

nectedness or imbalance among the experiments to make BLUP advantageous.

Conclusions

Methods of solving for GCA and SCA estimates for balanced (plot-mean basis) and unbalanced data have been presented along with the inherent assumptions of the analysis. The use of plot means and the matrix equations will produce sum-to-zero OLS estimates for GCA and SCA for all types of imbalance. Formulae in the literature which yield OLS solutions for balanced data can yield misleading solutions for unbalanced data because of the loss of orthogonality and also weightings on site means for crosses (or totals) are constants.

GCA's and SCA's obtained through sum-to-zero restriction are not truly estimates of parametric population GCA's and SCA's. There are an infinite number of solutions for GCA's and SCAs from the system of equations as a result of the overparameterized linear model. Yet, if the only comparisons of interest are among the specific parents on a particular site, then the estimates calculated by sum-to-zero restrictions are appropriate. Checklots may be used to provide comparability among estimates derived from disconnected sets.

Having discussed the innate mathematical features of OLS analysis, knowledge of these features should help the data analyst decide if OLS is the most desirable technique for the data at hand. It may be desirable to relax OLS assumptions, which are in all likelihood invalid for the variance-covariance matrix of the observations. This could lead to GLS, BLP or BLUP as better alternatives.

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Segregation and Linkage of Allozymes in Seed Tissues of the Hybrid Greek Fir *Abies borisii regis* Mattfeld

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Summary

Seed tissues (haploid megagametophyte and diploid embryo tissue) of *Abies borisii regis* were used in starch gel electrophoresis to study inheritance and linkage of isozyme variants. The 10 enzyme systems studied are coded

by a minimum of 15 isozyme loci. All loci code allozymes in both megagametophyte and embryo tissues. Mendelian segregation ratios were found for all enzyme systems except ACO and 6-PGD where distortion was observed. Segregation distortion could also exist in other enzyme systems (LAP, PGI2, MNR1, MNR2). Evidence of total linkage is provided for one pair of loci (GR/MNR1) that has never been tested before in conifers and tight linkage for another pair of loci (MNR1/PGI1).

Key words: *Abies borisii regis*, allozymes, inheritance, linkage, electrophoresis, seed tissues.

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Introduction

Genetic studies in conifers using isozyme techniques, whether investigating phylogeny (e. g. JACOBS et al., 1984; MITSOPOULOS and PANETOS, 1987; CONKLE et al., 1988; MILLAR et al., 1988; CRAWFORD, 1989), or genetic diversity on a large geographical scale (e. g. SCHILLER et al., 1986; CHELIAK et al., 1988; LI and ADAMS, 1989), among or within populations (e. g. COLES and FOWLER, 1976; KORMUTAK et al., 1982; LEDIG and CONKLE, 1983; SHEA, 1990), or estimating mating system parameters (e. g. MÜLLER, 1977; SHAW and ALLARD, 1979; RITLAND and JAIN, 1981; NEALE and ADAMS, 1985; BROWN, 1989), can only be truly meaningful if the Mendelian genetics of isozyme band patterns are known. Several studies have been performed on the inheritance of isozymes in conifers (e. g. CONKLE, 1971; HARRY, 1986; STRAUSS and CONKLE, 1986; ADAMS et al., 1990), but few have yet dealt with the species of *Abies* (NEALE and ADAMS, 1981; SHEA, 1988), and apparently none with *Abies borisii* regis.

Abies borisii regis MATTFELD is considered to be a natural hybrid between the European silver fir (*Abies alba* MILLER) and the Greek fir (*Abies cephalonica* LOUDON). Both *Abies cephalonica* and *A. borisii* regis are very promising plantation species for Mediterranean France where extensive areas need to be reforested after destruction by wildfires.

As a preliminary to analyzing the genetic structure of an *Abies borisii* regis population in the Pertouli forest of Thessaly, Greece, it was first necessary to determine the genetic control of electrophoretic variants in seeds of open-pollinated trees. In this paper, the band patterns of ten enzyme systems are reported: acid phosphatase (ACP, EC 3.1.3.2), aconitase (ACO, EC 4.2.1.3), catalase (CAT, EC 1.11.1.6), glutamate dehydrogenase (GDH, EC 1.4.1.3), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), glutathione reductase (GR, EC 1.6.4.2), leucine aminopeptidase (LAP, EC 3.4.11.1), menadione reductase (MNR, EC 1.6.99.2), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44) and phospho-glucose isomerase (PGI, EC 5.3.1.9). Both megagametophyte (haploid) and embryo (diploid) tissues of germinated seeds were examined. Evidence for polymorphic loci coding allozymes was based on segregation in megagametophytes of heterozygous mother trees. Linkage relationships between these loci were also investigated. Genetic control for enzymes found to be monomorphic in this *Abies borisii* regis population is discussed based on comparisons with band patterns of other *Abies* and conifers in which evidence of inheritance has already been demonstrated.

Materials and Methods

Wind-pollinated seeds were collected from 30 trees growing along a transect in the University of Thessaloniki forest of Pertouli (Central Pindos mountains, Greece, 39°30' N, 21°30' E) in the fall of 1988 and stored at the Laboratory of Tree Breeding, I.N.R.A. Bordeaux, France. Seeds were stratified for 21 days at 4°C and then germinated at room temperature on moist filter paper at the Institute of Forest Genetics in Placerville, California, in the fall of 1990. After stratification, a total of 403 seeds (6 to 41 progenies per mother-tree) could be used for analysis.

When the radicle had emerged 2 mm beyond the seed coat, megagametophytes and embryos were excised from their seed coats and crushed separately with a glass rod in wells of micro-plates filled with three drops of extrac-

tion buffer (0.2 M phosphate buffer, CONKLE et al., 1982). The extracts were absorbed onto 3 X 14 mm paper wicks (Whatman #3 paper, Northfork Products, Tumwater, WA, USA) and then inserted into a vertical slice on a 12.5% starch gel made with hydrolyzed potato starch (Sigma Chemical Company, St. Louis, MO, USA). Electrophoretic buffer systems were as follows:

+ system A (10 liter solution): gel stock (pH 8.3, 62.0 g trizma base and 14.6 g citric acid); electrode tray (pH 8.3, 12.0 g LiOH monohydrate and 118.9 g boric acid); gel buffer formulated with 90% stock and 10% tray buffer;

+ system B (10 liter solution): gel (pH 8.8, 121.1 g trizma base titrated with 0.2 M citric acid); electrode tray (pH 8.0, 185.5 g boric acid and 20.0 g NaOH titrated with 4 N NaOH solution).

Gel running conditions were described in CONKLE et al. (1982). The wicks were removed from the gels 10 min after the beginning of the run. Electrophoresis was then continued until the borate front had migrated 8 cm from the origin to the anode. The anodal portion of the gels was sliced horizontally into four to five sections (1 mm thick) and incubated in staining solutions at 37°C overnight. Stain recipes were modified slightly from CONKLE et al. (1982). The stain recipe of GR was as follows: 0.1 M Tris HCl pH 8.0 (50 ml), 2,6-dichlorophenolindolphenol (1 mg), oxidized glutathione (20 mg), NADPH (10 mg), MTT (20 mg). Slices from buffer system A gels were stained for MNR, ACO, LAP and PGI and B gels for CAT, GOT, GR, ACP, GDH and 6-PGD.

From the 30 mother trees studied, 20 were heterozygotes as indicated by their band patterns at one or more loci. To test hypotheses of inheritance, chi-square tests were calculated to determine the "goodness of fit" of segregating allozymes to the expected 1:1 ratio for megagametophytes from heterozygous mother trees. Chi square analyses were also used to test heterogeneity among trees and linkage relationship for pairs of segregating loci. Recombination values and standard errors were estimated using the maximum likelihood method (BAILEY, 1961): recombination frequencies $r = a/n$ and standard error $s = (r(1-r)/n)^{1/2}$, where a is the number of recombinant gametes and n is the number of total gametes. Wicks from embryos and megagametophytes were run side by side on gels in groups of five. Their band patterns were thus easy to compare to determine if the same genes were coding allozymes in both tissues.

Results and Discussion

For enzyme systems in which more than one zone of activity appeared or in zones of activity with more than one allozyme, the fastest migrating (most anodal) zone or allozyme was labelled 1 and the slower zone or allozyme was labelled 2, 3, etc.

All zones of activity were detectable at the same position in both megagametophyte and embryo tissues indicating that all enzymes were coded in both seed tissues. Megagametophyte segregation data and "goodness of fit" of allozyme segregation to the expected 1:1 ratio for each locus are summarized in table 1. Chi square heterogeneity tests indicated that the data scored for each allelic pair were homogeneous over all mother trees, except for GOT2. There, however, heterogeneity was due to a single tree of the seven used to test the hypothesis. Pooled data are thus presented in table 1. Figure 1 shows the segregation patterns of the isozymes.

Table 1. — Observed single locus segregation of allozymes from heterozygous mother trees: chi square tests of heterogeneity of mother trees and "goodness of fit" to the 1:1 ratio.

| enzyme locus | allele 1 | allele 2 | allele 3 | N | heterogeneity | | segregation | |
|--------------|----------|----------|----------|-----|---------------|-------|-----------------|-------|
| | | | | | χ^2 (df) | p* | χ^2 (df=1) | p** |
| LAP | 66 | 48 | - | 114 | 8.352 (6) | 0.213 | 2.842 | 0.092 |
| PGI1 | 126 | 119 | - | 245 | 19.605 (14) | 0.143 | 0.147 | 0.702 |
| PGI2 | 54 | - | 36 | 90 | 5.105 (4) | 0.277 | 3.6 | 0.058 |
| PGI2 | 52 | 43 | - | 95 | 2.356 (2) | 0.308 | 0.674 | 0.412 |
| GR | 62 | 61 | - | 123 | 3.206 (8) | 0.921 | 0.0 | 1.0 |
| ACO | 37 | - | 19 | 56 | 1.610 (2) | 0.447 | 5.786 | 0.016 |
| ACO | 62 | 50 | - | 112 | 8.529 (7) | 0.288 | 1.286 | 0.257 |
| GOT2 (1) | 35 | 37 | - | 72 | 12.821 (6) | 0.046 | 0.056 | 0.814 |
| GOT2 (2) | 34 | 27 | - | 61 | 4.762 (5) | 0.446 | 0.59 | 0.442 |
| GOT3 | 20 | - | 14 | 34 | 1.596 (3) | 0.660 | 1.059 | 0.304 |
| GOT3 | 43 | 39 | - | 82 | 0.566 (3) | 0.904 | 0.195 | 0.659 |
| MNR1 | 105 | 79 | - | 184 | 12.111 (9) | 0.207 | 3.674 | 0.056 |
| MNR2 | 26 | 15 | - | 41 | 1.775 (1) | 0.183 | 2.951 | 0.086 |
| ACP2 | 39 | 43 | - | 82 | 0.001 (1) | 0.974 | 0.195 | 0.659 |
| 6PGD | 32 | 11 | - | 43 | 1.296 (1) | 0.255 | 10.256 | 0.001 |

*) probability of heterogeneity between trees due to chance alone

**): probability of deviation from Mendelian expectations due to chance alone

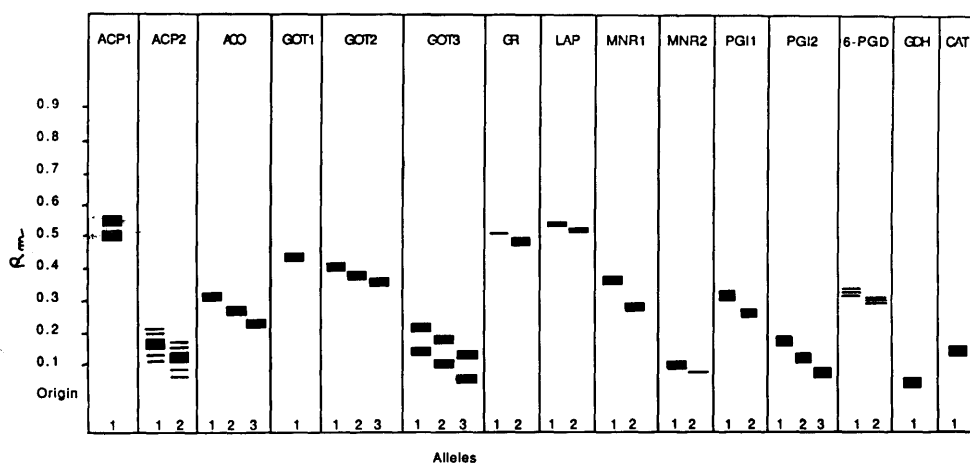


Figure 1. — Segregation patterns of enzyme systems in megagametophytes of *Abies borisii regis*. Rm is the mobility relative to the distance covered by the borate front (8 cm).

Enzymes will be presented individually and unless otherwise stated, allele segregation presented no significant distortion from the expected 1:1 ratio. Considering the limited size of our sample, only severe cases of distortion were detected in this study.

Acid phosphatase

Gels stained for ACP had two zones of activity in megagametophytes. Two ACP zones of activity have often been reported for conifers (ECKERT et al., 1981). ACP2 presented two variants consisting of five bands shifting together where the central band was the most strongly expressed. Their segregation in heterozygous mother trees was not significantly different from the expected 1:1 ratio, indicating that ACP2 is controlled by a single diallelic locus. ACP2 is coded by a single locus in *Picea glauca* (KING and DANCİK, 1983). The invariant ACP1 zone is double-banded and is probably controlled by a separate locus. ACP1 is controlled by one locus in *Pinus strobus* (ECKERT et al., 1981). ACP has been shown to be monomeric or dimeric (WEEDEN and WENDEL, 1989) and is monomeric in *Abies borisii regis*. ACP1 appeared weaker in megagametophytes than embryos, although the opposite was true for ACP2, suggesting a weak specialization of the isozymes, depending on the tissue.

Aconitase

Gels stained for ACO had one zone of activity with three single banded variants. A single zone of activity coded by a single locus has been reported in many conifer species (e. g. KING and DANCİK, 1983; STRAUSS and CONKLE, 1986; HARRY, 1986; SHEA, 1988). However, two zones were found in *Larix laricina* (CHELIAK and PITEL, 1985). Heterozygotes in embryos appeared double banded without a middle band. This could indicate a monomeric subunit structure. ACO is dimeric in *Picea abies* (MUONA et al., 1987) but monomeric in *Pseudotsuga menziesii* (ADAMS et al., 1990). Allele 3 appeared significantly deficient ($p = 0.016$) in the combination ACO(1)—ACO (3). A significant excess of fast alleles has also been reported in another *Abies*, *A. lasiocarpa* (SHEA, 1988).

Glutamate-oxaloacetate transaminase

Gels stained for GOT had three zones of activity. GOT1 appeared monomorphic and single-banded. GOT2 was polymorphic with three single-banded variants which segregated as alleles at one locus. The slowest zone (GOT3) showed three double-banded variants which segregated as alleles at one locus. In other conifers (O'MALLEY et al., 1979; NEALE and ADAMS, 1981; CHELIAK and PITEL, 1985; STRAUSS and CONKLE, 1986), a three locus genetic control

has been suggested when three zones of activity are present on zymograms. However, two loci systems have also been documented (BOYLE and MORGENSTERN, 1985; PERRY and KNOWLES, 1989) when the slow isozyme GOT3 was absent. GOT3 appears as either double or triple banded in other conifers (O'MALLEY et al., NEALE and ADAMS, 1981; BOYLE and MORGENSTERN, 1985; CHELIAK and PITEL, 1985; STRAUSS and CONKLE, 1986; PERRY and KNOWLES, 1989; ADAMS et al., 1990). Heterozygotes in embryos were triple-banded for GOT2 which suggests a dimeric subunit structure for GOT. GOT is dimeric in other species (e. g. WEEDEN and WENDEL, 1989; ADAMS et al., 1990). However, for isozyme GOT3, heterozygotes were double-banded at an intermediate position.

Glutathione reductase

Gels stained for GR had two zones of activity, but only the fastest migrating one was scorable. In this zone, two single banded variants were observed. Segregation data support the hypothesis of a diallelic locus. Embryos showed bands either at the same place as the megagametophyte or in between, revealing that the enzyme is dimeric. No studies on conifers were available for this enzyme. However, it was easy to resolve under our conditions and showed a high degree of polymorphism.

Leucine aminopeptidase

Gels stained for LAP had two migrating zones in megagametophytes. However, only the fastest zone, with two single-banded variants, was scorable. A third slow-migrating variant was observed once in an embryo. LAP appeared as a monomeric unit. It is also considered to be monomeric in other conifers (CONKLE, 1971; NEALE and ADAMS, 1981; MILLAR, 1985). In this species, no distortion ($p > 0.05$) appeared although it has frequently been observed in other conifers (HARRY, 1986; STRAUSS and CONKLE, 1986; ADAMS et al., 1990), including *Abies balsamea* (NEALE and ADAMS, 1981). Usually, two zones of activity have been observed (O'MALLEY et al., 1979; KING and DANCIG, 1983; CHELIAK and PITEL, 1985; STRAUSS and CONKLE, 1986), although 3 ones can also be found (CONKLE, 1971; ECKERT et al., 1981).

Menadione reductase

Gels stained for MNR had two zones of activity with two single-banded variants in each zone. Both MNR1 and MNR2 variants segregated as alleles at one locus. Data from embryos suggest that MNR could be dimeric, although resolution in MNR2 was very poor and heterozygotes in MNR1 often appeared smeared. Three zones coded by three loci have been found in *Pinus attenuata* (STRAUSS and CONKLE, 1986). No other reports of MNR in conifers were found, although this enzyme resolved easily under our conditions and demonstrated a high degree of polymorphism.

Phospho-glucose isomerase

Gels stained for PGI had two polymorphic zones of activity with single-banded variants. Segregation data support the hypothesis of one diallelic locus for PGI1 and one triallelic locus for PGI2. Two segregating zones appeared in other conifers, in most cases related to the activity of two loci, where the slowest one was highly polymorphic (O'MALLEY et al., 1979; NEALE and ADAMS, 1981; KING and DANCIG, 1983; BOYLE and MORGENSTERN, 1985; CHELIAK and PITEL, 1985; HARRY, 1986; STRAUSS and CONKLE, 1986; SHEA, 1988; PERRY and KNOWLES, 1989; ADAMS et al.,

1990). Triple-banded heterozygotes in embryos suggest that PGI is a dimeric enzyme. PGI has a dimeric subunit structure in other species (WEEDEN and WENDEL, 1989; ADAMS et al., 1990).

6-phosphogluconate dehydrogenase

Gels stained for 6-PGD had one polymorphic zone of activity with a triple-banded phenotype. NEALE and ADAMS (1981) observed one zone of activity in *Abies balsamea* as well. However, the most common 6-PGD phenotype in conifers has two segregation zones (O'MALLEY et al., 1979; KING and DANCIG, 1983; BOYLE and MORGENSTERN, 1985; CHELIAK and PITEL, 1985; HARRY 1986; SHEA, 1988; PERRY and KNOWLES, 1989; ADAMS et al., 1990) and sometimes three (STRAUSS and CONKLE, 1986). 6-PGD showed a significantly distorted segregation ($p = 0.001$): the slow 6-PGD(2) allele was deficient. Significant distortion in 6-PGD has been observed in several other conifers (O'MALLEY et al., 1979; STRAUSS and CONKLE, 1986; ADAMS et al., 1990). 6-PGD has a dimeric subunit structure in several species (NEALE and ADAMS, 1981; PERRY and KNOWLES, 1989; WEEDEN and WENDEL, 1989).

Other enzyme systems

Catalase and glutamate dehydrogenase appeared as monomorphic and single-banded systems. GDH is also monomorphic in *Abies balsamea* (NEALE and ADAMS, 1981) and *A. lasiocarpa* (SHEA, 1988). The hypothesis that these two enzymes are coded by a single locus each has been supported by MITTON et al. (1979), KING and DANCIG (1983) and ADAMS et al. (1990) for GDH and MILLAR (1985), HARRY (1986) and ADAMS et al. (1990) for CAT. Both enzymes showed multimeric subunit structures in other species (tetrameric in CAT and hexameric in GDH according to WEEDEN and WENDEL, 1989).

Segregation distortion

No resolution difficulties were found for ACO and 6-PGD, and heterogeneity over the few mother trees tested (3 trees for ACO and 2 for 6-PGD) was not significant. These results indicate that the cause for segregation distortion is genetic and suggest that, as only the surviving gametes are tested, selection has occurred against the under-represented alleles or against other deleterious alleles linked to them. Distortion was barely significant ($0.05 < p < 0.10$, table 1) for four other loci (LAP, PGI2, MNR1 and MNR2). *Abies borisii regis* is considered to be a hybrid species which emerged at the end of the last Ice Age. Parental *A. cephalonica* and *A. alba* types can be found together with intermediate types in the same stand (FADY et al., 1991). Thus, aberrant meiotic products or other causes linked with interpopulation crosses (STRAUSS and CONKLE, 1986), could be responsible for the appearance of deleterious effects leading to distortion in a number of enzyme systems. Deleterious effects could be amplified by the fact that the mating system of Greek firs seems to be highly consanguineous (FADY, 1990).

Linkage

All possible polymorphic pairs of loci except MNR(2) and 6-PGD were found in at least one tree. Genetic linkage was tested using chi-square tests (BAILEY, 1961). Two locus segregation was homogeneous for all pairs in which more than one mother tree was involved. Thus, only combined data are given in table 2. Strong evidence of linkage was observed for two pairs of loci: GR-MNR1 ($p = 10^{-4}$) and PGI1-MNR1 ($p = 0.028$). For all other

Table 2. — Two locus segregation patterns and chi-square tests for linkage analysis.

| enzyme locus (locus1 : locus2) | allelic combination | | | | N | heterogeneity | | segregation at locus 1 | | segregation at locus 2 | | joint segregation | |
|-----------------------------------|---------------------|----|----|----|-----|---------------|-------|---------------------------|-------|---------------------------|------------------|----------------------|------------------|
| | 11 | 12 | 21 | 22 | | χ^2 (df) | p* | χ^2 (df=1) | p** | χ^2 (df=1) | p** | χ^2 (df=1) | p** |
| ACO : ACP2 | 12 | 16 | 9 | 7 | 44 | - | - | 3.273 | 0.070 | 0.091 | 0.763 | 0.818 | 0.3657 |
| ACO : GOT3 | 13 | 13 | 8 | 7 | 41 | - | - | 2.951 | 0.086 | 0.024 | 0.876 | 0.024 | 0.876 |
| ACO : MNR1 | 6 | 8 | 11 | 8 | 33 | 7.667 (6) | 0.264 | 0.758 | 0.384 | 0.030 | 0.862 | 0.758 | 0.384 |
| ACP2 : GOT3 | 13 | 8 | 7 | 12 | 40 | - | - | 0.100 | 0.752 | 0.0 | 1.0 | 2.500 | 0.114 |
| GR : ACP2 | 11 | 9 | 6 | 9 | 35 | - | - | 0.714 | 0.398 | 0.029 | 0.866 | 0.714 | 0.398 |
| GR : MNR1 | 0 | 60 | 59 | 0 | 119 | 3.205 (21) | 1.000 | 0.008 | 0.927 | 0.008 | 0.927 | 119.0 | 10 ⁻⁴ |
| LAP : GOT3 | 12 | 2 | 8 | 0 | 22 | 0.573 (3) | 0.903 | 1.636 | 0.201 | 14.727 | 10 ⁻⁴ | 0.182 | 0.669 |
| LAP : GR | 17 | 14 | 12 | 13 | 56 | 10.840 (6) | 0.093 | 0.464 | 0.496 | 0.071 | 0.789 | 0.286 | 0.593 |
| LAP : MNR1 | 18 | 21 | 10 | 18 | 67 | 13.325 (9) | 0.148 | 1.806 | 0.179 | 1.806 | 0.179 | 1.866 | 0.172 |
| LAP : PGI1 | 18 | 24 | 13 | 19 | 74 | 12.415 (12) | 0.413 | 1.351 | 0.245 | 1.946 | 0.163 | 0.0 | 1.0 |
| PGI1 : ACO | 28 | 23 | 19 | 18 | 88 | 18.343 (18) | 0.433 | 2.227 | 0.136 | 0.409 | 0.522 | 0.182 | 0.670 |
| PGI1 : ACP2 | 9 | 7 | 9 | 12 | 37 | - | - | 0.676 | 0.411 | 0.027 | 0.869 | 0.676 | 0.411 |
| PGI1 : GR | 24 | 28 | 32 | 24 | 108 | 16.312 (18) | 0.571 | 0.148 | 0.700 | 0.148 | 0.700 | 1.333 | 0.248 |
| PGI1 : GOT2 | 8 | 14 | 15 | 16 | 53 | 17.203 (12) | 0.142 | 1.528 | 0.216 | 0.925 | 0.336 | 0.472 | 0.492 |
| PGI1 : GOT3 | 7 | 9 | 11 | 8 | 35 | 2.981 (6) | 0.811 | 0.257 | 0.612 | 0.029 | 0.866 | 0.714 | 0.398 |
| PGI1 : MNR1 | 42 | 26 | 31 | 41 | 140 | 15.204 (21) | 0.813 | 0.114 | 0.735 | 0.257 | 0.612 | 4.829 | 0.028 |
| PGI1 : PGI2 | 30 | 24 | 23 | 13 | 90 | 20.074 (12) | 0.066 | 3.6 | 0.058 | 2.844 | 0.092 | 0.178 | 0.673 |
| PGI2 : ACP2 | 11 | 16 | 8 | 7 | 42 | - | - | 3.429 | 0.064 | 0.381 | 0.537 | 0.857 | 0.355 |
| PGI2 : GOT3 | 11 | 13 | 11 | 5 | 40 | - | - | 1.600 | 0.206 | 0.400 | 0.527 | 1.600 | 0.206 |
| PGI2 : MNR1 | 19 | 15 | 13 | 12 | 59 | 7.667 (6) | 0.264 | 1.393 | 0.241 | 0.424 | 0.515 | 0.153 | 0.696 |

*) probability of heterogeneity between trees due to change alone

***) probability of deviation from Mendelian expectations due to chance alone

pairs, no linkage was detected. No recombinants were found for the GR-MNR1 group ($r = 0$) and linkage is total. For the PGI1-MNR1 linkage group, $r = 0.41 \pm 0.04$. Linkage between PGI and MNR has been described in STRAUSS and CONKLE (1986) for *Pinus attenuata*. Since no significant linkage was found between GR and PGI1, the loci are probably positioned in the following sequence: GR-MNR1-PGI1.

Linkage between GR and MNR1 has never been tested before in conifers, and since conifers are thought to be highly conservation in their gene arrangements (STRAUSS and CONKLE, 1986), this linkage group could be characteristic of Pinaceae in the same way as, for example, the common GOT1-PGI2 linkage group (e. g. GURIES et al., 1978; O'MALLEY et al., 1979; STRAUSS and CONKLE, 1986; BARRET et al., 1987; MUONA et al., 1987), and thus used for taxonomic and phyletic studies in the Pinaceae.

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The Hybrid Origin of *Paulownia taiwaniana* Hu and Chang – Evidence from Isozyme Gene Markers

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Abstract

Based on an investigation of 6 enzyme systems coding for 13 gene loci, no variation between 202 *Paulownia taiwaniana* HU and CHANG trees was detected at any locus. The high level of heterozygosity (7 out of the 13 gene loci) is explained by the supposed hybrid origin of *P. taiwaniana*; the genetic uniformity is due to its exclusively vegetative propagation. The silvicultural significance of the findings (heterosis, susceptibility to diseases) as well as aspects of gene conservation are discussed. The potential of isozyme studies for a better understanding of the systematics of the genus *Paulownia* as well as for future breeding programmes is indicated.

Key words: *Paulownia* spp., isozymes, natural hybrid, heterosis, genotypic uniformity, gene conservation.

Introduction

The tree genus *Paulownia* (*Scrophulariaceae*) is widely distributed in China. Out of the 9 species described by the Chinese Academy of Forestry Staff (1986), three species are found on Taiwan: While Taiwan forms the eastern border of the wide range of natural distribution of *P. fortunei* HEMS. and *P. kawakamii* ITO, *P. taiwaniana* HU and CHANG is endemic to Taiwan. The latter taxon was recently described for the first time (HU and CHANG, 1975). It is frequently assumed to be a natural hybrid between the former two species (LI, 1978, WANG and HONG, 1979), because many phenotypic features are reported to be intermediate between *P. kawakamii* and *P. fortunei*. The most prominent of these features are: Size and colour of flowers, mode of branching of inflorescences, size of fruits (capsules) (HU and CHANG, 1975); shape of trichomes (HU and LIN, 1975); and some features of the wood anatomy (WANG, 1985).

Both *P. kawakamii* and *P. fortunei* are very rare on Taiwan. In one year of intensive search by this author no more than 8 *P. fortunei* and 20 *P. kawakamii* trees could be found. It is reported that some of those trees are relics

of natural stands. The extreme rarity is the result of incidents — mainly intensive logging and typhoons — during the last decades. Natural stands of *P. taiwaniana* are not known. Cultivation of *Paulownias*, which are named *P. taiwaniana* since HU and CHANG's publication in 1975, started around the beginning of this century (LIN, 1979). *P. taiwaniana* plantations were established on a large scale during the 1970's, the total plantation area covering more than 19,000 ha in 1977 (LIN, 1979). In addition to these plantations for wood production, which were predominantly established in the eastern part of Taiwan (namely in Hualien county), *P. taiwaniana* is a frequently found roadside tree all over Taiwan. The trees have been seriously infected by mycoplasma like organisms (MLOs) causing witches-broom disease since the late 1970's. The disease caused a decline in the silvicultural importance of the genus on Taiwan, and no new plantations were established during the last ten years.

P. taiwaniana is propagated exclusively vegetatively by means of root cuttings. Plantations are thus clones or clone mixtures. However, due to the uncertain origin of the species, the degree of genetic differentiation within and between plantations is unknown. This study involving isozyme gene loci aimed at obtaining information on genetic differentiation patterns within and between the native *Paulownia* spp. on Taiwan and relating the findings to silvicultural features, breeding potential, and problems of gene conservation of the genus.

Materials and Methods

All trees belonging to the taxa *P. kawakamii* and *P. fortunei* according to their phenotypic appearance were included in the genetic inventory, as both species are extremely rare on Taiwan (see above). One *P. fortunei* tree near Liu-Kuei (Kaoshiung county) is reported to be the relic of a small natural stand; 7 more *P. fortunei* trees were obviously artificially propagated. Out of the 20