in *Larix decidua* and *L. kaempferi* macrogametophytes by Bergmann and Ruetz (1987). In our study four alleles were detected at this locus (*Figure 1*). Heterozygous embryos displayed two-banded monomeric patterns. This agrees with the structure of SHDH in *Pinus ponderosa* (Linhart et al., 1981), *P. sylvestris* (Szmidt and Yazdani, 1984) and *P. muricata* (Millar, 1985).

Clear and intensive staining of SHDH variants in macrogametophyte and embryo samples as well as the considerable high polymorphism makes this enzyme especially useful as a genetic marker in *Larix decidua*.

Acknowledgements

This work was supported by Polish Academy of Sciences, grant CPBP 0404.

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Genetics of Sweet Chestnut (Castanea sativa Mill.)

III. Genetic Analysis of Zymograms of Single Tree Offspring

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(Received 5th May 1989)

Summary

Genetic analysis of four enzyme systems (PGI, IDH, DIA, AP) in European sweet chestnut (*Castanea sativa* Mill.) is performed by a method utilizing single trees and their seed progenies, as offspring from controlled crosses were not available. A total of six variable gene loci with single-locus codominant modes of inheritance were intuitively postulated on the basis of adult zymograms alone. Subsequent genetic analysis supported the intuitive interpretation for only five of the six proposed loci. The results for the sixth, AP-A, demonstrate the necessity for genetic analysis of zymograms, the limitations of such analysis, and its usefulness for providing indications of distortive phenomena. These results are summarized as follows:

Observation of adult zymograms suggests complete codominance for AP-A, but qualitative analysis of the single tree progenies compels rejection of this hypothesis, instead suggesting the presence of a (recessive) null allele, A_0, in several maternal trees. The revised, null-allele-hypothesis is unequivocally supported by the qualitative tests in all maternal trees as well as by the quantitative tests in all maternal trees not possessing A_0. It must, however, be rejected on the grounds of the quantitative test for heterozygous maternal trees presumably possessing the null allele: A_0 was significantly overrepresented in two progeny sets, significantly underrepresented in a third, and showed no significant deviation from expectation for the remaining three. It is considered whether the null-allele-hypothesis must necessarily be false or whether other phenomena could have caused the seemingly erratic segregation distortion. Possible explanations are discussed in the light of the fact that, despite the apparently considerable frequency of A_0 in the population, no adult trees nor any seeds were found to be homozygous for it.

Key words: Electrophoresis, zymogram, genetic analysis, *Castanea*.

1. Introduction

Previous papers dealing with enzyme systems in sweet chestnut were limited to studies of the isoenzyme pheno-

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types (zymograms) (SAWANO et al., 1984; FINESCHI, 1986. 1988). Such phenotypes, which could be shown to be invariant over the environments under study, were used for the identification of sweet chestnut species, varieties, and individuals. The present paper is the third in a series of reports on a program, the objective of which is to use enzyme gene loci to study the genetic structure and the reproductive system of sweet chestnut populations. Of course, any study of either of these two aspects rests on the results of the genetic analysis of the prospective isoenzyme gene markers, which is the subject of this paper.

In principle, genetic analysis requires the investigation of offspring from controlled crosses, either self- or cross-pollinations, in order to unequivocally trace the inheritance of the observed phenotypes. However, in trees it is often not possible to obtain controlled cross offspring. In most tree species, it takes many years for an individual to reach the reproductive stage, and even then, the flowering intensity is often erratic over the seasons, incompatibility mechanisms are often not understood, and efficient methods of artificial pollination are difficult to develop. All of these problems arise with sweet chestnut.

We will report here on the determination of the modes of inheritance of four enzyme systems in European sweet chestnut *Castanea sativa* MILL. Sufficient numbers of offspring from controlled crosses were not available. Therefore, a method of genetic analysis is applied which utilizes only the isoenzyme phenotypes of single trees and of random samples of their respective offspring from open pollination, as specified by GILLET (1991), GILLET and HATTEMER (1989). Its advantages lie not only in circumvention of the need for controlled crosses but also in the ease of harvesting large numbers of single-tree offspring from open pollination, in the possibility that at each locus all of the allelic variants present within an investigated population may be represented in the offspring of a single tree, and in the fact that the same data used for genetic analysis can be utilized to study the realized mating system.

2. Material and Methods

2.1 Material

The material consisted of bud tissue of approximately 100 individual trees and of seeds from open-pollination collected from 20 of them. The individuals belong to an isolated clone orchard consisting of about 500 grafted sweet chestnut trees located near Grohnde, Lower Saxony, FRG*)

Table 1. — Qualitative and quantitative tests of a postulated single-locus, codominant mode of inheritance.

Proposed maternal genotype	Possible genotypes of offspring	Expected relationship between observed numbers of offspring phenotypes
A_iA_i	A_iA_i A_iA_k $(k \neq i)$	
A_iA_j $(i \neq j)$	A_iA_i A_jA_j A_iA_j A_iA_k A_jA_k $(k \neq i,j)$	$N_{ij} = N_{ii} + N_{jj}$ $N_{ik} = N_{jk}$ $(k \neq i,j)$

Following electrophoresis (see 2.2) of bud tissue from the ca. 100 trees, hypotheses on the possible modes of inheritance of the isoenzyme phenotypes exhibited by the zymograms were set up. Twenty of the trees, each belonging to a different clone, were then chosen for seed harvesting such that both prospective homozygotes and prospective heterozygotes were represented at each of six proposed gene loci.

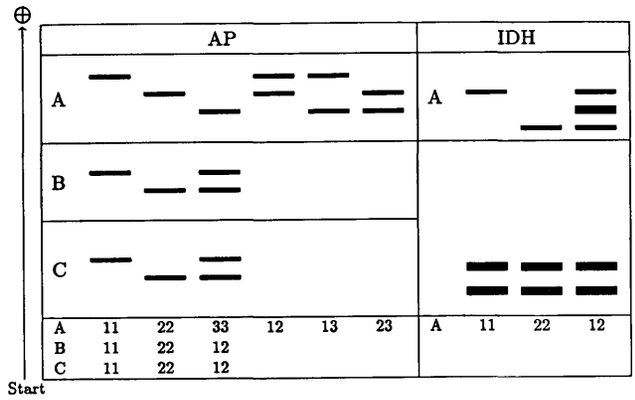
2.2 Electrophoresis

Starch gel electrophoresis was used to investigate the enzyme activity in bud tissue and seeds. Enzyme phenotypes were scored for four enzyme systems: PGI (phosphoglucose isomerase E.C. 5.3.1.9), IDH (isocitric dehydrogenase E.C. 1.1.1.42), DIA (diaphorase E.C. 1.6.4.3.), and AP (leucine and alanine aminopeptidase, E.C. 3.4.11.1 and E.C. 3.4.11.12, respectively). Details on laboratory methods have been previously described for chestnut (MALVOLTI and FINESCHI, 1987) and follow the methods proposed by BERGMANN (1971, 1974, 1981) and MÜLLER-STARCK (1985).

*) The authors are indebted to Dr. J. KLEINSCHMIT, Director of the Abteilung Forstpflanzenzüchtung, Niedersächsische Forstliche Versuchsanstalt, Escherode, for his kind permission to collect material in this orchard.

Table 2. — Qualitative and quantitative tests of a postulated mode of inheritance allowing for the presence of a null allele and codominance between active alleles.

Proposed maternal genotype	Possible phenotypes of offspring	Expected relationship between observed numbers of offspring phenotypes
A_0A_0	A_0A_0 A_k- $(k \neq 0)$	
A_iA_i $(i \neq 0)$	A_i- A_iA_k $(k \neq 0,i)$	
A_iA_0 $(i \neq 0)$	A_0A_0 A_i- A_k- A_iA_k $(k \neq 0,i)$	$N_{00} \leq N_{i-}$ $N_{k-} = N_{ik}$ $(k \neq 0,i)$
A_iA_j $(0 \neq i \neq j \neq 0)$	A_i- A_j- A_iA_j A_iA_k A_jA_k $(k \neq 0,i,j)$	$N_{ij} \leq N_{i-} + N_{j-}$ $N_{ik} = N_{jk}$ $(k \neq 0,i,j)$



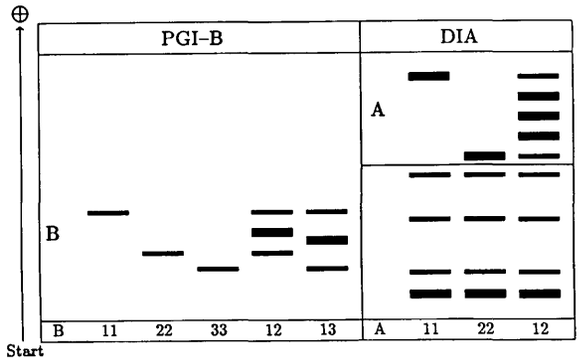


Figure 1. — Observed phenotypes of the four investigated enzyme systems.

2.3 Genetic analysis

Genetic analysis was carried out using the method specified by GILLET (1991), GILLET and HATTEMER (1989). It is based on three requirements on the mechanisms of meiosis and fertilization, which also underlie classical Mendelian analysis:

(i) regular meiotic segregation during egg production;
(ii) random fertilization of the ovules by each pollen (haplo)type;
(iii) absence of differential viability selection in the offspring prior to the investigation.

Given a postulated *single-locus codominant mode of inheritance* of an isoenzyme phenotype with alleles A_l ($l > 0$), the hypothesis can be tested qualitatively and quantitatively on each maternal tree and a random sample of its offspring from open pollination as summarized in *Table 1*, where N_{lm} is defined as the observed number of offspring with unordered genotype A_lA_m.

If the hypothesis postulates *the presence of a recessive null allele* A_0 and *codominance between all active alleles,* then the analogous qualitative and quantitative tests are as summarized in *Table 2*, where $N_{l\_}$ denotes the observed number of offspring with phenotype $A_{l\_}$ (i.e. unordered genotype A_lA_l or A_0A_l).

To test the fulfillment of the quantitative tests, standard goodness-of-fit tests can be used.

3. Results

3.1 Isoenzyme phenotypes

In this section, the zymograms of the various enzyme systems (see *Figure 1*) as observed in the bud tissue of the 100 investigated trees are discussed. Intuitive interpretations of the modes of inheritance are given, i.e. without comparison of the maternal individuals with the phenotypes of their offspring.

PGI: For the PGI system only the slower zone of activity can be sufficiently resolved. For this apparently dimeric zone, presumably three alleles are present. In the sample, three of the postulated homozygous phenotypes but only two of the three possible heterozygous phenotypes were found. A single-locus, codominant mode of inheritance is postulated for this locus, denoted PGI-B.

IDH: The IDH system reveals a slower zone of activity, which does not show any variation, and a faster zone with two electrophoretic variants. The faster zone appears to be controlled by a single locus with two alleles encoding a dimeric structure. Again, a single-locus, codominant mode of inheritance is proposed for this zone, referred to as IDH-A. The slower zone will not be considered further, since the number of loci controlling the two invariant bands is not discernible.

DIA: The only variable zone appears to be tetrameric. Individual trees supposed to be heterozygous can be detected by the presence of a five-banded electrophoretic phenotype. Two different phenotypes controlled by presumably homozygous genotypes and one phenotype presumably controlled by the corresponding heterozygous genotype were observed. A single-locus, codominant mode of inheritance is inferred.

AP: The aminopeptidase system seems to be controlled by three gene loci and to have a monomeric structure. This system has been studied in other angiospermous tree species of the same order, *Fagales.* It was found to be controlled by one gene locus in beech (KIM, 1980) and by two gene loci in alder (LINARES BENSIMÓN, 1984). As described by the latter author in alder, we suppose that the AP zymograms reveal the activity of both the LAP and AAP enzyme systems. However, staining solutions are not specific for either of the two systems in sweet chestnut. It is therefore impossible with the methods we have applied here to differentiate between the activity

Table 3. — Genetic analysis of *PGI-B*: Hypothesis of codominance. The twenty (maternal) trees are grouped by proposed genotype and the distribution of phenotypes within each progeny sample listed ($\sum$ denotes total sample size). Testing according to the method summarized in *Table 1* supports the hypothesis.

Maternal tree		Progeny					
No.	Type	$\sum$	N_{11}	N_{12}	N_{13}	N_{33}	$X^{2\,1)}$
5	B_1B_1	51	12		39		
100	B_1B_1	52	11	2	39		
450	B_1B_1	44	13		31		
84	B_3B_3	50			5	45	
93	B_3B_3	49			18	31	
95	B_3B_3	49			11	38	
172	B_3B_3	49			23	26	
174	B_3B_3	45			24	21	
245	B_3B_3	49			20	29	
320	B_3B_3	43			27	16	
471	B_3B_3	46			16	30	
472	B_3B_3	41			8	33	
488	B_3B_3	52			7	45	
511	B_3B_3	51			22	29	
4	B_1B_3	50	14		24	12	.08 ns
22	B_1B_3	53	3		24	26	.47 ns
255	B_1B_3	50	9		27	14	.32 ns
500	B_1B_3	50	7		27	16	.32 ns
515	B_1B_3	46	6		21	19	.35 ns
523	B_1B_3	51	10		24	17	.18 ns

[1]) χ^2 test of $N_{11} + N_{33} = N_{13}$: "ns" not significant at .05 level

Table 4. — Genetic analysis of IDH-A: Hypothesis of codominance. As in *Table 3*, testing supports the hypothesis.

Maternal tree		Progeny				
No.	Type	Σ	N_{11}	N_{12}	N_{22}	X^2 [1]
4	A_1A_1	50	22	28		
5	A_1A_1	51	12	39		
84	A_1A_1	50	30	20		
511	A_1A_1	51	31	20		
523	A_1A_1	51	14	37		
22	A_2A_2	53		18	35	
95	A_2A_2	49		22	27	
100	A_2A_2	52		11	41	
174	A_2A_2	45		24	21	
245	A_2A_2	49		14	35	
320	A_2A_2	43		24	19	
450	A_2A_2	44		15	29	
471	A_2A_2	46		26	20	
472	A_2A_2	41		28	13	
488	A_2A_2	52		3	49	
500	A_2A_2	50		11	39	
515	A_2A_2	46		27	19	
93	A_1A_2	49	9	22	18	.51 ns
172	A_1A_2	49	13	28	8	1.00 ns
255	A_1A_2	50	5	22	23	.72 ns

[1]) χ^2 test of $N_{11} + N_{22} = N_{12}$: "ns" not significant at .05 level

zones of the two different peptidases. For each of the three proposed loci, AP-A,B,C, a codominant mode of inheritance appears to be correct, with three alleles at locus A and two each at B and C. However, it will be shown below that for AP-A this hypothesis is false.

3.2 Results of the genetic analysis

Initially, for each of the six proposed single gene loci, the genotype of each of the 20 maternal trees was intuitively postulated solely on the basis of the zymograms of the 100 adult trees, as described in the previous section. *Tables 3* to *5*, *6a*, *7*, and *8* show these trees, grouped

Table 5. — Genetic analysis of *DIA-A:* Hypothesis of codominance. As in *Table 3*, testing supports the hypothesis.

Maternal tree		Progeny				
No.	Type	Σ	N_{11}	N_{12}	N_{22}	X^2 [1]
100	A_1A_1	52	9	43		
245	A_1A_1	49	20	29		
450	A_1A_1	44	9	35		
471	A_1A_1	46	11	35		
84	A_2A_2	50		11	39	
95	A_2A_2	49		19	30	
255	A_2A_2	50		8	42	
472	A_2A_2	41		6	35	
488	A_2A_2	52		10	42	
511	A_2A_2	51		11	40	
523	A_2A_2	51		9	42	
4	A_1A_2	50	12	23	15	.32 ns
5	A_1A_2	51	9	25	17	.02 ns
22	A_1A_2	53	8	26	19	.02 ns
93	A_1A_2	49	2	27	20	.51 ns
172	A_1A_2	49	9	26	14	.18 ns
174	A_1A_2	45	8	25	12	.55 ns
320	A_1A_2	43	6	27	10	2.81 ns
500	A_1A_2	50	10	22	18	.72 ns
515	A_1A_2	46	1	26	19	.78 ns

[1]) χ^2 test of $N_{11} + N_{22} = N_{12}$: "ns" not significant at 0.05 level

Table 6a. — Genetic analysis of *AP-A*: Erroneous hypothesis of (complete) codominance. Application of the method summarized in *Table 1* leads to rejection of the qualitative tests for trees 5, 172, 523, 84, 93, and 511, and of the quantitative tests for trees 100, 22, 472, and 515.

Maternal tree		Progeny							
No.	Type	Σ	N_{11}	N_{12}	N_{22}	N_{13}	N_{23}	N_{33}	X^2 (d.f.)
5	A_2A_2	78			43		14	21 (!)	
95	A_2A_2	49			40		9		
172	A_2A_2	73			56		13	4 (!)	
174	A_2A_2	45			35		10		
245	A_2A_2	49		2	39		8		
255	A_2A_2	50			30		20		
471	A_2A_2	46			38		8		
500	A_2A_2	50			40		10		
523	A_2A_2	99	1 (!)	2	60		12	24 (!)	
84	A_3A_3	97			37 (!)		2	58	
93	A_3A_3	99			26 (!)		25	48	
450	A_3A_3	44				1	24	19	
511	A_3A_3	51			11 (!)		7	33	
100	A_1A_3	52	7	13		6	12	14	8.37 * (2)[1]
4	A_2A_3	50			15		21	14	1.28 ns (1)[2]
22	A_2A_3	53			20		19	14	4.25 * (1)[2]
320	A_2A_3	47			16		27	4	1.04 ns (1)[2]
472	A_2A_3	41			19		11	11	8.80 ** (1)[2]
488	A_2A_3	52			26		20	6	2.77 ns (1)[2]
515	A_2A_3	46			24		13	9	8.70 ** (1)[2]

(!) signifies that offspring of this phenotype cannot occur.
[1]) χ^2 test of $N_{11} + N_{33} = N_{13}$ and $N_{12} = N_{23}$;
[2]) χ^2 test of $N_{22} + N_{33} = N_{23}$.
Levels of significance: "ns" not significant, *0.05, **0.01.

Table 6b. — Genetic analysis of *AP-A*: Null-allele-hypothesis. Application of the method summarized in *Table 2* leads to rejection only of the quantitative tests for trees 172, 523, and 84 (also see Discussion).

Maternal tree		Progeny							
No.	Type	$\sum$	N_{1-}	N_{12}	N_{2-}	N_{13}	N_{23}	N_{3-}	X^2
95	A_2A_2	49			40		9		
174	A_2A_2	45			35		10		
245	A_2A_2	49		2	39		8		
255	A_2A_2	50			30		20		
471	A_2A_2	46			38		8		
500	A_2A_2	50			40		10		
450	A_3A_3	44				1	24	19	
5	A_2A_0	78			43		14	21	1.40 ns [2]
172	A_2A_0	73			56		13	4	4.74 * [2]
523	A_2A_0	99	1	2	60		12	24	4.33 * [1]
84	A_3A_0	97			37		2	58	31.41 *** [3]
93	A_3A_0	99			26		25	48	.02 ns [3]
511	A_3A_0	51			11		7	33	.89 ns [3]
100	A_1A_3	52	7	13		6	12	14	× .04 ns [4]
4	A_2A_3	50			15		21	14	×
22	A_2A_3	53			20		19	14	×
320	A_2A_3	43			16		23	4	×
472	A_2A_3	41			19		11	11	×
488	A_2A_3	52			26		20	6	×
515	A_2A_3	46			24		13	9	×

[1]) χ^2 test of $N_{1-} + N_{3-} = N_{12} + N_{23}$;
[2]) χ^2 test of $N_{23} = N_{3-}$;
[3]) χ^2 test of $N_{2-} = N_{23}$;
[4]) χ^2 test of $N_{12} = N_{23}$;
Significance levels: "ns" significant, *0.05, **0.01, ***0.001. × indicates fulfillment of $N_{ij} \leq N_{i-} + N_{j-}$ or non-significant deviation from equality (tree 320 only).

by proposed genotype, together with the corresponding phenotypes of their offspring. The method of genetic analysis described in 2.3 was applied in each case to test the hypothesis of a single-locus, codominant mode of

Table 7. — Genetic analysis of *AP-B*: Hypothesis of codominance. As in *Table 3*, testing supports the hypothesis.

Maternal tree		Progeny				
No.	Type	$\sum$	N_{11}	N_{12}	N_{22}	X^2 [1]
22	B_1B_1	53	44	9		
93	B_1B_1	99	69	30		
100	B_1B_1	52	44	8		
172	B_1B_1	73	47	26		
174	B_1B_1	45	29	16		
245	B_1B_1	49	32	17		
255	B_1B_1	50	41	9		
320	B_1B_1	43	41	2		
450	B_1B_1	44	31	13		
471	B_1B_1	46	33	13		
488	B_1B_1	52	36	16		
500	B_1B_1	50	31	19		
4	B_2B_2	50		36	14	
5	B_1B_2	78	26	46	6	2.51 ns
84	B_1B_2	97	27	53	17	.84 ns
95	B_1B_2	49	21	25	3	.02 ns
472	B_1B_2	41	15	16	10	1.98 ns
511	B_1B_2	51	10	30	11	1.58 ns
515	B_1B_2	46	12	26	8	.78 ns
523	B_1B_2	99	36	47	16	.25 ns

[1]) χ^2 test of $N_{11} + N_{22} = N_{12}$: "ns" not significant at 0.05 level

Table 8. — Genetic analysis of *AP-C*: Hypothesis of codominance. As in *Table 3*, testing supports the hypothesis.

Maternal tree		Progeny				
No.	Type	$\sum$	N_{11}	N_{12}	N_{22}	X^2 [1]
4	C_1C_1	50	50			
5	C_1C_1	78	72	6		
22	C_1C_1	53	27	26		
95	C_1C_1	49	38	11		
100	C_1C_1	53	41	12		
172	C_1C_1	73	64	9		
174	C_1C_1	45	40	5		
245	C_1C_1	49	42	7		
255	C_1C_1	50	43	7		
320	C_1C_1	43	41	2		
450	C_1C_1	44	35	9		
471	C_1C_1	46	34	12		
488	C_1C_1	52	49	3		
511	C_1C_1	51	40	11		
515	C_1C_1	46	37	9		
84	C_1C_2	97	32	51	14	.26 ns
93	C_1C_2	99	42	55	2	1.22 ns
472	C_1C_2	41	9	24	8	1.20 ns
500	C_1C_2	50	20	27	3	.32 ns
523	C_1C_2	99	48	42	9	2.27 ns

[1]) χ^2 test of $N_{11} + N_{22} = N_{12}$: "ns" not significant at 0.05 level

inheritance. It will be shown that, with the one exception of AP-A, the results support these intuitive hypotheses.

PGI-B, IDH-A, DIA-A, AP-B, AP-C: The above qualitative and quantitative conditions for a codominant mode of inheritance are fulfilled for each of these prospective gene loci: Each offspring possesses at least one maternal allele, and the offspring phenotypic structures show no significant deviations from the expected 1:1 ratios of homozygous:heterozygous offspring. Therefore, in all cases there is no apparent reason to reject the hypothesis of a single-locus, codominant mode of inheritance.

AP-A: At the locus AP-A (*Table 6a*), however, the qualitative test of the hypothesis of complete codominance leads to its rejection, since six phenotypically homozygous maternal trees (trees 5, 172, 523 and 84, 93, 511) have offspring which are phenotypically homozygous for a different, non-maternal allele and thus do not appear to possess a maternal allele. This is impossible under a codominant mode of inheritance. Additional grounds for rejection are provided by the quantitative test of four of the seven maternal trees supposedly heterozygous for two active alleles.

The results of the qualitative test of codominance strongly suggest that the six phenotypically homozygous maternal trees listed above are actually heterozygous for a (recessive) null allele, subsequently denoted by A_0. Thus maternal trees 5, 172, and 523, phenotypically A_{2-}, would be of genotype A_2A_0; this would explain the phenotypes A_{1-} and A_{3-} among their offspring. Analogously, trees 84, 93, and 511 would be of genotype A_3A_0. Likewise, those of their offspring which seemed to be homozygous for a non-maternal allele would be heterozygous for A_0 and an active allele contributed by the pollen. Thus the hypothesis of a codominant mode of inheritance was revised to include the presence of a null allele A_0 (see *Table 6b*) and will be referred to as the "null-allele-hypothesis". Note that no maternal tree nor any seed offspring was observed to be homozygous for A_0.

Null alleles in various enzyme systems have also been detected in other deciduous tree species, e.g. ACP (acid phosphatase, E.C. 3.1.3.2) and 6-PGDH (6-phosphogluconate dehydrogenase, E.C. 1.1.1.44) (RAJORA, 1986) and MDH (malate dehydrogenase, E.C. 1.1.1.37) (MALVOLTI et al., 1991) in poplar, PER (peroxidase, E.C. 1.11.1.7) in Siberian elm (FERET and STAIRS, 1971) and beech (THIEBAUT et al., 1982), and LAP (leucine aminopeptidase, E.C. 3.4.11.1) in beech (KIM, 1979).

The qualitative test for each maternal tree supports the null-allele hypothesis, since each offspring of each maternal tree can be assigned a maternal allele. Now consider the quantitative tests for the heterozygous maternal trees (for homozygous maternal trees, none is given nor necessary):

(a) For all heterozygous maternal trees which do not possess A_0, i.e. those of genotype A_iA_j ($i \neq j$, $i,j \neq 0$), the quantitative test supports the null-allele-hypothesis: For six of these seven trees, $N_{ii} + N_{jj}$ was (in four of them significantly) greater than N_{ij}, while in the seventh tree $N_{ii} + N_{jj}$ was not significantly less than N_{ij}. (Testing was done against the hypothesis of $N_{ii} + N_{jj} = N_{ij}$.) Only offspring of tree 100 possess an additional, non-maternal allele to allow quantitative testing of $N_{ik} = N_{jk}$, and the ratio $N_{12} : N_{13} = 13 : 12$ for tree 100 shows no significant deviation from the expectation. The null-allele-hypothesis is thus supported for all maternal trees which do not posses A_0.

(b) On the contrary, the test of $N_{k\_} = N_{ik}$ ($k \neq i$) for three of the six maternal trees which are postulated as being heterozygous for A_0, i.e. of genotype A_iA_0 ($i \neq 0$), lead to rejection of the null-allele-hypothesis: Two (172, 523) of the three maternal trees of presumed genotype A_2A_0 and one (84) of the three trees of presumed type A_3A_0 show significant deviations from this condition. On the other hand, $N_{00} \leq N_{i\_}$ holds in all cases, since $N_{00} = 0$ in all of the offspring sets (see below).

Thus the null-allele-hypothesis, based on the requirements (i)—(iii) above, must be rejected on the grounds of the quantitative test in the progeny of three of the six maternal trees which are heterozygous for A_0.

4. Discussion

As offspring from controlled crosses were unavailable, the above method of genetic analysis using single tree progenies was applied. For all but one of the six postulated single gene loci, the results of the genetic analysis supported our *a priori* intuitive interpretation based only on the zymograms of 100 trees in the population. However, at the sixth locus, AP-A, both the qualitative and the quantitative tests of the hypothesis of codominance called for its rejection. The qualitative test suggests that six of the seemingly homozygous maternal trees are actually heterozygous for a null allele A_0. This allele escaped detection during investigation of the zymograms of only the adult trees, since none of these trees was homozygous for it. The null-allele-hypothesis was subsequently not rejected by the qualitative tests for all of the maternal trees. It was furthermore not rejected by the quantitative tests for all of the maternal trees which do not contain A_0, but it was rejected for three of the six trees which do.

The fact that the null-allele-hypothesis is rejected only on the basis of the quantitative tests of three of the six heterozygous carriers of A_0 (i.e. of genotype A_iA_0, $i \neq 0$) raises the question as to whether all components of the null-allele-hypothesis must necessarily be false. Possible causes of rejection other than incorrectness of the hypothesized mode of inheritance are erroneousness of the maternal genotype A_iA_0 or violation of the requirements (i)—(iii). Since the qualitative considerations strongly support the correctness of the postulated maternal genotype A_iA_0, violation of requirements (i)—(iii) appears to be the likelier cause of rejection and will be pursued in the following.

Suppose that violation of the requirements (i)—(iii), in the form of segregation distortion, non-random fertilization of egg cells, or differential viability selection, resulted solely from the presence of A_0. Then the ratios $N_{k\_} : N_{ik}$ should have been distorted to a similar degree and in the same direction for all maternal trees of genotype A_iA_0 ($i = 2$ or 3). However, this is decidedly not the case, since significant deviations were observed for only three of six trees, and of these three, two result from overrepresentation and one from underrepresentation of the phenotype $N_{k\_}$. As a consequence, it appears that variation in the genetic background must be invoked in order to explain this pattern of segregation distortion.

An additional observation which must be accounted for is the following: All investigated adult trees and seeds showed some activity at AP-A. Thus none could be assigned the genotype A_0A_0. However, if the observed proportion 6/20 of trees containing A_0 among the investigated trees (A_0 in a heterozygous state cannot be detected in trees not chosen for offspring study) is representative of the total population, then offspring of genotype A_0A_0 certainly should have been found among the large offspring sets of the A_0A_i-maternal trees. This observation suggests that A_0-homozygotes may have reduced viability.

Thus the results not only present a clear demonstration of the necessity for genetic analysis of zymograms. They also indicate that limitations are imposed on genetic analysis using single tree progenies, in that mechanisms operating during reproduction can significantly distort the expected ratios of offspring phenotypes. This phenomenon is, however, certainly not unique to this type of analysis, since it can equally well occur within controlled cross progenies. In fact, the observability of such distortions can lead to the discovery of new phenomena.

Acknowledgements

The authors are much obliged to F. BERGMANN, G. MÜLLER-STARCK and K. RADLER for discussions on isoenzyme analysis, to H.-R. GREGORIUS and H. H. HATTEMER for discussions on genetic analysis, and to S. KRAKUHN for excellent technical assistance. This study was financially supported by a grant from Consiglio Nazionale delle Ricerche to S. FINESCHI.

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Geographic Variation in Specific Gravity and Fiber Length of Green Ash (Fraxinus pennsylvanica Marsh.) in East Texas

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(Received 20th July 1989)

Abstract

Specific gravity and fiber length were evaluated in three, ten-year-old green ash genetic tests in East Texas which contained 42 open-pollinated families representing seven East Texas provenances. Fiber length was significantly affected by family within provenance and the plantation × provenance interaction. No consistent geographic pattern of variation was discernible for this interaction. Specific gravity was significantly affected by plantation, provenance, family within provenance, and the plantation × family within provenance interaction. Family heritability estimates for specific gravity and fiber length were 0.80 and 0.73, respectively. Coefficients of genetic prediction between fiber length and growth parameters were small; those between specific gravity and growth parameters were near 0. Based on these data, a tree improvement program could be expected to produce moderate gains in specific gravity. Among family variation in fiber length was too small to warrant attempts at genetic improvement.

Key words: Progeny testing, heritability, provenance, genotype × environment interaction.

Introduction

Green ash (*Fraxinus pennsylvanica* MARSH.) is a widely distributed North American species whose range encompasses the eastern half of the United States and southern Canada. Uses for its wood include handles, cabinetry, furniture and cooperage (VINES, 1960). Green ash is also used to produce paper (BARKER, 1974), although it makes a weaker pulp than American sycamore (*Platanus occidentalis* L.) or sweetgum (*Liquidambar styraciflua* L.).

Geographic variation in green ash has been investigated in Mississippi by WELLS (1986). At age 10 he found differences in height growth attributable to latitude of provenance, provenances within the same latitude, and individual trees within a provenance. Variation was great enough to suggest significant potential for genetic improvement. HENDRIX (1986) found similar results from four genetic tests in Texas, Arkansas, Mississippi and Louisiana. Both provenance and family within provenance differences existed for growth rate and specific gravity. Heritability values for growth parameters were intermediate (0.56), while heritability for specific gravity was high (0.89).

Fiber length and specific gravity affect the economics of making paper and the quality of the product (CLARK, 1978). The fineness of the fiber and its length determines paper smoothness and strength. If high wood density results from thick-walled fibers, paper quality suffers; however, the yield of pulp from a given volume of wood increases as specific gravity increases (CLARK, 1978).

The potential for genetic improvement of fiber length and specific gravity has been demonstrated for few hardwood species. Significant genetic variation in specific gravity was documented for American sycamore (NEBGEN and LOWE, 1982), based on data from three genetic tests in southeast Texas and southeast Louisiana. Genetic differences in fiber length have been found in genetic tests of white ash (*Fraxinus americana* L.) (ARMSTRONG and FUNK, 1980), and hybrid *Populus* clones (MURPHEY *et al.*, 1979).

In an earlier paper, STAUDER and LOWE (1983) reported data on height, diameter and volume from three green ash genetic tests in eastern Texas. The present study is a continuation of that work, examining specific gravity and fiber length data from the same plantations.

The objectives of this study were to determine the effects of provenance, family within provenance, and plantation location on fiber length and specific gravity of green ash; to estimate the narrow sense heritability for specific gravity and fiber length in East Texas; and to estimate the degree of correlation among these traits and growth parameters.

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