Genetic Control of Growth of Young Eucalyptus globulus Clones in Portugal

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(Received 5th August 1991)

Summary

Clonal (broad-sense) heritability and clone-site interactions for height and sectional area at two to three years after planting were investigated for five clonal trials of *Eucalyptus globulus* in central Portugal. These trials included clones from phenotypically superior trees selected in genetically unimproved stands of more than 10 years old in Portugal.

Clone-site interaction effects were significant for sectional area, however, some clones had good general performance across sites. Clonal heritabilities ranged between 0.87 and 0.91 for height and 0.79 and 0.88 for sectional area. Expected genetic gains of 17% in sectional area at three years can be expected from selecting good general performing clones at an intensity of one in 10. Comments are made in the paper about how these heritabilities, and hence genetic gains, may be increased by improving nursery techniques to reduce within-clone variability.

Key words: Clonal heritability, clone-site interaction, clonal selection.

Introduction

Many *Eucalyptus* breeding programmes are presently concentrating on improving clonal plantations propagated by stem cuttings or tissue culture (Campinhos and Ikemori, 1989; Cameron et al., 1989; Lambeth et al., 1989; Ikemori, 1990). However, despite the increasing interest in clonal forestry, basic genetic information such as clonal genetic variation, clonal (or "broad-sense") heritabilities and correlations, and interactions between clones and environments are not well known for commercially important species such as *Eucalyptus globulus* Labill. This basic information is absolutely essential in determining optimum strategies for breeding for clonal forestry and calculating expected genetic gains. There is also a lack of information on effects due to types of "mother" (or stock) plants used to take cuttings (Libby and Jund, 1962; Burdon and Shelbourne, 1974; Foster *et al.*, 1984; Farmer et al., 1988), propagation techniques, and how these factors interact with genotype.

Borralho *et al.* (1992) give estimates of additive genetic parameters for *E. globulus* progeny trials in central Portugal. This present paper extends the knowledge on genetic control of growth of *E. globulus* by presenting estimates of clonal heritability and also clone-site interactions for height and sectional area at two to three years, from five first-generation clonal trials of *E. globulus* in central Portugal. These clonal parameters include therefore the combined effects of both additive and non-additive genetic variances. There appear to be no other published estimates of clonal genetic parameters for *E. globulus*. The only other reported estimates of clonal genetic parameters for any species of *Eucalyptus* seem to be the results of *E. grandis* clonal trials in Brazil (Kageyama and Kikuti, 1989; Ikemori, 1990), Zambia (Mikkola, 1982) and Colombia (Lambeth and Endo, 1990).

Materials and Methods

Clones

The ortets represented in clonal trials were superior phenotypes for growth, selected between 1969 and 1976 from mature (between 20 and 30 years) genetically unimproved stands of *E. globulus* in northern and central Portugal.

Propagation Techniques

Typical cuttings were 10 cm long shoots with one to three pairs of leaves. These shoots were collected from "hedged" mother-plant ramets (less than 2 years old) growing either in the field or 10 litre pots at Celulose Beira Industrial (CELBI) S. A., Quinta do Furadouro Research Centre. The mother plants growing in pots had originally been established as coppice shoot cuttings from ortets. Cuttings were collected all year but more material was available in spring and autumn. Each cutting was dipped in a rooting hormone (indole-3-butyric acid) before being set in trays of "paper-pots" filled with 1:1 (volume) sterilised peat and styrofoam.

The cuttings were held in a greenhouse during a 30 to 40 day rooting period, with inside air humidity maintained above 80%. After the rooting phase surviving clones (still in paper pots) were moved outside to a "holding area" and grown under a travelling boom irrigation/fertilization system. The cuttings remained in holding area until taken to the field for planting.

The cuttings established in any particular clonal trial were from different rooting campaigns in the greenhouse, and hence were different ages. For instance, some ramets of a particular clone may have been rooted in early spring and held in the outdoor holding area for over one year until field planting the following spring. Other ramets of the same clone may have been rooted in late winter and planted after only about two months in holding area. The age of ramets at planting, average rooting percentages of clones, and details regarding mother plants were not recorded.

Location and Environment

The five clonal trials, known locally as C316, C449, C795, C653 and C225, were established by CELBI between spring 1986 and autumn 1988 across a range of sites in central Portugal (*Table 1*). Trial C316 is located *ca.* 20 km east of Lisbon, C499 *ca.* 15 km west from Abrantes, C795 *ca.* 25 km north-west Tomar, C653 *ca.* 15 km east of Penamacor, and C225 *ca.* 10 km west of Obidos. Soil preparation was similar across trials and involved discing, ripping to a depth of *ca.* 60 cm, and finally ploughing along 4 m spaced contours. Cuttings were planted by hand along contours at spacing of 2 m between trees. Following

Table 1. — Experimental details of *E. globulus* clonal Trials C316, C499, C795, C653 and C225 in Portugal.

Details	Clonal Trial				
	C316	C499	C795	C653	C225
Location and Environment					
Location	Lisboa	Abrantes	Tomar	Penamacor	Obidos
Soil Fertility	low	moderate	moderate	moderate	moderate
Annual rainfall (mm)	550	600	500	450	600
Previous vegetation	shrubs	pines	pines	grass	eucalypts
Date planted	March 86	March 86	March 86	April 87	Oct. 88
Measurements					
Diameter	June 89	June 89	July 89		
Height	June 89	June 89		July 89	Dec. 90

planting 150 g Foskamonia 7:21:7 (i.e. 7% N, 21% P_2O_5, 7% K_2O) fertilizer was applied to each cutting.

Trial Design

Each trial involved between 11 and 15 clones, of which 11 clones were common across trials C316, C449 and C795. Trial C653 involved a somewhat different set of clones (*Table 2*). Only one clone was common across all five trials.

Table 2. — List of the *E. globulus* clones represented in the five clonal trials in Portugal.

Clone	Trials				
	C316	C499	C795	C653	C225
A1	X	X	X		X
A2	X	X	X		X
A3	X	X	X		X
A4	X	X	X		X
A5	X	X	X		
A6				X	
A7	X	X	X	X	
A8				X	X
A9	X	X	X		X
A10				X	
A11				X	X
A12				X	
A13				X	
A14				X	
A15					X
A16				X	X
A17	X	X	X		X
A18				X	X
A19				X	X
A20	X	X	X	X	X
A21	X	X	X		X
A22	X	X	X		X

All trials were established as 10 randomised complete blocks of 5-tree row plots. The data reported here came from all blocks, except in the case of Trial C795 where only seven blocks were measured.

Measurements

Diameter at breast-height (1.3 m) over-bark was measured at *ca.* 3 years in Trials C316, C499 and C795 as the average of two diagonal caliper measurements (*Table 1*). The diameter measurements (denoted DIA) were converted to sectional area of stem (SA = π (DIA/2)^2). Height was assessed at *ca.* three years in Trials C316 and C499 using a hypsometer, and at *ca.* two years in Trials C225 and C653 using a telescopic rod (*Table 1*). Height was not assessed in Trial C795. At time of measurements the number of surviving trees was recorded.

Statistical Analyses

Statistical analyses were carried out using a generalised least-squares program (Harvey, 1970, 1990). The data from each clonal trial were analysed separately according to the following random model —

$$Y_{ijk} = \mu + c_i + b_j + (cb)_{ij} + e_{ijk} \quad (1)$$

where Y_{ijk} represents an individual tree observation, μ overall trial mean, c_i effect of the ith clone, b_j effect of the jth block, $(cb)_{ij}$ clone-block interaction and e_{ijk} within-plot error.

In another analysis sectional area data from trials C316, C499 and C795 were pooled and analysed according to the model

$$Y_{ijkl} = \mu + g_i + t_j + b(t)_{jk} + gt_{ij} + gb(t)_{ijk} + e_{ijkl} \quad (2)$$

where Y_{ijkl} is the individual tree observation, μ overall mean across trials, g_i effect of the ith clone, t_j effect of the jth trial, $b(t)_{jk}$ effect of the kth block nested within jth trial gt_{ij} interaction between ith clone and jth trial, $gb(t)_{ijk}$ interaction between ith clone and kth block within jth trial and e_{ijkl} within-plot error. In preliminary analysis of pooled data, log transformation was used to remove any scale

effects due to heterogeneity of variance across trials. However, the transformation had little influence on significance levels or genetic parameter estimates and results presented here are, therefore, only for analysis of raw untransformed data.

Parameter Estimates

The expectations of mean squares for Equations 1 and 2 are listed in *table 3*. Variance components were determined by equating appropriate mean squares with their expectations. In the case of analysis of separate clonal trials (*i.e.* using Equation 1) heritabilities were estimated on an individual tree basis as —

$$H^2_{I_i} = \frac{\sigma^2_c}{\sigma^2_{P_i}} \qquad (3)$$

where H^2_{Ii} denotes the heritability on an individual tree basis which is relevant to estimating gains from selecting best individual ramets from a particular clonal trial, σ^2_c variance due to clonal effects (*i.e.* including additive and non-additive genetic variances) for the particular trial, and σ^2_{Pi} phenotypic variance among ramets in the trial. The σ^2_{Pi} is calculated as

$$\sigma^2_{P_i} = \sigma^2_c + \sigma^2_{cb} + \sigma^2_w \qquad (4)$$

where σ^2_{cb} represents variance due to clone-block interaction and σ^2_w within-plot error. Standard errors of H^2_{Ii} were estimated according to SWIGER *et al.* (1964).

The H^2_{Ii} is not usually important in forestry because tree breeders seldom select the best individual ramets from clonal trials. The objective of clonal trials is mostly to select the best clones for mass propagation in commercial forests. The broad-sense heritability relevant to estimating genetic gains from selecting best clones is the "clonal heritability" (denoted H^2_{Ci}) which can be calculated from H^2_{Ii} using an appropriate equation in FALCONER (1986, *Table 13.4*) —

$$H^2_{C_i} = \frac{nr}{1+(n-1)t} . H^2_{I_i} \qquad (5)$$

where n is the number of ramets per clone in the particular trial, r the coefficient of relationship (r = 1 for clones), and t the intraclass correlation (t = H^2_I for clones).

In the case of analysis of pooled data across trials (*i.e.* using Equation 2) the individual heritability (denoted H^2_{Ip} where "p" subscript indicates pooled) was estimated as

$$H^2_{C_p} = \frac{\sigma^2_g}{\sigma^2_{P_p}} \qquad (6)$$

where σ^2_g represents variance due to clonal effects (*i.e.* including additive and non-additive genetic variances) across trials. The phenotypic variance σ^2_{Pp} in the case of pooled data is estimated as —

$$\sigma^2_{P_p} = \sigma^2_g + \sigma^2_{gt} + \sigma^2_{gb(t)} + \sigma^2_w \qquad (7)$$

where σ^2_{gt} represents variance due to clone-trial interactions and $\sigma^2_{gb(t)}$ clone-block within trial interactions. The H^2_{Ip} can be converted to clonal heritability H^2_{Cp} using Equation 5 but with n equal to total number of ramets per clone across trials.

The difference between the clonal variances σ^2_c (Equation 3) and σ^2_g (Equation 6) is that the σ^2_c for each separate trial is confounded with clone-trial interaction effects. In

Table 3. — Expected mean squares for separate trial and across trial analyses. The variance components for analyses of one trial are σ^2_c due to clones, σ^2_{cb} clone-block interactions and σ^2_w within-plot error. For analyses across trials σ^2_g is due to clones, $\sigma^2_{b(t)}$ block nested within trial, σ^2_{gt} clone-trial interaction and $\sigma^2_{gb(t)}$ clone-block within trial interaction. The constant estimates are k_1 = 4.5, 4.5, 2.9, 4.2, 4.1; k_2 = 43.5, 44.2, 23.2, 41.4, 39.6 for Trials C316, C499, C795, C653 and C225 respectively; and k_3 = 4.3; k_4 = 38.7; and k_5 = 124.2 for across site analyses.

Source of Variation	Expected Mean Squares
<u>Separate Clonal Trials (following Equation 1)</u>	
Clone	$\sigma^2_w + k_1 \sigma^2_{cb} + k_2 \sigma^2_c$
Clone-block	$\sigma^2_w + k_1 \sigma^2_{cb}$
Within plot	σ^2_w
<u>Across Trials (following Equation 2)</u>	
Clone	$\sigma^2_w + k_3 \sigma^2_{gb(t)} + k_4 \sigma^2_{gt} + k_5 \sigma^2_g$
Clone-trial	$\sigma^2_w + k_3 \sigma^2_{gb(t)} + k_4 \sigma^2_{gt}$
Clone-block (trial)	$\sigma^2_w + k_3 \sigma^2_{gb(t)}$
Within-plot	σ^2_w

Table 4. — Overall means (± standard deviations) for height (HT) and sectional area (SA) of ramets at two to three years in *E. globulus* Trials C316, C499, C795, C653 and C225.

Trial	Age	Survival (%)	HT (m)	SA (cm^2)
C316	3	77	6.7 ± 1.1	30 ± 14
C499	3	85	9.3 ± 1.0	63 ± 24
C795	3	75		50 ± 22
C653	2	87	2.6 ± 0.6	
C225	2	82	4.4 ± 0.7	

this sense the σ^2_c are appropriate for estimating genetic gains from selecting best clones at one site and then reforesting that particular site only with the selected clones. In the case of σ^2_g the effects due to clone-site interactions have been isolated into a separate σ^2_{gt} component (see expectations of mean squares, *Table 3*). The σ^2_g, and associated H^2_{Cp} in Equation 6, are appropriate for estimating gains from selecting best clones on average performance across sites and then reforestating a range of sites with selected clones.

Genetic Gains

Genetic gains from clonal selection were estimated as — (8)

$$\Delta G = i H^2_{C_p} \sigma^2_{P_{p(C)}}$$

where ΔG represents expected gain from selecting superior clones, i standardized selection intensity for infinite population size (Becker, 1985), H^2_{Cp} clonal heritability, and $\sigma^2_{Pp(C)}$ phenotypic standard deviation among clonal means. The $\sigma^2_{Pp(C)}$ is calculated as (Falconer, 1986, *Table 13.3*) — (9)

$$\sigma^2_{P_{p(C)}} = \sqrt{\frac{1+(n-1)t}{n}}\,\sigma^2_{P_p} \qquad (9)$$

Results and Discussion

Growth and Survival

Overall means for height (denoted HT) and sectional area (SA), and survival of ramets at two to three years after planting is presented in *table 4* for the five clonal trials studied. Survival ranged from 87% at two years in Trial C653 to 75% at three years in Trial C795. These values are somewhat lower than normally expected for E. *globulus* seedlings in Portugal (Borralho et al., 1992).

Among the three older clonal trials, SA growth was best for Trial C499 (with sectional area at age 3 year, SA3 = 63 cm^2), followed by Trial C795 (SA3 = 50 cm^2) and Trial C316 (SA3 = 30 cm^2). The slow initial growth at Trial C316 is probably a consequence of poorer soil fertility and low rainfall (*Table 1*). Of the younger trials, C225 is located on a reasonably good site for *E. globulus* and height growth and survival were good (4.4 m and 82%, respectively). This contrasts with the modest growth performance of trees, despite good survival, in Trial C653 at the same age (HT2 = 2.6 m, survival = 87%). Trial C653 is located in the interior east of Portugal on a marginal site for *E. globulus*, with long dry summers and several days of frost in the winter. For example, in January 1988 (nine months after planting) severe frost damage causing leaf and branch necrosis were observed in this trial.

Table 5. — Mean squares from analyses of variance of pooled data for sectional area (SA3) measured at three years across *E. globulus* Trials C316, C499 and C795.

Source of Variation	d.f.	M.S. (cm^2)
Clone	10	3721 **
Clone-trial	20	964 *
Clone-block (trial)	236	605 **
Within-plot	982	425

** $P < 0.005$
* $P < 0.05$

Clone-Site Interactions

Table 5 presents mean squares from analyses of variance of SA3 across Trials C316, C499 and C795. It is apparent that clonal effects and all interactions were significant at the 5% level. The mean squares for clonal effects and clone-block interactions were also significant for HT and SA traits in analysis of separate clonal trials (not reported here).

The fact that the clone-trial interaction is statistically significant across the three trials evaluated does not necessarily mean the interaction variance should be utilized in clonal forestry of *E. globulus* in Portugal. This point is better illustrated by studying mean SA3 values presented in *table 6* for individual clones across trials. Some clones, such as Clones A3, A21 and A22, show considerable changes in ranking across trials. However, other clones are either consistently good (*e.g.* A20) or consistently poor (A9) performers. These good general performing clones suggest that it is possible to select genotypes which are suitable for a wide range of site environments. Utilizing one population of good general performers has the obvious practical advantage of simplicity in breeding programs, and also in nursery management.

Bulmer (1980, Chapter 2) proposed the ratio $\sigma^2_g/(\sigma^2_g + \sigma^2_{gt})$ as a guide to the importance of genotype-environment interactions. The value of this ratio for the present study, 0.68, indicates that the variance due to σ^2_{gt} is reasonably substantial at about one-thirds the level of the clonal variance σ^2_g. However, this ratio can be misleading because it is unlikely that anywhere near the full level of σ^2_{gt} given in *table 5* could ever be utilized by selecting different clones of *E. globulus* for particular sites in Portugal. Unless, prior to planting, sites can be classified as suitable for particular clones (*i.e.* in statistical terms the site effects must be "fixed", not "random"; Matheson and

Table 6. — Mean sectional area (cm^2) and rankings (bracketed) of the 11 *E. globulus* clones represented in Trials C316, C499 and C795.

Clone	Trial		
	C316	C499	C795
A1	30 (7)	63 (4)	47 (8)
A2	29 (8)	59 (5)	53 (6)
A3	36 (2)	56 (8)	47 (9)
A4	31 (4)	58 (7)	59 (2)
A5	30 (6)	59 (6)	54 (5)
A7	27 (9)	67 (2)	56 (3)
A9	23 (11)	50 (10)	31 (11)
A17	35 (3)	54 (9)	48 (7)
A20	30 (5)	78 (1)	63 (1)
A21	22 (10)	64 (3)	54 (4)
A22	37 (1)	50 (11)	36 (10)

Table 7. — Clonal heritabilities (± standard errors) estimated on an individual tree (H^2_{Ii}) and clonal mean (H^2_{Ci}) basis for height (HT) and sectional area (SA) at two to three years in the five *E. globulus* clonal trials. Also presented are heritabilities estimated on an individual (H^2_{Ip}) and mean (H^2_{Cp}) basis from pooled analyses across Trials C316, C499 and C795.

Trial	H^2_I		H^2_C	
	HT	SA	HT	SA
Separate Clonal Trials (H^2_{Ii} and H^2_{Ci})				
C316	0.15±0.08	0.08±0.06	0.87	0.79
C499	0.15±0.08	0.11±0.06	0.88	0.86
C795				
C653	0.18±0.08		0.91	
C225	0.15±0.06		0.91	
Across Trials (H^2_{Ip} and H^2_{Cp})				
		0.06±0.05		0.88

COTTERILL, 1990), σ^2_{gt} cannot be utilized in breeding or clonal programs.

Many of the clone-trial interactions are due to changes in rankings of clones from Trial C316 to Trials C499 and C795 (*Table 6*). Trial C316 has, as already mentioned, different soil fertility and rainfall (*Table 1*). It is therefore tempting to classify Trial C316 as say a "Type-A" site, and Trials C499 and C795 as "Type-B" sites. However, it seems almost certain that there would be as much clone-trial interaction within site types (this is apparent by significant clone-block within site interactions, *Table 5*) as there are across these site classifications, as noted by MATHESON and COTTERILL (1990) for *Pinus radiata*. Uncontrollable or unpredictable environmental factors such as climate can vary as much from year to year at any particular site, as they vary between different site classifications. These factors make classification of sites as suitable or not for particular clones difficult (*i.e.* the site effects are actually more "random" than "fixed").

There are, of course, examples of environmental effects (such as altitude or latitude) which are dominant and can make it possible to classify sites reliably and objectively (RAYMOND and LINDGREN, 1986). However, in the case of *E. globulus* in Portugal such clear environmental effects do not seem to be present. It is, nevertheless, necessary to continue testing *E. globulus* clones across a range of sites in Portugal. These tests provide information on both average performance of genotypes and genetic parameters, and also help to assemble data which in future may allow some sites to be classified according to suitability for particular clones.

Heritabilities

Heritabilities estimated on individual tree and clonal mean basis are presented in *table 7*. The H^2_{Ci} values estimated following analyses of separate sites ranged from 0.87 to 0.91 for height and 0.79 to 0.86 for sectional area measured between two and three years. This trend for height to be somewhat more heritable than sectional area at early ages was also reported by BORRALHO *et al.* (1992) for progeny trials grown from seedlings of *E. globulus* in Portugal. The pooled clonal heritability for SA3 across clonal trials was a high $H^2_{Cp} = 0.88$. Although the pooled individual tree heritability H^2_{Ip} for SA3 is slightly lower than corresponding H^2_{Ii} for each individual trials, the H^2_{Cp} is higher than corresponding H^2_{Ci} because the value of n (number progeny per clone; Equation 5) is higher for pooled analyses.

The genetic gain which can be expected from selecting say the best one clone out of every 10 on across trial performance is 7.8 cm^2 (or 17% change in mean) for sectional area at around three years. The calculation is based on selection intensity i = 1.539 (for one in 10) and $H^2_{Cp} = 0.88$. This expected gain is high despite obvious silvicultural shortcomings of this present study such as holding clones for widely different periods in nursery. Recent improvements in propagation and nursery techniques for *E. globulus* should provide more uniformity within clones (Dr. P. J. WILSON, CELBI, personal communication) giving higher levels of H^2_C and, therefore, higher expected gains.

Conclusions

Clones of *E. globulus* propagated from stem cuttings exhibited highly significant clone-site interactions for growth across a range of sites in Portugal. However, some clones perform either consistently well or consistently poorly across sites. In practice it may be better to concentrate on selection for good general performance rather than attempting to classify sites in Portugal into different categories and develop specific clones for each site category. This question requires further investigation.

The high clonal mean heritability $H^2_{Cp} = 0.88$ for sectional area at there years can lead to expected gains of around 17% from selection of one in 10 clones on good general performance. Recent improvements in propagation and nursery techniques may considerably increase this heritability and expected genetic improvement.

Acknowledgements

The clonal Trials C316, C499 and C795 were established by Celulose Beira Industrial (CELBI) S. A., under supervision of BENGT CARLSSON and Trials C653 and C225 under supervision of MATS BRINDBERGS. We are grateful to Drs. D. G. NIKLES, D. L. ROCKWOOD, P. J. KANOWSKI and P. J. WILSON for helpful comments on earlier drafts and to Messrs. LEONEL SANTOS, JOAO ALEXANDRE SILVA and JOAO REIS for recent measurements of the trials. This

paper is part of CELBI's Project D95 and the senior author's postdoctoral studies at Oxford Forestry Institute.

Literature Cited

BECKER, W. A.: Manual Quantitative Genetics. Fourth Edition. Academic Enterprise, Pullmann, Washington, USA (1985). — BORRALHO, N. M. G., KANOWSKI, P. J. and COTTERILL, P. P.: Genetic control of growth of *Eucalyptus globulus* in Portugal. I. Genetic and phenotypic parameters. Silvae Genet. **41**: 39—45 (1992). — BULMER, M. G.,: The Mathematical Theory of Quantitative Genetics. Clarendon Press, Oxford (1980). — CAMERON, J. N., COTTERILL, P. P. and WHITEMAN, P. H.: Key elements of a breeding plan for temperate eucalypts in Australia. *In:* Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry. Proc. IUFRO Conf., Pattaya, Thailand. (GIBSON, G. L., GRIFFIN, A. R. and MATHESON, A. C., eds.). Oxford Forestry Institute, U. K. and Winrock Int., Arlington, Virginia, U. S. A., 159—168 (1989). — CAMPINHOS, E. and IKEMORI, Y. K.: Selection and management of the basic population of *Eucalyptus grandis* and *E.urophylla* established by Aracruz for the long term breeding programme. *In*: Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry. Proc., IUFRO Conf., Pattaya, Thailand. (GIBSON, G. L., GRIFFIN, A. R. and MATHESON, A. C., eds.). Oxford Forestry Institute, U. K. and Winrock Int., Arlington, Virginia, U. S. A., 169—175 (1989). — FALCONER, D. S.: Introduction to Quantitative Genetics. Second Edition. Longman, London (1986). — HARVEY, W. R.: Estimation of variance and covariance components in the mixed model. Biometrics **26**: 485—504 (1970). — HARVEY, W. R.: User's Guide for LSMLMW and MIXMDL, PC-2 Version. Mimeographed Paper, 89pp. Ohio State University, Columbus, Ohio (1990). — IKEMORI, Y. K.: Genetic Variation in Characteristics of *Eucalyptus grandis* (HILL) MAID. Raised from Micro-Propagation, Macro-Propagation and Seed. Unpublished D. Phil. Thesis, University of Oxford (1990). — KAGEYAMA, P. Y. and KIKUTI, P: Comparison between clones and open-pollinated progenies originating from a population of *Eucalyptus grandis* (HILL) MAIDEN in Brazil. *In*: Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry. Proc., IUFRO Conf., Pattaya, Thailand. (GIBSON, G. L., GRIFFIN, A. R. and MATHESON, A. C., eds.). Oxford Forestry Institute, U. K. and Winrock Int., Arlington, Virginia, U. S. A., 227—235 (1989). — LAMBETH, C. C. and ENDO, M.: Growth and quality of 460 clones of *Eucalyptus grandis* and gains over commercial seed lots. Forestry Research Report No. 2. Smurfit Group, Callahan, Florida, USA. 9pp. (1990). — LAMBETH, C. C., Lopez, J. L. and EASLEY, D. F.: An accelerated *Eucalyptus grandis* clonal tree improvement program for the Andes mountains of Colombia. *In*: Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry. Proc., IUFRO Conf., Pattaya, Thailand. (GIBSON, G. L., GRIFFIN, A. R. and MATHESON, A. C., eds.). Oxford Forestry Institute, U. K. and Winrock Int., Arlington, Virginia, U. S. A., 259—266 (1989). — MATHESON, A. C. and COTTERILL, P. P.: Utility of genotype x environment interactions. Forest Ecol. Manag. **30**: 159—174 (1990). — MIKKOLA, L.: Result from a trial of *Eucalyptus grandis* cuttings assessed after seven years. Zambia Forest Department Division of Forest Research. Research Note No. **29**: 1—12, (1982). — RAYMOND, C. A. and LINDGREN, D.: A model for genetic flexibility. *In*: Provenances and Forest Tree Breeding for High Latitudes. (Ed. D. LINDGREN). Proc. Franz Kemp Symp., 10 to 11 June 1986 Umea. Swedish Univ. Agric. Sci. Rep., 6, Umea, SWEDEN: 159—177 (1986). — SWINGER, L. A., HARVEY, W. R., EVERSON, D. O. and GREGORY, K. E.: The variance of intraclass correlation involving groups with one observation. Biometrics **20**: 818—826 (1964).

Allozyme Differentiation in the Genus Pinus

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(Received 15th August 1991)

Abstract

Needle samples from 17 species of pine taken from the collection of botanical gardens were analyzed at 11 enzyme loci. In all 57 alleles were found among 156 trees of the analyzed species, and only 7 of them were common to the two subgenera, *Pinus* and *Strobus*. The values of NEI's distance between species belonging to these subgenera vary from 1.42 to 2.66 (with average value 2.17) and are very great for intra-genus gene differentiation (maximal estimates of the distance in plants do not exceed 1.5). Large samples taken from natural populations of *Pinus sibirica* and *P. sylvestris* were analyzed at 22 loci to estimate bias in genetic distances influenced small samples from the botanical gardens.

Key words: subgenus *Pinus*, subgenus *Strobus*, alloyzme polymorphism, genetic divergence, genetic distance, phylogeny.

Introduction

The genus *Pinus* includes more than 100 species, more than any other gymnosperm. Pines are one of the main forest tree species and account for as much as 70% of all woodland areas. Rational management and usage of forests should be combined with measures aimed to preserve the biological diversity (NAMKOONG, 1983). One of these measures is the study of taxonomy and phylogenetic relationships of species. The systematics of pines has been extensively studied in this century and has been repeatedly reconstructed (MIROV, 1967). The classification of LITTLE and CRITCHFIELD (1969) is now widely recognized.

At the same time there are some problems in the taxonomy of pines. One needs to estimate genetic differentiation among pines, especially between two main subgenera, *Strobus* (white pines) and *Pinus* (hard pines). In addition, the taxonomic status of some species in the subsection *Sylvestris* needs to be verified (KORZUBOV and MURATOVA, 1986). Similar problems occur for the subsection *Contortae* (WHEELER et al., 1983; CRITCHFIELD, 1985).

The analysis of allozyme variability can allow an estimation of genetic differentiation among pine species, since the genetic variability of pine is high (MITTON, 1983). However, studies of allozyme variation in pine have dealt mainly with species of the subgenus *Pinus* (WHEELER et al., 1983; CONKLE et al., 1988; MILLAR et al., 1988; KARAMANGALA and NICKRENT, 1989). The other big subgenus, *Strobus*, is little studied (KRUTOVSKII et al., 1990). Therefore the aim of this study was to estimate genetic variability within the genus *Pinus*, especially to emphasize the genetic difference between these two subgenera.