). — EL-KASSABY, Y. A., MEAGHER, M. D., PARKINSON, J. and PORTLOCK, F. T.: Allozyme inheritance, heterozygosity and outcrossing rate among *Pinus monticola* near Ladysmith, British Columbia. Heredity **58**: 173–181 (1987). — ENNOS, R. A. and CLEGG, M. T.: Effect of population substructuring on estimates of outcrossing rate in plant populations. Heredity **48**: 283–292 (1982). — FERET, P. P.: Isozyme variation in *Picea glauca* (MOENCH) VOSS seedlings. Silvae Genet. **20**: 46–50 (1971). — GEBUREK, T., STEPHAN, B. R. and WANG, J. Y.: Genetics of six enzyme systems in Henryi pine (*Pinus Henryi* MAST). J. Genetics and Breeding **44**: 269–275 (1990). — GEBUREK, T. and WUEHLISCH, G.: Linkage analysis of isozyme gene loci in *Picea abies* (L.) KARST. Heredity **62**: 185–191 (1989). — HUANG, Q. Q., TOMARU, N., WANG, L. H. and OHBA, K.: Genetic control of isozyme variation in masson pine, *Pinus massonia* LAMB.. Silvae Genet. **43**: 285–292 (1994). — LEWANDOWSKI, A.,

BURCZYK, J. and MEJNARTOWICZ, L.: Inheritance and linkage of some allozymes in *Taxus baccata* L.: Silvae Genet. **41**: 342–347 (1992). — LIU, T.: Study on the phytogeography of the conifers and taxads of Taiwan. Bull. Taiwan For. Res. Inst. no. 122, (in Chinese) (1966). — MATHER, K.: The measurement of linkage in heredity. Methuen and Co. Ltd., London. 149 pp. (1951). — MITTON, J. B.: The dynamic mating systems of conifers. *In*: Population genetics of forest trees. ADAMS, W. T., STRAUSS, S. H., GOPES, D. L. and GRIFFIN, A. R. (Eds.). Proc. of the international symposium on population genetics of forest trees, Corvallis, Oregon, USA, Jul. 31 to Aug. 2, 1990. Kluwer Academic Publishers, Dordrecht. pp. 197–216 (1992). — MÜLLER-STARCK, G. and LIU, Y. Q.: Genetics of *Cunninghamia lanceolata* HOOK. 1. Genetic analysis. Silvae Genet. **37**: 236–243 (1988). — MÜLLER-STARCK, G. and LIU, Y. Q.: Genetics of *Cunninghamia lanceolata* HOOK. 2. Genetic variation within and between two provenance samples. Silvae Genet. **38**: 172–177 (1989). — MUONA, O. and HARJU, A.: Effective population sizes, genetic variability, and mating system in natural stands and seed orchards of *Pinus sylvestris*. Silvae Genet. **38**: 221–228 (1989). — NA'IEM, M., TSUMURA, Y., UCHIDA, K., NAKAMURA, T. and OHBA, K.: Linkage of allozyme loci in Japanese red pine (*Pinus densiflora*). Can. J. For. Res. **23**: 680–687 (1993). — RITLAND, K.: A series of FORTRAN computer programs for estimating plant mating system. J. Hered. **81**: 235–237 (1990). — SHAW, D. V. and ALLARD, R. W.: Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor. Appl. Genet. **62**: 113–120 (1982). — SHAW, D. V., KAHLER, A. L. and ALLARD, R. W.: A multilocus estimator of mating system parameters in plant populations. Proc. Natl. Acad. Sci. (USA) **78**: 1298–1302 (1981). — 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). — SURLES, S. E., HAMRICK, J. L. and BONGARTEN, B. C.: Mating systems in open-pollinated families of black locust (*Robinia pseudoacacia*). Silvae Genet. **39**: 35–40 (1990). — YEH, F. C., BRUNE, A., CHELIAK, W. M. and CHIPMAN, D. C.: Mating system of *Eucalyptus citriodora* in a seed production area. Can. J. For. Res. **13**: 1051–1055 (1983). — YEH, F. C., SHI, J., YANG, R., HONG, J. and YE, Z.: Genetic diversity and multilocus association in *Cunninghamia lanceolata* (LAMB.) HOOK. from The People's Republic of China. Theor. Appl. Genet. **88**: 465–471 (1994).

Genetic Variation in Cone and Seed Characteristics in a Clonal Seed Orchard of Aleppo Pine Grown in Greece

By D. MATZIRIS[1])

(Received 6th October 1997)

Summary

Cone and seed characteristics of Aleppo pine (*Pinus halepensis* MILL.) were investigated in a clonal seed orchard for two successive years, 1994 and 1995. The orchard was established in January 1987 in an area of 10 ha, at Amphilochia, west Greece and includes 76 clones. The results showed that significant genetic variation exists among clones for cone wet and dry weight, number and volume of seeds extracted, weight and volume of 1000 seeds, percentage of full seeds and cone, seed and wing lengths and widths. Only cone moisture content was predominantly influenced by the environment. Cone weight at the time of harvesting (June) varied among clones from 30 g to 77.2 g ($\bar{x}$ = 48.99 g), while the dry weight varied from 27.2 g to 70.2 g ($\bar{x}$ = 44.1 g). Cone length varied from 6.6 cm to 11.6 cm with overall mean 9.3 cm. Year to year correlation coefficients for seed characteristics were varied from moderate (0.46) to strong (0.81). These correlations indicated that the clones are quite stable from year to year, in production and in seed quality and size. Broad sense heritability (H^2) estimates were variable. Cone length and width were strongly inherited with H^2 values 0.74 and 0.73 respectively. The respective H^2 values for wet and dry cone weight were 0.79 and 0.78. The number of seeds per cone and the number of full seeds were moderately inherited characteristics with H^2 values 0.47 and 0.36 respectively. Seed volume and weight based on a 1000 seed sampling were strongly inherited (H^2 = 0.75 and 0.73, respectively). The percentage of full seed had a H^2 value 0.41.

Key words: *Pinus halepensis*, Aleppo pine, seed orchard, heritability, correlation, maternal effect.

FDC: 164.7/8; 165.3; 165.51; 232.311.3; 232.312.31; 174.7 (*Pinus halepensis*; (495).

[1]) National Agricultural Research Foundation, Forest Research Institute, Terma Alkmanos, Athens, Greece

Introduction

Aleppo pine (*Pinus halepensis* MILL.) is a circum-Mediterranean species with discontinous geographic distribution and is very well adapted to dry sensitive to fire ecosystems. The species flowers at an early age and produces a large number of serotinous cones, that persist on branches for many years. Cone and seed characteristics vary among species, provenances and individual genotypes in *Pinus* L.. Cones vary in size from 2 cm to 3 cm in length (*P. montana* MILL.) to 50 cm to 60 cm (*P. lamberteana* DOUGL.) (MIROV, 1967). In weight the variation is even greater (2 g in *P. montana* MILL., 1100 g in *P. coultery* D. DON.). Cone and seed sizes vary also widely among seed orchard clones (BERGMAN, 1968). It is well known that double fertilization in gymnosperms does not take place (cf. CHAMBERLAIN, 1966) as in the angiosperms and the endosperm (female gametophyte) has a haploid number of chromosomes (n = 12) instead of being diploid (2n = 24). The endosperm is enclosed in a seed coat which is diploid, developed also from maternal tissue. Therefore the characteristics of the seed coat including seed wings are inherited from the female parent alone. CLAIR and ADAMS (1991) stated that the variation in seed weight of different female parents of coniferous species are the result of three factors: (i) the diploid gametophyte of embryo, (ii) the haploid genotype of the megasporophyte (endosperm), and (iii) the enviromental effect on the mother tree during seed development. As the endosperm in coniferous seeds, that is the nutrient source of embryo, is exclusively maternal in origin, the female parent determines the characteristics "e. g., endosperm weight" of the nutrient source. The maternally inherited diploid seed coat also determines phenomena related to seed dormancy and germination (EL-KASSABY et al., 1992).

The purpose of the present study is to investigate the variation and inheritance pattern in cone and seed characteristics of