large number of trees when they eventually start producing viable seeds.

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Genetics of Cunninghamia lanceolata Hook.

1. Genetic Analysis

By G. Müller-Starck and Y.-Q. Liu*)

Abteilung für Forstgenetik und Forstpflanzenzüchtung,
Universität Göttingen,
Büsgenweg 2, D-3400 Göttingen, Fed. Rep. Germany

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Summary

As a first step in the study of genetics of *Cunninghamia lanceolata,* the genetic control and the inheritance of the polymorphism revealed by gel electrophoresis for six enzyme systems is elucidated. By analysing endosperm and embryo of individual seeds from single maternal clones, genetic analyses are based on the segregation of enzyme phenotypes among the haploid female gametes and the association of phenotypes in diploid zygotes. The latter method turned out to be an efficient tool for inferring on the inheritance of the observed enzyme phenotypes where offspring from controlled crossings are not available. It is concluded that the six enzyme systems are coded by ten enzyme gene loci, which show a considerable number of codominant alleles in addition to the phenomenon of lacking enzyme activity ("null alleles"). Interactions between 26 pairs of gene loci were studied on the basis of gametic segregation among single tree progenies. Considerable variation with respect to the estimated recombination frequencies can be observed for the same pair of loci among some progenies. In such cases inferences on linkage should be drawn with care. Analogously to other coniferous species, linkage between GOT- and PGI-loci is evident.

Key words: Enzyme, gene marker, genetic control, inheritance, linkage, seed, *Cunninghamia lanceolata.*

Zusammenfassung

Als erster Schritt in den genetischen Untersuchungen an *Cunninghamia lanceolata* Hook. wurde die genetische Kontrolle und der Vererbungsmodus für den von 6 Enzymsystemen in der Gel-Elektrophorese gezeigten Polymorphismus nachgewiesen. Ausgehend von Untersuchungen an Endosperm und Embryo der Samen aus Nachkommenschaften einzelner Bäume, stützte sich die genetische Analyse auf die Aufspaltung der Enzym-Phänotypen zwischen den haploiden weiblichen Gameten und die Assoziation der Phänotypen in den diploiden Zygoten. Die zuletzt genannte Methode erwies sich als geeignetes Instrument zum Nachweis des Vererbungsmodus gegebener Enzym-Phänotypen für Fälle, in denen Nachkommenschaften aus kontrollierten Kreuzungen nicht zur Verfügung stehen. Es wird gefolgert, daß die 6 Enzymsysteme von 10 Genorten kodiert werden, welche eine beträchtliche Anzahl von kodominanten Allelen aber auch das Phänomen der fehlenden Enzymaktivität ("Nullallele") zeigen. Auf der Basis der gametischen Segregation innerhalb von Einzelbaum-Nachkommenschaften wurden Interaktionen für 26 Paare von Genorten untersucht. Zwischen einigen Nachkommenschaften besteht eine beträchtliche Variation der Schätzwerte für Rekombinationshäufigkeiten bezüglich derselben paarweisen Kombination von Genorten. In solchen Fällen sollten Rückschlüsse auf Kopplung mit Vorsicht gezogen werden. Analog zu anderen Nadelbaumarten zeigt sich Kopplung zwischen GOT- und PGI-Genorten.

Introduction

Cunninghamia lanceolata is a coniferous tree species which is widespread in the subtropical regions of the southern part of China. The economic importance of this species is substantial, due to its fast growth, the high quality of its timber and its multiple use potential. It constitutes approximately one-quarter of the total marketed timber production of China.

Cunninghamia lanceolata is known as a species which shows considerable variation in morphological characters and in growth (e.g. Ye Peizhang, Shen Xihuan, Chen Yuewu, pers. comm.). A genetic characterization of provenances of this species has not as yet been attempted, nor has, to our knowledge, any study dealing with environmentally independent markers been published. Studies in this field are in progress at the Beijing Forestry University (Shen, pers. comm.). The lack of studies concerns the whole family of *Taxodiaceae* — the only population genetic investigations by means of genetic markers in this family deal with *Cryptomeria japonica* (e.g. Sakai and Park, 1971) and *Sequoiadendron giganteum* (Fins and Libby, 1982).

*) Permanent address: Dept. of Forestry, Sichuan Agricultural University, Yaan, Sichuan, People's Rep. of China

As genetic markers, we used enzyme systems, since this type of marker has been proven by many authors to be highly efficient in monitoring genetic variation and other genetic parameters in forest trees (for survey see e.g. El-Kassaby and White, 1985). The aim of our study is to elucidate the genetic control of several enzyme systems in seeds of *Cunninghamia lanceolata* and thus to enable the identification of genotypes. This information will be used for an intra- and interpopulational genetic characterization of two provenances as well as for inferences on components of the reproductive system (Müller-Starck and Liu, in preparation).

Material and Methods

Seed Samples

Two categories of seed were analyzed: The first comprises seeds which were collected separately from each of 23 single clones for the purpose of elucidating the genetic control of selected enzyme systems. Particular attention was given to the offspring of seven selected clones, the sample sizes of which ranged between 36 and 60 seeds. The second category of seeds consists of material of two provenances, the one being located in the district of Hungya in west-central China and the other in the district of De-Chang in southwestern China. Both seed samples were drawn randomly from the respective mixtures of crops from several stands of *Cunninghamia lanceolata*. Clonal offspring was supplied by the Fujian College of Forestry in Nanpin, provenance seed samples by the Sichuan Agricultural University, Forestry Department, in Yaan.

Enzyme Systems

Genetic analysis was focussed on those enzyme systems which exhibited the greatest phenotypic variability and clearly visible enzyme phenotypes. The selected systems are: Glutamate dehydrogenase (GDH, E.C. 1.4.1.2), glutamate oxalacetate transaminase (GOT, E.C. 2.6.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25).

Electrophoretic Methods

Before the routine assays, it was proven whether or not the enzyme phenotypes in the gels (see subsequent topic) in fact refer to the enzyme systems under consideration: The respective enzyme substrates were omitted in one gel slice and the obtained phenotypes compared with those in the regularly stained second slice. The IDH and 6PGDH substrates reveal additional unspecific enzyme phenotypes ("nothing dehydrogenases", Shaw and Koehn, 1964), which are not included in this study.

Seeds were pretreated by a 24-hour soaking in water Endosperm and embryo were homogenized separately in a 0.08 M tris — 1.00 M HCl buffer pH 7.3 with the addition of selected agents from Rhodes (1977) (see Müller-Starck, 1982). The enzymes were separated by horizontal starch gel electrophoresis by applying the following buffer systems (electrode buffer/gel buffer): 0.06 M NaOH — 0.30 M boric acid pH 8.2/0.07 M tris — 1.00 HCl pH 8.7 (Poulik, 1957, modified) for GDH and GOT; 0.135 M tris — 0.045 M citric acid pH 7.0/0.038 M tris — 0.013 M citric acid pH. 7.0 (Shaw and Prasad, 1970, modified) for IDH, 6PGDH, and for PGI, SKDH with pH 6.5 in both buffer systems. Gel-concentration was 11%, voltage distribution 20 to 30 mV/cm, bridge distance 12 cm. Staining solutions were slight modifications (Bergmann, 1974, and pers. comm.; Müller-Starck, unpubl.) from Henderson, 1965 (IDH), Shaw and Koehn, 1968 (GDH), Brewer, 1970 (6PGDH, PGI), Siciliano and Shaw, 1976 (GOT), Linhart *et al.*, 1981 (SKDH).

In electrophoresis, starch gel was preferred to polyacrylamide gel, because the former method is less hazardous for health and could be proven to result in a similar or even better visualization of enzyme phenotypes than the latter method (horizontal polyacrylamide gel electrophoresis with a 5.5% stacking gel (1 cm) and a 8% separation gel (8 cm) with pH-values ranging between 7.7 and 8.9).

Genetic Analysis

Identification of enzyme gene loci and the corresponding allelic polymorphism was based on the segregation of enzyme phenotypes among the haploid tissue of the endosperm (macrogametophyte) of individual seeds from single clones (each seed of a clonal seed lot could be supposed to originate from the same maternal genotype).

Because offspring from controlled crossings were not available, the interaction of alleles in the diploid tissue could only be studied by means of the enzyme phenotypes in the embryos of the respective clonal seed lots. This was done in combination with the endosperm analysis by a separate preparation of each tissue from each individual seed. Thus allowing the female and the male contribution to each embryo to be detected as an ordered pair (Müller (-Starck, 1976).

For linkage analysis, the observed two-locus segregation among endosperms of double heterozygous maternal clones were compared statistically with the expected segregations by means of the χ^2-test of goodnees of fit and the log likelihood ratio test (G-test) of heterogeneity in contingency tables. By this, uniformity of the frequency distribution of the respective four gametic types and of the segregation within each gene locus was tested. Because the parental genotypes of each maternal clone were unknown, there were two possibilities to assign the gametic types to the recombinant and the non-recombinant pair. In case of deviating frequencies between the pairs the less frequent was considered to contain the recombinant types, the more frequent one the non-recombinants. Uniform pairwise segregation was tested by means of χ^2- and G-tests. The recombination frequency was estimated by means of a maximum likelihood estimation resulting from the use of the "folded binomial" probability function (Nordheim *et al.*, 1983).

Results and Discussion

Survey of Enzyme Phenotypes

The enzyme systems of GDH and PGI showed at most one band in the zymograms of the endosperms, 6PGDH and SKDH two bands, and GOT three bands. The bands in the latter three systems appeared in various combinations as endospermic and as embryonic phenotypes within certain sections of the gel and are considered for the present as "independent" from each other. The respective zones in the zymograms are designated by capital letters (A, B, C). The above also holds for the IDH system, but the enzyme polymorphism in the faster of the two zones had to be excluded from further interpretation because of insufficient enzyme activity.

The array of enzyme phenotypes, one of which was detectable in the endosperm and the embryo of seeds from the maternal clones and the two provenance samples, is illustrated schematically in *Figure 1*. This also includes

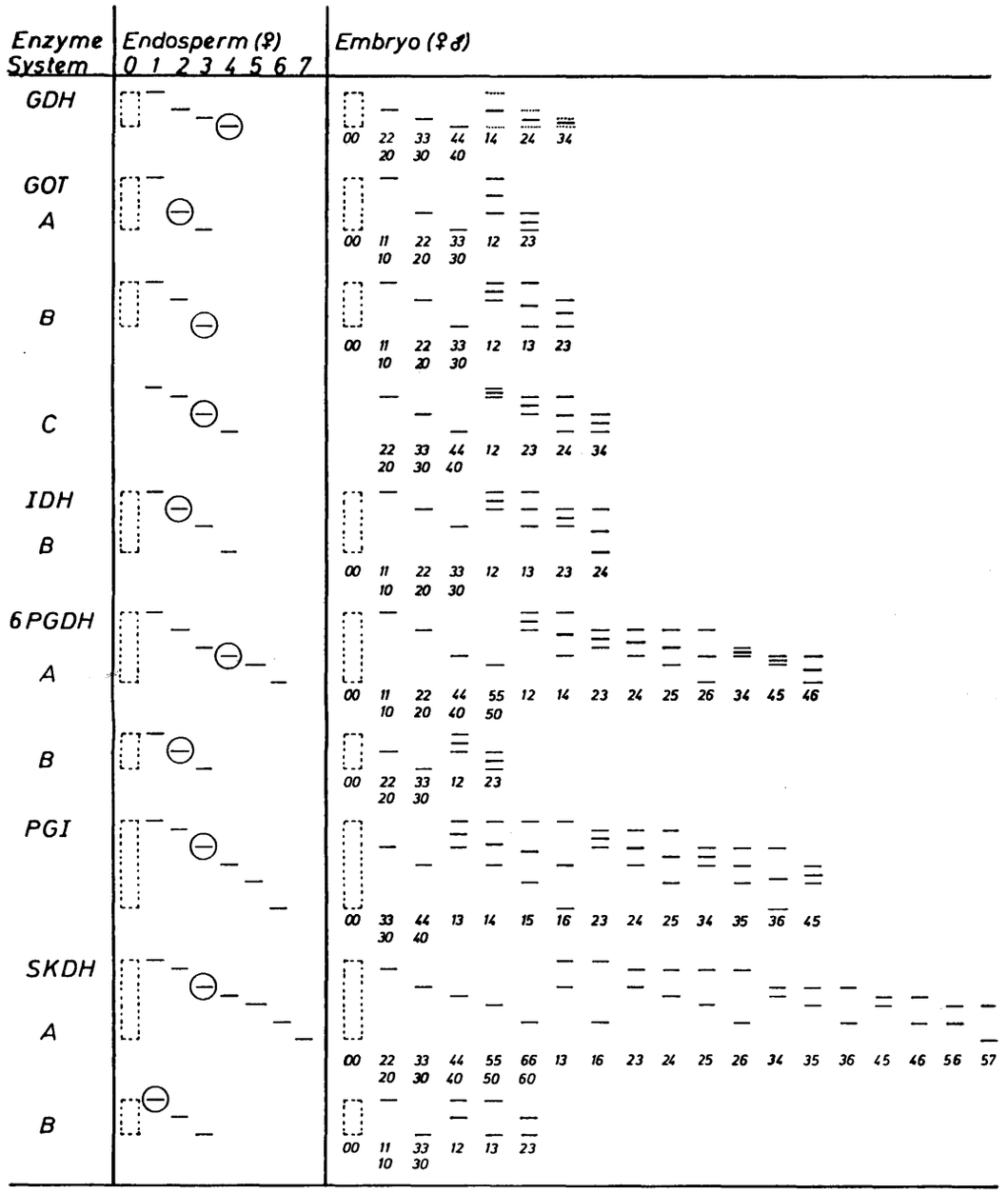


Figure 1. — Array of enzyme phenotypes in endosperm and embryo from seven maternal clones and two provenance samples. The polymorphism is illustrated independently for each of the two seed tissues and separately for each enzyme system or each zone (A, B, C) with apparently independent variation of enzyme phenotypes. Ordering of zones and intrazonal phenotypes is proportional to the anionic relative mobility. The only overlap occurs in GOT: B_1 is located between A_2 and A_3. Dotted frames indicate lacking enzyme activity, and circles mark phenotypes with a frequency equal to or greater than 50% in provenance samples. Designation of embryonic phenotypes is unordered.

cases in which enzyme activity is lacking in one or both tissues. Discrimination between a lack of activity due to technical biases and the phenotypic expression *per se* is possible, even in the case of enzyme systems with only one zone in the zymogram: Such systems are combined with those with bi- or tri-zonal zymograms by horizontal slicing of the gel and simultaneous staining (e.g. GDH-GOT, PGI-SKDH), so that only phenotypes with lacking activity in all of the respective zones may be misinterpreted as being due to technical deficiency.

Single-banded phenotypes in the embryo may reflect either identical contributions from the endosperm and the pollen or, in the case of lacking enzyme activity in the pollen, different contributions. (Entire absence of pollen cannot result in seed formation in *Cunninghamia*). This becomes evident when embryos are found which entirely lack enzyme activity in at least one zone of the zymogram.

The observed combinations in the embryos of the endosperm enzyme phenotypes seem to fulfill the requirements for a one-to-one correspondence between zonal enzyme polymorphism and controlling gene locus. In the case of the SKDH-system, the variety of phenotypes in the diploid embryos can be explained without exception as simple combinations of the single bands appearing in the haploid tissue, i.e. the endosperm. The same holds for the other enzyme systems, but there are additional bands with greater

Table 1. — **Enzyme phenotypes of the maternal clones 4, 5, 8, 12, 13, 21, 22 and their offspring: A test of the segregation among the endosperms and between such pairs of corresponding embryonic phenotypes which both contain the most frequent pollen phenotype. ($♀_i♂_{max}$, $♀_j♂_{max}$ for $i \neq j$). For further explanations see article. Phenotypes with identical indices may in fact represent two different types, if one type refers to lacking enzyme activity.**

Enzyme System	Maternal Clones: Designation	Maternal Clones: Enzyme Phenot.	Endosperm($♀_i : ♀_j$)	χ^2 (1)	Pollen Phenot. ($♂_k$)	Segregation among the offspring: $♀_i♂_{max} : ♀_j♂_{max}$	χ^2 (1)
GDH	4,5,8, 12,13, 21,22	A_4A_4	Uniformity A_4		A_2, A_4		
GOT	12	A_2A_3	$A_2 : A_3 = 27 : 33$	0.60 ns	A_2, A_3	$A_2A_2 : A_3A_2 = 15 : 17$	0.13 ns
	13	B_1B_2	$B_1 : B_2 = 28 : 19$	1.72 ns	B_1, B_3	$B_1B_3 : B_2B_3 = 16 : 11$	0.93 ns
	22	B_1B_3	$B_1 : B_3 = 17 : 19$	0.11 ns	B_1, B_3	$B_1B_3 : B_3B_3 = 9 : 13$	0.73 ns
	12	B_2B_3	$B_2 : B_3 = 32 : 28$	0.27 ns	B_1, B_2, B_3	$B_2B_3 : B_3B_3 = 25 : 13$	3.79 ns
	5	C_2C_3	$C_2 : C_3 = 20 : 22$	0.10 ns	C_1, C_2, C_3	$C_2C_3 : C_3C_3 = 14 : 17$	0.29 ns
	13	C_2C_3	$C_2 : C_3 = 22 : 25$	0.19 ns	C_2, C_3, C_4	$C_2C_2 : C_3C_3 = 18 : 12$	1.20 ns
	22	C_2C_3	$C_2 : C_3 = 16 : 20$	0.44 ns	C_2, C_3, C_4	$C_2C_2 : C_3C_2 = 10 : 8$	0.22 ns
	12	C_3C_4	$C_3 : C_4 = 36 : 24$	2.40 ns	C_2, C_3	$C_3C_3 : C_4C_3 = 24 : 19$	0.82 ns
IDH	12	B_2B_3	$B_2 : B_3 = 32 : 28$	0.27 ns	B_1, B_2, B_3	$B_2B_2 : B_3B_2 = 31 : 23$	1.19 ns
PGI	12	A_1A_3	$A_1 : A_3 = 25 : 35$	1.67 ns	A_1, A_3, A_4, A_5, A_6	$A_3A_3 : A_4A_3 = 11 : 14$	0.36 ns
	4	A_3A_4	$A_3 : A_4 = 25 : 23$	0.08 ns	A_3, A_4, A_6	$A_3A_4 : A_4A_4 = 24 : 14$	2.63 ns
	8	A_3A_4	$A_3 : A_4 = 21 : 16$	0.68 ns	A_3, A_4, A_5, A_6	$A_3A_4 : A_4A_4 = 13 : 14$	0.04 ns
	21	A_3A_4	$A_3 : A_4 = 14 : 22$	1.78 ns	A_1, A_2, A_3, A_4, A_5	$A_3A_1 : A_4A_1 = 5 : 13$	3.56 ns
	13	A_3A_5	$A_3 : A_5 = 18 : 29$	2.57 ns	A_1, A_2, A_3	$A_3A_1 : A_5A_1 = 8 : 13$	1.19 ns
6PGDH	4	A_2A_4	$A_2 : A_4 = 26 : 33$	0.19 ns	A_2, A_3, A_4, A_6	$A_2A_4 : A_4A_4 = 16 : 17$	0.03 ns
	5	A_2A_4	$A_2 : A_4 = 16 : 26$	2.38 ns	A_2, A_4, A_5, A_6	$A_2A_2 : A_4A_2 = 10 : 13$	0.39 ns
	8	A_2A_4	$A_2 : A_4 = 21 : 16$	0.68 ns	A_2, A_3, A_4, A_6	$A_2A_4 : A_4A_4 = 14 : 12$	0.15 ns
	13	A_2A_4	$A_2 : A_4 = 26 : 21$	0.53 ns	A_1, A_2, A_4	$A_2A_4 : A_4A_4 = 17 : 12$	0.86 ns
	22	A_2A_4	$A_2 : A_4 = 21 : 15$	1.00 ns	A_1, A_2, A_4, A_5, A_6	$A_2A_4 : A_4A_4 = 12 : 10$	0.18 ns
	5	B_2B_3	$B_2 : B_3 = 21 : 21$	0.00 ns	B_1, B_2, B_3	$B_2B_2 : B_3B_2 = 19 : 21$	0.10 ns
	21	B_2B_3	$B_2 : B_3 = 15 : 23$	1.68 ns	B_2, B_3	$B_2B_2 : B_3B_2 = 14 : 22$	1.78 ns
SKDH	8	A_2A_5	$A_2 : A_5 = 16 : 21$	0.68 ns	A_2, A_3, A_4, A_5, A_6	$A_2A_3 : A_5A_3 = 6 : 15$	3.86 *
	4	A_3A_5	$A_3 : A_5 = 22 : 26$	0.33 ns	A_2, A_3, A_4, A_5, A_6	$A_3A_3 : A_5A_3 = 16 : 20$	0.44 ns
	5	A_3A_5	$A_3 : A_5 = 21 : 21$	0.00 ns	A_1, A_3, A_4, A_5	$A_3A_3 : A_5A_3 = 13 : 5$	3.56 ns
	21	A_3A_5	$A_3 : A_5 = 20 : 16$	0.44 ns	A_1, A_3, A_5, A_6, A_7	$A_3A_3 : A_5A_3 = 16 : 11$	0.93 ns
	22	A_3A_6	$A_3 : A_6 = 18 : 16$	0.12 ns	A_1, A_3, A_4, A_5, A_6	$A_3A_3 : A_6A_3 = 9 : 7$	0.25 ns
	13	A_5A_6	$A_5 : A_6 = 24 : 23$	0.02 ns	A_1, A_3, A_4, A_5, A_6	$A_5A_3 : A_6A_3 = 18 : 11$	1.69 ns
	5	B_1B_3	$B_1 : B_3 = 19 : 23$	0.38 ns	B_1, B_3	$B_1B_3 : B_3B_3 = 19 : 18$	0.03 ns
	21	B_1B_3	$B_1 : B_3 = 17 : 19$	0.11 ns	B_1, B_3	$B_1B_3 : B_3B_3 = 14 : 16$	0.13 ns

(1) χ^2-test of goodness of fit; * significance level 0:05; ns not significant

staining intensity in the hybrid position. These facts support the assumption of a monomeric enzyme structure for SKDH and a dimeric structure for GDH, GOT, IDH, 6PGDH, and PGI.

Segregation among Gametes

Segregation was studied among the endosperms of seeds from each of seven genotypes ("maternal clones"). The haploid endosperm originates from the macrogametophyte and represents the female gamete which is obtained also as successful gametic contribution to the embryo. The results of these endosperm analyses are summarized in *Table 1*: For a better overview, the phenotypes of the maternal clones are always written first, although they were not detected directly in diploid tissue but were inferred from the gametophytic phenotypes of the endosperm of the respective seed lot. For results dealing with the corresponding embryos see subsequent topic.

In all of the seed lots from maternal clones and in each zone, either uniformity or only two alternating phenotypes were always found in the endosperm. With the exception of GDH, all enzyme systems show segregation among the endosperms of at least one maternal clone. The remaining cases of 29 segregations were all compared statistically with the expected one-to-one ratio according to the Mendelian mode of genetic control. As can be seen from *Table 1*, none of these deviate at the 5% significance level from the expectation.

This result does not refute the hypothesis of one-locus control for each of the segregating enzyme systems and zones (for a test of linkage between loci see separate topic). This may also be true for GDH, but this cannot yet be verified. Although the procedure applied here is commonly accepted as sufficient proof of the one-locus genetic control, several questions are still open:

(1) Not all of the endospermic phenotypes given in *Figure 1* are represented in *Table 1*, and neither are all possible combinations between them. Only in GOT-B are all possible phenotypes and all combinations realized, whereas the other extreme is represented by 6PGDH-A with only two out of six visible phenotypes. Lacking enzyme activity was not found in any endosperm of the seven seed lots.

(2) The statement of one-locus control for each enzyme system or zone with apparently independent segregation can be upheld on the grounds that the results do not contradict it. Nevertheless, it cannot be ruled out that other gene loci share in the genetic control of the detected enzyme polymorphism. However, the statement of "one gene locus control" is considered as the

simplest among a multitude of hypotheses which are not refuted by the results, since the results do not permit discrimination between them.

In this sense, the one gene locus control is indicated for each of the polymorphic zones, although it is verified here exemplarily only with respect to a certain part of that polymorphism. Therefore, it can be stated that, at the least, each of the segregating phenotypes in the endosperm represents an allele at one of the following gene loci: GOT-A, GOT-B, GOT-C, IDH-B, 6PGDH-A, 6PGDH-B, PGI-A, SKDH-A, and SKDH-B. For the present, there is no indication that this statement may not hold for additional phenotypes which are represented among the endosperms of the two provenance samples but not among the seven maternal clones.

The genetically controlled enzyme phenotypes in the haploid gametophytes allow the identification of the respective diploid maternal clone. For simplicity, the designations of clonal phenotypes in *Table 1* correspond to the clonal genotypes. The genetic control of the segregation among the female gametes (represented by the endosperms) should result in a corresponding segregation among the male gametes (represented by the viable pollen), the successful proportion of which is traceable as the male contribution to an embryo in the case of unspecific expression of enzyme phenotypes in these tissues.

Segregation among Zygotes

The interaction of alleles in diploid tissue could be studied here only by identifying the female and the male gametic contributions to the embryos of the maternal clones. These clones belong to different populations, so that any simultaneous testing of the composition of the successful male gametophytic contributions to each clonal set of embryos is not feasible,

The presumable phenotypes of the successful male gametophytes ("pollen phenotypes" in *Table 1*) result from the analyses of endosperm and corresponding embryo of those seeds which were utilized for the study of the segregation among endosperms. This implies the assumption of a tissue-unspecific expression of the female and male contribution in the embryo. The numerical designation of phenotypes refers to *Figure 1*. As can be seen in *Table 1*, in most cases the assumed male gametophytic phenotypes show a larger variation than the identified female ones. This trend is especially pronounced at those gene loci which reveal more than average enzyme polymorphism (6PGDH-A, PGI-A, SKDH-A — see *Figure 1*). The GDH system appeared to be monomorphic in the female gametophyte but show variation among the male gametophytes. This extension of the polymorphism could easily be explained by the greater genetic heterogeneity of the pollen pool, since the samples do not include all potential paternal clones.

In most cases, the additional phenotypes of the male contributions to the embryos occur in combination with each of the two maternal alleles which were identified alternatively in the corresponding endosperms. This is true for all frequently appearing additional phenotypes in the embryo. Only rare ones which occur in three or fewer of the embryos of the maternal seed set were found in combination with only one of the alleles in the endosperm. A testing of the observed segregation among the embryonic phenotypes is based on the following assumptions:

Firstly, the one-to-one segregation detected among the female gametophytes also holds for the male gametophytes, and secondly, there is no preferential fusion between a certain pollen type and one of the female gametophytic types of the respective maternal clone. Finally, the viability of the resulting zygotes should be the same up to the stage of census.

In *Table 1*, the statistical comparison is focussed on those presumable pollen phenotypes, denoted by δ_{max}, which occur most frequently as additional types in embryo. Thus the ratio $♀_i\delta_{max} : ♀_j\delta_{max}$ for the maternal clonal genotype ij ($i \neq j$) is compared to the expected ratio of 1 : 1. The resulting X^2-values indicate deviations at the 5% significance level from the one-to-one ratio only in the case of SKDH-A for clone 8 and deviations close to this level in three other cases (GOT-B for clone 12, PGI-A for clone 21, SKDH-A for clone 5). These are explainable, if it is taken into consideration that five to ten percent of the pregerminated seeds had to be excluded from electrophoretic analysis because they appeared to be mouldy. Nevertheless, in all remaining cases there is no indication that the most frequent presumable pollen phenotype does not occur equally often in combination with each of the two female gametophytes. This is also true for the pollen phenotypes with lower frequencies among the embryos (occurrence in at least five embryos), but the statistical test results could not be included in *Table 1*.

In summary, it can be stated that the revealed allelic polymorphism in the endosperms corresponds to the embryonic phenotypes, since one of the alleles is always contained in the embryo. There is much evidence that the additional phenotypes in the embryo (including the stronger staining intensity in the case of identical phenotypes in endosperm and corresponding embryo) represent the alleles of the respective pollen contribution. This assumption is supported not only by the results of the segregation test but also by the fact that, in all cases, explainable combinations of phenotypes were obtained. The higher variation among the assumed male contributions to the embryo also confirm the expectation. In none of the embryos were any phenotypes observed which were incompatible with the diploid state of this tissue.

Consequently, for the present, the obtained phenotypes in the embryo are considered to represent the alleles originating from a female and a male gametophyte and as such can be designated as an ordered pair. This statement holds for the gene loci GOT-A, GOT-B, GOT-C, IDH-B, 6PGDH-A, 6PGDH-B, PGI-A, SKDH-A, and SKDH-B.

The obtained embryonic types do not indicate interactions among the alleles which are represented by visible enzyme phenotypes: The monitored alleles at each of the studied gene loci appear to be codominant. The lacking activity in endosperm and in some cases also in the corresponding embryo of the seeds from provenance samples (Müller-Starck and Liu, in preparation) is conform with a recessive allelic state ("null allele"), but this could not be included in the genetic analyses.

To simplify the nomenclature, the designation of enzyme phenotypes were retained for the enzyme genotypes.

Interactions between Gene Loci

Out of the 36 possible two-locus combinations which can be formed from the nine polymorphic gene loci (GDH-A excluded), 26 double heterozygous pairs of loci were represented by at least one of the studied maternal clones. As can be seen from the upper right half of *Table 2*, the missing pairs mostly concern the gene loci GOT-A and IDH-B. Half of the 26 realized combinations were found in only one maternal clone, the remaining 13 pairs in two to five

Table 2. — Number of single tree progenies per two-locus combination (upper right half) and results of a statistical testing of the pairwise segregation between recombinant and non-recombinant female gametic types (lower left half; ns = not significant, * = significant at least at 5% level in at least one progeny).

	GOT			*IDH*	*PGI*	*6PGDH*		*SKDH*	
	A	*B*	*C*	*B*	*A*	*A*	*B*	*A*	*B*
GOT-A		1	1	1	1	–	–	–	–
-B	*		3	1	2	2	–	2	–
-C	ns	ns		1	2	3	1	3	1
IDH-B	ns	ns	*		1	–	–	–	–
PGI-A	*	*	ns	ns		3	1	4	1
6PGDH-A	–	ns	ns	–	*		1	5	1
-B	–	–	ns	–	ns	ns		2	2
SKDH-A	–	ns	ns	–	*	*	ns		2
-B	–	–	ns	–	ns	ns	ns	ns	

Table 3. — Segregation among gametic types at two loci *A*, *B* and a testing of the frequency distribution and of the recombinant vs. the non-recombinant pair of genotypes (for designation and intralocus segregation see *Table 1*). Recombination frequency $\hat{\Theta}$ (maximum likelihood) is estimated separately for each single tree progeny.

Pair of gene loci	Design. matern. clone	Frequency of two-locus types $\binom{A_i}{B_i}$	$\binom{A_i}{B_j}$	$\binom{A_j}{B_i}$	$\binom{A_j}{B_j}$	All two-locus types χ^2	Recomb. vs. non-recomb. χ^2	Recomb. frequ. $\hat{\Theta}$
GOT-A/GOT-B	12	26	1	1	32	[1])25.20***	60.00***	0.033
GOT-A/PGI-A	12	15	2	7	14	11.90**	10.53**	0.237
GOT-B/PGI-A	12	13	7	3	15	9.60*	8.53**	0.263
	13	23	4	6	12	19.45***	13.89***	0.222
GOT-C/IDH-B	12	22	10	4	14	13.68**	9.68**	0.280
PGI-A/6PGDH-A	4	25	0	1	22	[1])21.38***	44.08***	0.021
	8	12	9	9	7	1.38	0.27	0.500
	13	14	3	12	17	9.48*	5.57*	0.326
PGI-A/SKDH-A	4	18	7	8	15	7.17	6.75**	0.313
	8	14	7	7	9	3.54	2.19	0.381
	13	10	7	13	16	3.91	0.78	0.500
	21	9	5	7	15	6.22	4.00*	0.333
6GPDH-A/SKDH-A	4	19	7	7	15	9.00*	8.33**	0.292
	5	11	5	10	16	5.81	3.43	0.357
	8	13	8	8	8	2.03	0.68	0.500
	13	17	9	6	14	6.35	5.57*	0.326
	22	10	10	6	8	1.29	0.12	0.500

Significance levels 0.05 (*); 0.01 (**); 0.001 (***)
1) pooled

clones. The total number of genotyped clonal progenies is seven (clones No. 4, 5, 8, 12, 13, 21, 22). The number of studied endosperms per progeny is the same as given *Table 1* or is smaller in several cases because of blurryness of enzyme phenotypes which occurred non-simultaneously in the respective pair of gene loci (see also *Table 3*).

For clarity, the results of linkage tests are given comprehensively in the lower left half of *Table 2*. The gametic segregations at those pairs of gene loci which revealed statistically significant deviations are presented in *Table 3*. In this table only the results of a testing of the frequency distribution of the four two-locus gametic types and of the recombinant and the non-recombinant pair are included. The test of the segregation within each gene locus did not reveal any significant deviations (see *Table 1*).

An additional calculation of G-values proved both statistical methods, i.e. the χ^2-test and the G-test, to result in similar values. In most cases, the χ^2-values were smaller than the G-values but indicate deviations at the same significance level, e.g. 25.20*** vs. 34.51***, 60.00*** vs. 83.18***, 11.90** vs. 14.05**, 10.53** vs. 11.08***, 9.60* vs. 10.67*, 8.53** vs. 8.88** (values for the first three lines in *Table 3*). For each progeny, the testing of the two hypotheses revealed small deviations with respect to the χ^2-or the G-values. Consequently, the greatest part of the non-uniformity of the two-locus frequency distribution of gametes traces back to the distorted segregation between recombinant and non-recombinant types.

The applied maximum likelihood estimation ($\hat{\Theta}_1$ according to Nordheim *et al.*, 1983) is equal to the quotient of the number of recombinants to the total number in all cases with statistically significant deviations between the frequencies of the recombinant and the non-recombinant pair. In cases with low χ^2-values, the used estimation method results in $\hat{\Theta}$-values of 0.5. Cases with χ^2-values close to the significant level may nevertheless indicate non-random recombination (clone 8 for PGI-A/SKDH-A and clone 5 for 6PGDH-A/SKDH-A).

Outstandingly low estimates for the recombination frequency are obtained in the case of clone 12 for the pair GOT-A/GOT-B and of clone 4 for PGI-A/6PGDH-A (values between 0.021 and 0.033). But also in the case of the remaining pairs of loci, results mostly suggest non-random segregation of gametic types due to linkage. The variation of those estimates between trees could be studied only with respect to four pairs of loci.

As can be seen from *Table 3*, the combination GOT-B/PGI-A show very similar recombination frequencies in clone 12 and clone 13 (0.263 vs. 0.222), while particularly PGI-A/6PGDH-A but also PGI-A/SKDH-A and 6PGDH-A/SKDH-A reflect a great variety of estimates. For each of these combinations, the given frequency distributions of two-locus gametic types were compared statistically between all pairs of clonal progenies. There are no significant deviations exept in the case of PGI-A/6PGDH-A with respect to the frequency distributions of clone 4 vs. clone 8 and 4 vs. 13. In the first case, the ratio of recombinants to non-recombinants is 1:47 for clone 4 and 18:19 for clone 8.

These deviations between single tree progenies with respect to the same pair of loci can no longer be discussed exclusively on the basis of non-random sampling. The inter-individual variation in recombination frequency may be caused by genetic effects (e.g. modifer genes, gametic selection) or possibly by various environmental impacts (e.g. temperature, see Rudin and Ekberg, 1978). There is not much evidence that this phenomenon might be a consequence of the different clonal genotypes at the same pair of loci. For instance, there are two clones with identical two-locus genotypes, only one of which shows statistically significant deviations in the gametic segregation (6PGDH-A/SKDH-A, clones 4 and 5), and two clones with different genotype which show very similar results (GOT-B/PGI-A, clones 12 and 13). It has to be kept in mind that even in the case of two-locus identity there are still two possibilities for the arrangement of gametic types in pairs of recombinants and non-recombinants. If different alleles are involved, up to four different kinds of such arrangements can occur. Anyway, in the present study, the available data do not allow detailed interpretations of the discribed inter-individual variation in recombination frequencies. This phenomenon has also been reported by several authors, especially in the case of pine species (e.g. Rudin and Ekberg, 1978; O'Malley *et al.*, 1986; Strauss and Conkle, 1986), although the observed deviations were less pronounced.

For the present the estimated recombination frequencies should primarily be related to the individual level. Therefore we did not pool the recombination data. Having in mind that inferences on linkage should be drawn with care, the assumption is supported that the gene loci GOT-A,

GOT-B, PGI-A are located on the same chromosome. This is in accordance with the results of linkage studies in seven pine species, and in *Abies balsamea, Pseudotsuga menziesii,* and *Picea glauca* (for review see STRAUSS and CONKLE, 1986). It cannot be ruled out that 6PGDH-A is also located on this particular chromosome. The same hold can hold for GOT-C and IDH-B.

Conclusions

The genetic analysis could not be based on offspring from controlled crossings. However, the study of the offspring from single maternal clones allowed verification of the segregation among the female gametes and elucidation of the genetic control for nine gene loci. In addition, there was evidence for another gene locus (GDH-A), which did not reflect polymorphism among the studied endosperms. The analysis of the corresponding embryos turned out to be an efficient tool in supplying essential information on the inheritance of the observed enzyme phenotypes. Unfortunately, not all phenotypes known from provenance samples were present among the maternal clones.

The observed enzyme phenotypes among the male gametic contributions to the embryos also suggest the inclusion of GDH-A among the identified gene loci. No evidence could be found against this hypothesis nor against those concerning the other eight loci among the observed combinations of enzyme phenotypes with the embryos of the two provenance samples.

Consequently, the following statements on the genetic control and inheritance of enzyme phenotypes in *Cunninghamia lanceolata* can be made for the present:

Glutamate dehydrogenase (GDH):

Dimeric enzyme system which is coded by one gene locus (GDH-A) with four codominant alleles in addition to the state of lacking enzyme activity ("null allele") which supposedly interacts recessively (preliminary statement). Only one GDH-locus was also observed in various other coniferous species (e.g. CHUNG, 1981; CONKLE, 1981; SHEN, 1981; YEH, 1981; ALLENDORF *et al.*,1982; GURIES and LEDIG, 1982; MILLAR, 1988).

Glutamate oxalacetate transaminase (GOT)

Dimeric enzyme system coded by three gene loci with three codominant alleles each in the case of GOT-A and GOT-B and four at GOT-C. Additionally, the loci GOT-A and GOT-B reveal "null alleles" in endosperm and embryo tissue which appear to interact recessively.

Isocitrate dehydrogenase (IDH)

Dimeric enzyme system coded by at least one gene locus (IDH-B) with four codominant alleles and "null alleles" in endosperm and embryo. There is evidence for another gene locus (IDH-A), the enzyme polymorphism of which is coded independently of IDH-B, but it had to be excluded from further interpretations because of ambiguities due to faint staining and inferences by artifacts.

6 Phosphogluconate dehydrogenase (6PGDH)

Dimeric enzyme system coded by two gene loci with six codominant alleles at 6PGDH-A and three at 6PGDH-B. In addition, "null alleles" are apparent at 6PGDH-A and B in endosperm and embryo tissue. Artifacts are visible in all zymograms, but with faint staining intensity, and are easy to distinguish from the original bands.

Phosphoglucose isomerase (PGI)

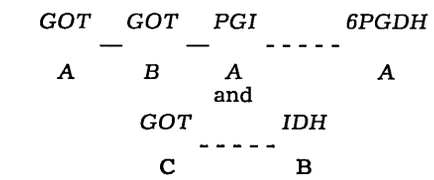
Dimeric enzyme system coded by one gene locus (PGI-A) with six codominant alleles. Also in this system, "null alleles" are obtained in the endosperm and embryo tissue. The statement on artifacts in 6PGDH also holds for PGI.

Shikimate dehydrogenase (SKDH)

The only monomeric system among those studies is coded by two gene loci with seven codominant alleles at SKDH-A and three at SKDH-B. The polymorphism at SKDH-B is generally less intensively stained than at SKDH-A. "Null alleles" are observed at SKDH-B in both endosperm and embryo tissue. The existence of the second gene locus (SKDH-B) was monitored also in other coniferous species (e.g. FINESCHI, 1984; GURIES and LEDIG, 1982; NEALE *et al.*, 1984; SZMIDT and YAZDANI, 1984; MÜLLER-STARCK, 1985).

With the exception of GOT-C, at all loci "null alleles" were observed, although they occur in the studied provenance samples in very low frequencies (mostly around 1%, only in SKDH up to 5.6%, MÜLLER-STARCK and LIU, in preparation). Unfortunately, there was no lacking enzyme activity among the maternal clones, so that unequivocal clarification of the interitance of this phenomenon has to remain open. However, studies on the inheritance of such "null alleles" in other coniferous species suggest the above-mentioned recessive allelic status (e.g. ALLENDORF *et al.*, 1982).

The study of *recombination* frequencies is preliminary in the sense that nor all pairs of loci are represented and that the number of studied progenies as well as the sample sizes are fairly small. All data refer to the recombination among the female gametes, which must not necessarily be identical to that among the males (e.g. MORAN *et al.*, 1983). The inter-individual variation of recombination frequencies suggest that one differentiate between recombination at the individual and at the species level (possibly also at the population level). In some cases linkage might be feigned or exist in reality but be compensated by genetic or environmental impacts as pointed out before. Generally, conclusions from genetic two-locus segregations on linkage should be drawn with care in the case of substancial individual variation between the ratio of the assumed recombinants to the non-recombinants. This concerns some pairs of loci in the present study. Surveying the obtained results, the following two associations of gene loci are suggested, which must not necessarily be located on different chromosomes:



Further studies are required to obtain precise information on linkage relations and to calculate chromosomal map distances in *Cunninghamia lanceolata.* The results in this study demonstrate that such interactions between some of the identified gene loci exist.

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Estimates of Genetic Parameters and Gains expected from Selection in Hoop Pine in south-east Queensland

By C. A. DEAN[1]), D. G. NIKLES[2]) and K. J. HARDING[2])

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Summary

Genetic parameters were estimated for height, diameter, stem straightness, internode length and wood density between five and 16 years after planting in four open-pollinated progeny tests of hoop pine in south-east Queensland. All traits appear to be moderately heritable and favourably genetically correlated. Selection indices were formulated by combining the economically important traits of diameter, stem straightness and internode length. The implications of the genetic parameters estimated are discussed with reference to breeding strategies represented by these indices. It seems that substantial improvements can be made in these traits using straightforward breeding procedures such as individual selection.

Key words: Hoop pine, heritability, genetic and phenotypic correlations, selection indices.

[1]) CSIRO, Division of Forest Research, The Cunningham Laboratory, 306 Carmory Road, St. Lucia, Brisbane, 4067, Australia.

[2]) Queensland Department of Forestry, P.O. Box 631, Indooroopilly, Brisbane, 4068, Australia.

Introduction

The natural distribution of hoop pine (*Araucaria cunninghamii* AITON ex D. DON) is wide and extends from around latitude 7° S in Irian Jaya and Papua New Guinea down to 32° S on the east coast of Australia (FISHER, 1980). The major commercial plantings of the species are concentrated in south-east Queensland (Australia) where over 43,000 hectares of plantation have been established since the early 1920's.

As a timber species hoop pine has considerable commercial value because it produces high grade, even-density wood suitable for use as veneer, joinery, particle board and structural products. A program for genetically improving growth and form traits in the species has been undertaken in south-east Queensland with a number of first generation progeny tests being established over the last two decades. There is, however, a lack of reliable genetic information required for the efficient operation of a breeding program. The only documented estimates of genetic parame-