THE DEMOGRAPHIC GENETICS OF AN APPALACHIAN STAND OF *Liriodendron tulipifera* L.

by

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Approved By

Chairman of Advisory Committee

...is dedicated to

Dr. Gene Namkoong,
and all who lead by
respecting and supporting
the contributions of others...

BIOGRAPHY

Milton Kanashiro was born on August 30, 1954 in Morro Agudo - SP, Brazil. He received his elementary and secondary education in Itapecerica da Serra - SP.

The author attended the Superior School of Agriculture "Luiz de Queiroz", University of São Paulo at Piracicaba and graduated with a Bachelor of Science degree in Forestry Engineering in January 1979.

In May 1979, he started working with EMBRAPA, the Brazilian Agency for Agriculture Research, at the Center of Agriculture Research for Humid Tropics (CPATU), carrying out forestry research projects.

In Fall of 1984 he came to North Carolina State University to continue his education. In May 1987, he was awarded with a Master of Science degree in Forestry with minor in Genetics. Afterwards he pursued further education, seeking his Doctoral degree in the same field of study.

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ABSTRACT

KANASHIRO, MILTON. The demographic genetics of an Appalachian stand of *Liriodendron tulipifera* L. (under the direction of Dr. Gene Namkoong).

Temporal and spatial genetic comparisons were made in an Appalachian stand of *Liriodendron tulipifera* L.(tulip tree). The population was stratified into five age classes, and allele frequencies were tested by homogeneity chi-square tests within and among plots. The homogeneity in allele frequencies were also evaluated for plot and subplot differences, at different age levels.

Results revealed that the population of tulip tree is structured in time and space; however, neither one was recognized as a result of the natural selection process. The lack of consistent patterns for differences in allele frequencies for age classes does not support evidence for selection. One possible constraint may be the presence of rare alleles. Another explanation may be the divergence between genetic and statistical sampling at different life cycle stages.

The substructure in space is much stronger than in time. Significant differences in allele frequencies at different loci for plots within age classes suggest that the population is substructured at the subplot level. The substructure is more likely to be associated with the species' reproductive biological characteristics such as mixed mating

systems, and/or limited gene flow.

Statistical analyses either in space or time for the two most common alleles show the probability levels to be always higher than when all alleles are included. Although rare alleles may mask the presence of selection, it is important to consider them because depending upon how they are grouped (space or time), their frequencies change considerably.

Average heterozygosity for age classes over loci revealed that there are significant changes from age class 0 (newly germinated seedlings) to age class 1 (saplings of 3 - 5 years old), and from age class 1 to age class 2 (saplings of 7 - 10 years old). This change seems to be related with elimination of homozygous genotypes originated from selfing or from limited gene flow. The elimination occurs at an early stage in the life cycle. The fixation indices do not indicate that genotypic frequency distributions go beyond the frequencies expected by Hardy-Weinberg law. Thus the data do not support the heterozygote superiority hypothesis.

Linkage disequilibrium tests showed no evidence that alleles of different loci are selectively pairing together more frequently than expected by random association.

1. INTRODUCTION

It is not a novelty to state that plant and animal populations exhibit considerable amounts of genetic variation. Much information has been published, encompassing many diverse organisms, most notably after the zymogram technique was developed in the 1950's (Smithies 1955, Hunter and Markert 1957).

The important question is not simply how much genetic variation there is in a population, but what the nature of the variation for fitness is in that population (Roughgarden 1979). In other words, what is the meaning of the genetic variation in a natural population (Hamrick, 1982)? An accurate answer can be found if measures of genetic variation are reliable. Reliability may be questioned, because comparatively few isozymes are used in studies designed to assess genetic variation within a population, and it is assumed that these isozymes are representative of the entire genome.

When allozyme variation is detected, the question arises: What is the association between the variation and the evolutionary forces that maintain this polymorphic condition? The neutralist hypothesis states that a considerable proportion of amino acid substitutions in proteins are irrelevant to their functions, and therefore they are selectively neutral. The selectionist hypothesis states that a high proportion of polymorphism is maintained through a selection process. The controversy remains unresolved (Lewontin 1974, Roughgarden 1979, Forsyth 1986).

Hamrick (1982) suggests that an intermediate position between the extreme selectionist and neutralist viewpoints is desirable. He states that "...some alleles at some loci in some species are not acted upon by detectable levels of selection. Other alleles at other loci in other species will be shown to be under the influence of intense selection pressure".

Roughgarden (1979) recommends two approaches to evaluate this controversy: a) to derive predictions from the neutrality hypothesis and test this prediction against the data; b) to search for direct evidence of the kind of natural selection which produces polymorphism. Lewontin (1974, p.261) discusses the possibility of checking the neutralist or neo-classical theory by demonstrating *in vitro* that the kinetics of different allozymic forms are indeed different. He says,

> If a large proportion of allozyme variants were detectably different from each other in their *in vitro* kinetics, it would be difficult although not impossible to maintain that the organism could not detect the difference. This certainly would put the neo-classical theory in a shaky position. Conversely, the failure to find a kinetic difference would not mean much, since the demands on a molecule *in vivo* are certainly much more complex than *in vitro*. Thus, there is some difficult to probe this hypothesis.

Lewontin cites several examples which infer heterozygosity superiority. He mentions findings by Koehn which relates the significant allozyme activity differences to cline in nature for *Catostomus clarkii* at a polymorphic esterase

locus. However, Lewontin is aware of constraints that are inherent to balancing selection (*e.g.* huge inbreeding depression predicted and not observed, heterozygosity does not seem to be sensitive to ecological stringency).

Mitton (1983) presents evidence that supports the heterozygosity superiority model. His enzyme kinetics studies in *Pinus ponderosa* revealed that a genotype heterozygous at the peroxidase locus has a broader thermal spectrum efficiency between cold and warm temperature, compared to different homozygous genotypes which were more efficient in temperatures that were either cold or warm. He concludes that a single protein polymorphism can profoundly influence fitness, and there are often advantages experienced by highly heterozygous individuals. The classic example of this phenomenon is the resistance to malaria found in humans heterozygous for sickle-cell gene (Friedman and Trager 1981).

When considering gene action in tree breeding programs Namkoong (1984) questions the existence of general heterozygosity superiority and whether its effect can be simultaneously captured in multiple loci . He discusses among other things situations in which heterozygous superiority could be the causal agent for observed departures from Hardy-Weinberg expectations. However, he does not rule out the possibility that those observations may reflect random genotypic distributions in populations which had different initial genotypic frequencies, since the evidence to support heterozygosity superiority are generally limited to trees within older

stands which are of post-reproductive age and of unknown initial frequency distributions. Bush *et al.* (1987) searching for evidence to support the positive correlation between heterozygosity and fitness, recall the importance of rejecting the neutralist hypothesis not necessarily assuming that a locus-specific selection is taking place for the loci markers under consideration.

Although heterozygosity estimates are very important measures to understand population dynamics, is important to be aware of how this measure can be affected to avoid misinterpreting the genetic data. Different genotypes may reproduce in different years and natural selection operating at different life cycle stages may involve different forces in different years; therefore, generations of different genetic contributions may exist within a multiaged stand (Linhart *et al.* 1981a). As a consequence, this leads to a selectively structured population in time and space. However, mating structure (selfing *vs.* outcrossing) or random mating with limited gene flow can also affect heterozygosity due to population subdivision without selection (Hamrick 1982, Namkoong 1984, Namkoong *et al.* 1988). If there are consistent trends in selection, then consistent changes in age classes should be observed for the selected alleles. If seedlings initially have a uniform allelic distribution, the trends could be observed most easily, but even if they initially varied, directional selection should produce

consistent trends. However, if no trends are observed and if initial allele distributions are mixed or random, in spite of any mortality, then the weight of evidence would support neutrality. Therefore, seeking evolutionary forces that explain the polymorphism present in populations, requires information on mating systems as well as a well designed study to help differentiate effects which might help to elucidate the interpretation of genetic information.

Roughgarden (1979) maintains that greater insight into population structures and their ecology is needed if one is to search for selection pressure other than heterozygosity superiority. Evidence is increasing to support the idea that plant populations are not randomly arranged assemblages of genotypes but are actually structured in space and time. Such evidence is shared by Marshall and Allard (1970), Bradshaw (1972), Hamrick and Allard (1972), Allard *et al.* (1972), Clegg and Allard (1973), Kahler *et al.* (1975), Schaal (1975), Schaal and Levin (1976), Mitton *et al.* (1977), Clegg *et al.* (1978a, b), Brown (1979), Hamrick (1982), Mitton (1983), Loveless and Hamrick (1984), Rice and Jain (1985).

Plant studies concerned with genetic variation patterns show that variation is associated with certain life history characteristics. Forest tree populations generally maintain higher levels of variability than populations of shorter-lived species (Hamrick *et al.* 1979, 1981, Linhart *et al.* 1981a,b).

Loveless and Hamrick (1984) identified several ecological and life history traits which are likely to be particularly important in determining genetic structure: floral morphology, mode of reproduction, pollination mechanisms, seed dispersal, seed dormancy and phenology, life cycle, timing of reproduction, successional stage, geographical range, population size, population density and population structure. Despite their importance, there is little descriptive or experimental data that permit separation of the multiple effects of ecological traits. Loveless and Hamrick (1984) emphasize the need for comprehensive samples on different geographical scales (hierarchical sampling design). They also recognized the need to understand the temporal genetic structure of a population (*i.e.* stability) or how it is related to population growth or to demographic changes over time. More comprehensive and comparative studies are needed in which the effects of life history features are isolated and maximized within a single group or in related taxa.

Unlike tropical tree species which show a wide range of variation in pollination systems, trees from temperate zones are predominantly wind-pollinated. Wind pollination permits pollen from a particular tree to find its way far from the source of release. However, the bulk of pollen is distributed leptocurtically around the father resulting in tree populations which might consist of clusters of related

individuals. Such clusters include individuals which may suffer from inbreeding depression (Tigerstedt *et al.* 1982).

Natural forest tree populations in the temperate zone, often regenerate through bursts of highly dense and relatively even-aged seedling stands, a phenomenon more pronounced in conifers than in hardwoods which can regenerate from stump sprouts or seedling sprcuts (Oliver 1980). Establishment of relatively even-aged stands can be caused by ecological disruptions such as forest fires or storms, or they can result from having favorable environmental conditions for flowering and seed set in a given year. Tigerstedt *et al.* (1982) consider selection intensity of 10^{-3} to 10^{-6} is normally involved due to random or directional selection, from seedling stage to mature trees. The array of genotypes in a population would be affected differently by drastic reductions in population density if the reductions were driven by genetically associated causes rather than if selection were genetically random (stochastic elimination). Even small differences in selection coefficients would result in large differences in genotypic survival with such heavy mortality.

That there is a higher proportion of homozygote genotypes among seedlings than expected on the basis of random mating has been shown in studies with populations of *Pseudotsuga menziensii* var. *menziensii* (Shaw and Allard 1982), *Pinus ponderosa* (Farris and Mitton 1984), and *Pinus sylvestris* (Yazdani *et al.* 1985, Muona *et al.* 1987). Such excesses

of homozygosity generally disappear by the time a stand has reached maturity, and in some cases a slight excess of heterozygosity has been observed (Farris and Mitton 1984; Yazdani *et al.* 1985). Changes in genotypic frequency may occur before germination due to embryonic lethals (Koski 1982). Tigerstedt *et al.* (1982) found that 100-year old trees in a regenerated stand of *P. sylvestris* exhibited a proportion of homozygotes which departed significantly from Hardy-Weinberg equilibrium.

Although it is an insect-pollinated broadleaved species, the tulip tree (*Liriodendron tulipifera* L.), a member of the Magnoliaceae family seems to be one of the cases which fits the model described by Tigerstedt *et al.* (1982). Described by Brotschol (1983) as having a mixed mating system, tulip tree is a unique species among eastern United States' hardwoods because it is a dominant tree in both pioneer stands on good sites and in old-growth stands that are otherwise climax (Buckner and McCracken 1978). Approximately 99% of the tulip trees present in a stand regenerated subsequent to a clearcutting operation are of seedling origin (Minckler and Woerheide 1965). Pure stands are considered to be temporary and gradually tulip trees are expected to be replaced by more tolerant shade species (Fowells 1965). A study conducted by Della-Bianca (1983) shows that the population density from the seedling stage decrease considerably with age. The reduction in number, while associated with life

history characteristics, may be driven by both deterministic and stochastic elimination processes.

Some genetic variation studies have been done with tulip tree (Kellison 1966, 1970, Brotschol 1983; Parks *et al.* 1983, 1990). Parks *et al.* The species has a moderate degree of heterozygosity (H=.192, Parks, personal communication). Brotschol (1983) reported an excess of homozygotes in the seed population of a tulip tree stand, but the adult population showed no evidence of departure from Hardy-Weinberg equilibrium. It is unknown if populations ever achieve an excess of heterozygotes or when or if major genotypic frequency shifts occur.

Information of this sort is important, for recognizing the structure among and/or within populations. Insights into a species' biology and an ability to genetically manipulate a species is important whether one's interests are forest management, breeding, and/or conservation programs. The following questions are of special concern to understand the genetic architecture of a natural tulip tree population:

1) Are there differences in population genetic structure in time, and/or space?

2) Are differences observed over short distances?

3) Are changes detectable at an early stage of the life cycle? Are all periods during the life cycle equally responsive to selection pressure?

4) If changes in population genetic structure are observed, do they involve random or directional selection?

5) Does average heterozygosity change in time?

6) Is there any evidence for heterozygote superiority?

7) Is there any evidence for correlation among alleles at different loci?

2. MATERIAL AND METHODS

2.1. The species

Liriodendron tulipifera L., called tulip tree throughout this report is one of the two extant species of the genus *Liriodendron*. The other is *L. chinense* (Hemls.). Paleobotanical evidence indicate that the genus used to include many more species and was more widely distributed in the northern hemisphere during the late Cenozoic than it is at present (Parks *et al.* 1983, 1990). This genus represents a classic bitypic distribution pattern.

The tulip tree ranges in the United States from Louisiana and Florida to southern New England (28° to 42° N latitude) and from the Atlantic Ocean to the West the Mississipi River (Della-Bianca 1983). The species is found from near sea level to elevations of 1400 meters in the Appalachian Mountains. Although best growth is attained on moist, well-drained, loose-textured soils, tulip tree may be found on shallow-soil ridges (Kellison 1970). Tulip tree is a component of 16 forest cover types, and is a major species in 4 of these (Fowells 1965). The forest cover types where the species figures as a major component are: Tulip tree, tulip tree - hemlock (*Tsuga canadensis*), tulip tree - white oak (*Quercus alba*) - northern red oak (*Q. rubra*), and tulip tree - sweetgum (*Liquidambar styraciflua*).

Information on the life history and silvicultural perpectives of tulip tree is provided in several reports (Clark and Boyce 1964, Fowells 1965, Minckler and Woerheide 1965,

Beck and Della-Bianca 1981, and Della-Bianca 1983).

2.2. Site characterization

The site used in this study is located in Pisgah National Forest, near Brevard, Transylvania County, North Carolina.

The natural population study is at Cove Creek, (*ca.* 1000 m elevation), and is characterized by cove type formation or mixed mesophitic forest. This cove formation shelters species such as sweet buckeye (*Aesculus octandra* Marsh.), basswood (*Tilia heterophylla* Vent.), sugar maple (*Acer sacharum* Marsh.), silver bell (*Halesia monticola* Sarg.), beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britton), and hemlock [*Tsuga canadensis* (L.) Carr.].

The population covers an area of approximately 4 ha and is a result of previous clearcutting operations. The oldest tulip trees are now over 60 years old. The access to this area has been closed since the last thinning operation which occurred about 10 years ago. Thinning has allowed more light to penetrate the canopy so tulip tree saplings can be found throughout the cove. The saplings are concentrated in areas that receive greater sunlight.

2.3. Design of the study

Since the main objective was to evaluate how the population is genetically structured, temporally and

spatially, the population area was divided into plots A, B, and C with areas of 1.75 ha, 1.16 ha, and 1.10 ha, respectively. Each plot was subdivided, providing a total of 10 subplots, (A1, A2, A3, B1, B2, B3, B4, C1, C2, and C3), with sizes ranging from 500 to 1200 m^2.

The population in each plot could be divided into age classes. Fifty adult trees were sampled in each large plot and this constituted age class "3". It is assumed that all age class 3 trees have reached the reproductive age. Other age classes hereoften designated as "2", "1", "0", and "S" were sampled at the subplot level. Fifty individual plants were sampled in each subplot per age class resulting in 40 sub-populations.

Saplings were classified into age class either 2 or 1 according to height. A previous investigation revealed that age was predicted more accurately by height than by diameter. Plants that were included in age class "2", are all 7 - 10 years old, and are taller than 2.0 m. Age class "1", consisted of trees 3 - 5 years old, and were between 0.40 - 1.0 m. in height. Caution was taken during the sorting phase to use only plants of seedling origin.

The age classes "0" and "S" contain plants which originated from the same seedfall (fall, 1987). The age class "0" comprises seedlings that germinated in the natural field conditions following one winter season. The age class "S" is represented by seedlings grown from seeds that were

stratified and sown in controlled conditions. Seeds were stratified at 3 °C for a period of three months and then were sown in flats containing vermiculite, peat moss, and perlite in proportion 2:2:1 (metromix). Flats were placed inside the greenhouse at 25 °C during seed germination and early growth. At the cotyledon leaf stage, seedlings were transplanted into individual tubes. After the conditioning period of transplanting, seedlings were grown exposed direct to sunlight for four months.

Seed counts from 20 small squares (0.25 m^2) demarcated on the ground were recorded to estimate the number of newly fallen seeds per hectare. The collected seeds were stratified in a cold room (same conditions described above) and sown in flats containing a metromix medium and covered with vermiculite. This germination test was done to estimate the potential number of seedlings that might grow following a winter season provided that there are conditions conducive to successful seed germination.

The small square areas used to collect and record number of seeds were also used to evaluate the potential contribution of stored seeds. Soil samples from small squares were collected to a depth of 80 mm , washed, and the residual material was sown to evaluate the presence of viable seeds. Tulip tree seeds could not be seen in the washed sample residues because the samaras had deteriorated. The number of seedlings germinated from the sample residues material was used to estimate the potential contribution of residual

seeds in the soil to a new seedling stock, provided the environment supplies conditions which permit effective germination.

2.4. Collection and handling of leaf sample.

Enzymes were extracted from leaf tissue which was collected late in the summer (August 1988). One or two leaves from each plant, were placed in a sealable plastic bag with moist towel paper, and immediately stored in a cooler until the samples were taken to the laboratory. Leaves could be stored, maintaining their freshness, for up to 10 days. The condition of the leaves at the time of collection, and storage conditions between field and laboratory cold room greatly influenced the length of time the leaves remained fresh.

Preparation of the samples, was done by the use of mortars which were placed in a tray filled with ground ice for cooling before processing the samples in order to preserve the enzymes intact. A 2.5 cm X 2.5 cm piece of leaf tissue was cut into smaller pieces and placed in a mortar. Approximately 0.5 ml extraction buffer (Appendix A) was added with a spatula tip of polyvinylpolypyrrolidone (PVPP) and sand. The leaf tissue was ground until it became a thick solution.

A 2.5 cm x 2.5 cm piece of Kimwipe paper was placed on each ground sample, and wicks were placed on top of the

paper. Since wicks were not to be used immediately they were wrapped in the piece of kimwipe paper which was used as a filter, placed into small vials and frozen immediately. Once in an ultra low freezer (-60 °C) samples remain in good conditions for at least 6 months (based on my own experience) without seriously affecting enzyme activity or resolution.

2.5. Eletrophoresis

Two gel systems were used to evaluate allozyme variation: a) lithium borate tricitrate (LBTC) with a pH=8.3, and b) morpholine citrate (MC) with a pH=6.5. Each system used gel matrices of the same volume, but they used different concentrations of sucrose and gel buffer (Appendix A).

Gels were subjected to a direct current of approximately 14.5 watts for 6 hours in the LBTC system and 19 watts for 7 hours in the MC system.

Seventeen isozymes were scored. Only twelve exhibited sufficient resolution of banding patterns to be included in the statistical analysis. The twelve are: catalase (CAT), superoxide dismutase (SOD2), phophoglucose isomerase (PGI2), glutamate oxalo acetate transaminase (GOT1), malate dehydrogenase (MDH1, MDH2, and MDH5), phosphoglucomutase (PGM1 and PGM2), triosephosphate isomerase (TPI1 and TPI2), and aconitase (ACO). Numbers following the capital letters refer to isozyme mobility. The most anodal is denoted number 1, and higher numbers are given when more than one zone of

activity is detected for isozymes as they appear farther from the anode (Cheliak and Pitel, 1985; O'Malley and Bawa, 1987; Parks *et al.*, 1990). Likewise, allele identification follows in order of decreasing eletrophoretic mobility within a given active zone (allele 3 more mobile than 5 in a given zone).

2.6. Gel interpretation

Gel interpretation was based on previous work of Parks *et al.* (1990).

Of the twelve isozymes assayed for polymorphism only PGM1 and TPI2 are undoubtedly monomorphic. CAT and MDH5 show variant alleles in a few cases and are considered in the discussion. MDH2 and TPI1 are diallelic loci, and SOD2, PGI2, GOT, MDH1, PGM2, and ACO are multiallelic with 3 or 4 segregant alleles (Fig. 1).

2.7. Statistical analysis.

The Biosys-1 computer program (Swofford and Selander 1981) and Statistical Analysis System (SAS) program were used for statistical analysis of the samples.

The allele frequency was calculated for age classes and chi-square (χ^2) tests were used to test for homogeneity or independence among age classes. Statistical analyses were conducted across plots, by plots, and within plots. Similarly, the location effect was evaluated across age classes,

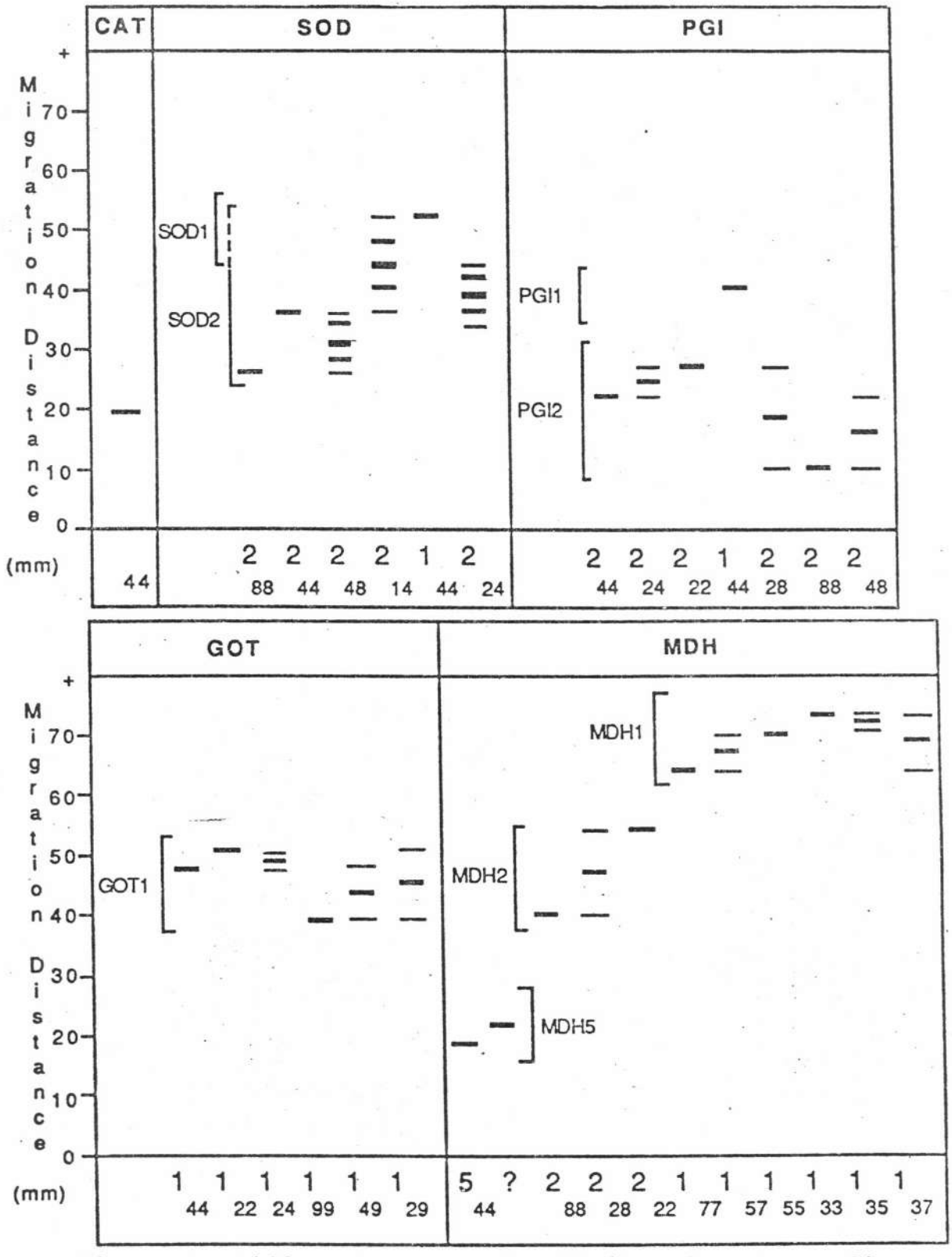
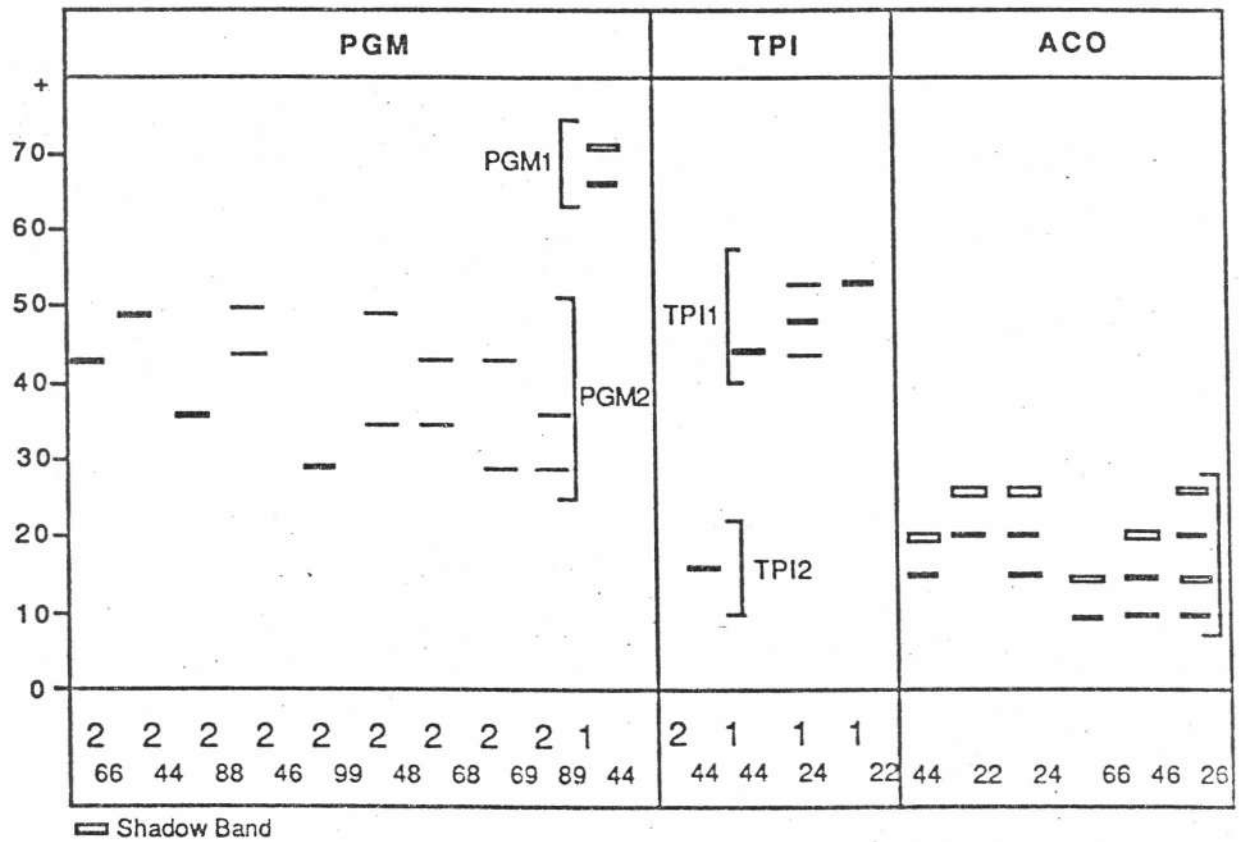


Figure 1. Different zones of activity of enzymes. The major genotypes of isozymes are represented according to their migration distance.

cont.



and by age class. For each age class the location effect was evaluated within plots. Tests were conducted including all alleles of polymorphic loci as well as only for the two most common alleles.

Reported X^2 values are likelihood ratio chi-square (G^2). The likelihood ratio chi-square statistic involves the ratios between the observed and the expected frequencies (SAS/STAT Guide 1985).

Average heterozygosity by age classes was estimated over all twelve loci following Weir *et al.* (1990a). Analysis of

variance was also conducted to evaluate the contribution of different sources of variation affecting the average heterozygosity. A mixed model was adopted, and all sources of variation, except loci were considered random variables.

Deviations of genotypic distribution from Hardy-Weinberg expectations were tested by X^2 tests for goodness of fit. The simplest approach is to measure departures from Hardy-Weinberg equilibrium with a set of disequilibrium coefficients, one for each heterozygous class at the locus in question (Weir 1990b). Both, estimates of deviation from Hardy-Weinberg expectation (fixation index) and linkage disequilibrium coefficients were obtained through procedures discussed in Weir and Cockerham (1989).

3. RESULTS AND DISCUSSION

3.1. Population demography

The vertical age structure of the population in the study averaged: age class 3, 38 trees/ha; age class 2, 1,100 saplings/ha; age class 1, 1,270 saplings/ha; age class 0, 718,414 seedlings/ha; and age class S, 8.5 million seeds/ha.

The average total seed and seedling density is higher compared to that of Fowells (1965) and Beck and Della-Bianca (1981), but it is not clear if those references refer to filled samaras or total samara production. The average percentage of seed germination using stratified seeds was 8.50% (± 0.758), higher than the 5% given by Clark and Boyce (1964).

Residual seeds in the ground produced an average of 2.9 (± 0.41) seedlings per 0.25 m^2. Although the extrapolation should be taken cautiously, this result implies that a considerable number of seedlings (120,000/ha) may be established if adequate ecological conditions prevails, even in the absence of fresh seeds. Clark and Boyce (1964) suggest management strategies if one wishes to rely on seeds stored in the ground. Seedlings from residual seed were not assessed for their allozymic variation because of the small sample size in this study.

3.2. Temporal structure

Allele frequencies by age classes across plots are presented in table 1, and statistical results are summarized

Table 1. Allele frequencies by age class across plots.

Locus	Allele	Age Class S	0	1	2	3
CAT	2	.005	.013			
	4	.995	.987	1.00	1.00	1.00
SOD2	1	.039	.014	.036	.040	.040
	2	.050	.030	.033	.040	.033
	4	.890	.934	.891	.885	.873
	8	.023	.021	.039	.036	.053
PGI2	2	.450	.460	.430	.436	.500
	4	.540	.530	.560	.556	.497
	8	.010	.010	.008	.008	.003
GOT	2	.329	.241	.258	.273	.237
	4	.458	.561	.521	.510	.438
	9	.213	.197	.221	.216	.325
MDH1	3	.832	.834	.837	.816	.787
	5	.077	.088	.087	.087	.097
	7	.090	.078	.075	.097	.117
MDH2	2	.137	.135	.135	.148	.153
	8	.863	.865	.865	.852	.847
MDH5	2	.005	.003	.001	.005	
	4	.995	.997	.999	.995	1.00
PGM1	4	1.00	1.00	1.00	1.00	1.00
PGM2	4	.452	.444	.420	.424	.426
	6	.102	.113	.142	.152	.144
	8	.404	.395	.373	.370	.376
	9	.042	.048	.065	.054	.054
TPI1	2	.057	.054	.053	.042	.053
	4	.943	.946	.947	.958	.947
TPI2	4	1.00	1.00	1.00	1.00	1.00
ACO	2	.130	.121	.148	.121	.087
	4	.780	.844	.783	.811	.870
	6	.090	.035	.069	.068	.044

Table 2. Change in allele frequencies due to age effect across plots.

Locus	df	χ^2	p
CAT	4	31.224	.000
SOD2	12	33.026	.001
PGI2	8	7.225	.513
GOT	8	38.990	.000
MDH1	8	8.445	.391
MDH2	4	1.407	.843
MDH5	4	5.421	.247
PGM2	12	21.587	.042
TPI1	4	2.595	.628
ACO	8	38.035	.000

in table 2. Age was a significant effect ($p<0.05$) for five of the ten polymorphic loci scored: CAT, SOD2, GOT, PGM2, and ACO. Allele 6 of the PGM2 locus is the only allele which exhibits a consistent frequency change pattern; *i.e.*, a slight increase with age. Otherwise, no pattern of change was found associated with age class. Allele frequencies of SOD2 and ACO change among age classes, but do not show a consistent directional change associated with age. Allele 4 at the GOT locus, shows a pattern of decreasing frequency as the population gets older but only if age class S is ignored, and there is no pattern for the other alleles at the GOT locus.

The CAT locus was shown to have highly significant changes in allele frequency, but all subpopulations sampled except B4S, C1S, and B40 (Appendix B, Table 1) were found to

be monomorphic. Because the variant allele 2 at the CAT locus has been scored in only 3 of 43 populations, this pattern cannot be distinguished from random variation.

A similar situation was found for MDH5. This locus shows a band pattern that was scored as a variant allele but also with a low frequency (≤.02). However, while rare, it is spread across the population and does not seem to be associated with any age class.

Other isozymes seemed to show trends for some alleles (MDH1 alleles 3 and 5 and MDH2, alleles 2 and 8), but the differences in allele frequencies were not statistically significant.

If any selective differences are associated with age the results support the assumption that these genes act independently, since no changes in allele frequency follow the same pattern among ages or areas.

Significant differences in allele frequency among plots exist for CAT, SOD2, GOT, PGM2, and ACO (Table 3). These are the same loci found to display significant differences across plots. Although the age class effect was found for the same five loci, the alleles that are significantly different are not the same as those displaying significant differences among plots. SOD2 and ACO are significant in plots A and B while GOT and PGM2 show significant differences only in plot B and C respectively.

Table 3: Change in allele frequencies due to (S 0 1 2 3) by plot.

Plot	Locus	df	X^2	p
A	SOD2	12	29.913	.003
A	PGI2	8	8.994	.343
A	GOT	8	14.684	.066
A	MDH1	8	8.539	.383
A	MDH2	4	3.314	.507
A	MDH5	4	3.022	.554
A	PGM2	12	15.796	.201
A	TPI1	4	1.991	.737
A	ACO	8	18.974	.015
B	CAT	4	31.488	.000
B	SOD2	12	22.052	.037
B	PGI2	8	3.001	.934
B	GOT	8	33.375	.000
B	MDH1	8	13.485	.096
B	MDH2	4	.622	.961
B	MDH5	4	2.459	.652
B	PGM2	12	14.900	.247
B	TPI1	4	4.906	.297
B	ACO	8	8.198	.414
C	CAT	4	6.658	.155
C	SOD2	12	15.810	.200
C	PGI2	8	4.064	.851
C	GOT	8	10.353	.241
C	MDH1	8	3.494	.900
C	MDH2	4	.701	.951
C	MDH5	4	5.181	.269
C	PGM2	12	30.376	.002
C	TPI1	4	4.360	.359
C	ACO	8	21.840	.005

No trends appear when considering pattern of allozyme distribution, by age class within plots. The only consistent allele frequency change with age in plot A (Appendix B, Table 2) was found for MDH2, but this was not statistically

significant. Allele 2 at the SOD2 locus and allele 9 at the GOT locus in plot B (Appendix B, Table 3) showed an increase in frequency from age class 0 to age class 3. There is no other pattern of change in plot B. A consistent increase in the frequency of allele 6 at the PGM2 locus is found in plot C as age increases from age class S to age class 3 (Appendix B, Table 4).

Chi-square tests, conducted using only the two most common alleles per locus showed an increase in *p* values, decreasing the number of loci in which changes in allele frequency by age class are significant (Table 3a). Hence, the analyses that include rare alleles (<*p*=0.01 *sec.* Brown, 1978) are more sensitive indications of population subdivisions. Of the isozymes studied, all but GOT and MDH2 have at least one rare allele.

Chi-square tests for homogeneity in allele frequency at the subplot level showed significant differences among age classes but not for the same loci.

Although analyses for age differences at the plot level showed PGM2 to have significantly changing allele frequencies only in plot C (Table 3), analyses at the subplot level revealed differences in allele frequency in subplot A3 (*p*=0.044 Table 4) and subplot B1 (*p*=0.011 Table 5). This finding could be explained by the two subpopulations having shared the same genetic background because of their proximity. This also may be interpreted as a subdivision of the population at the subplot level. When subplots were pooled

Table 4. Change in allele frequencies due to age effect (S 0 1 2) at subplot level for plot A.

Subplot	Locus	df	X^2	p
1	SOD2	9	21.662	.010
1	PGI2	6	6.602	.359
1	GOT	6	1.993	.920
1	MDH1	6	18.444	.005
1	MDH2	3	5.803	.122
1	MDH5	3	2.677	.444
1	PGM2	9	10.305	.326
1	TPI1	3	3.766	.288
1	ACO	6	15.561	.016
2	SOD2	9	19.359	.022
2	PGI2	6	7.588	.270
2	GOT	6	5.141	.526
2	MDH1	6	6.974	.323
2	MDH2	3	.107	.991
2	MDH5	3	2.770	.428
2	PGM2	9	13.162	.155
2	TPI1	3	6.935	.074
2	ACO	6	14.051	.029
3	SOD2	9	17.214	.045
3	PGI2	6	5.738	.453
3	GOT	6	21.652	.001
3	MDH1	6	17.770	.007
3	MDH2	3	7.124	.068
3	PGM2	9	17.339	.044
3	TPI1	3	4.228	.238
3	ACO	6	7.228	.300

Table 5: Change in allele frequencies due to age effect (S 0 1 2) at subplot level for plot B.

Subplot	Locus	df	X^2	p
1	SOD2	9	12.204	.202
1	PGI2	6	5.367	.498
1	GOT	6	10.434	.108
1	MDH1	6	5.965	.427
1	MDH2	3	.703	.873
1	MDH5	3	2.577	.462
1	PGM2	9	21.414	.011
1	TPI1	3	6.763	.080
1	ACO	6	11.827	.066
2	SOD2	9	19.890	.019
2	PGI2	6	7.172	.305
2	GOT	6	12.550	.051
2	MDH1	6	7.863	.248
2	MDH2	3	7.324	.062
2	MDH5	3	3.026	.388
2	PGM2	9	10.589	.305
2	TPI1	3	6.165	.104
2	ACO	6	17.980	.006
3	SOD2	9	18.024	.035
3	PGI2	6	5.618	.467
3	GOT	6	18.648	.005
3	MDH1	6	6.700	.349
3	MDH2	3	4.111	.250
3	MDH5	3	1.893	.595
3	PGM2	9	9.608	.383
3	TPI1	3	1.374	.712
3	ACO	6	11.177	.083
4	CAT	3	30.976	.000
4	SOD2	9	11.680	.232
4	PGI2	6	6.306	.390
4	GOT	6	16.871	.010
4	MDH1	6	16.948	.009
4	MDH2	3	4.107	.250
4	MDH5	3	2.811	.422
4	PGM2	9	12.684	.177
4	TPI1	3	.529	.912
4	ACO	6	4.824	.567

Table 6. Change in allele frequencies due to age effect (S 0 1 2) at subplot level for plot C.

Subplot	Locus	df	X^2	p
1	CAT	3	6.272	.099
1	SOD2	9	9.011	.436
1	PGI2	6	.756	.993
1	GOT	6	10.681	.099
1	MDH1	6	9.001	.174
1	MDH2	3	1.779	.620
1	MDH5	3	2.677	.444
1	PGM2	9	22.176	.008
1	TPI1	3	3.182	.364
1	ACO	6	4.313	.634
2	SOD2	9	9.836	.364
2	PGI2	6	5.711	.456
2	GOT	6	12.402	.054
2	MDH1	6	7.293	.295
2	MDH2	3	1.083	.781
2	MDH5	3	2.667	.446
2	PGM2	9	13.196	.154
2	TPI1	3	7.529	.057
2	ACO	6	15.843	.015
3	SOD2	9	8.480	.487
3	PGI2	3	1.422	.700
3	GOT	6	6.215	.400
3	MDH1	6	7.305	.294
3	MDH2	3	1.573	.666
3	MDH5	3	3.251	.354
3	PGM2	9	9.253	.414
3	TPI1	3	3.391	.335
3	ACO	6	21.151	.002

plots the differences in subplot A3 and B1 are not reflected in the other subplots, and a significant difference at the PGM2 locus at plot level was detected only in plot C. Within plot C, subplot C1 seems to account for all the difference (p=0.008, Table 6), since it is the only subplot exhibiting a significant X^2 value.

Significant differences at locus SOD2 were found in plot A (p=0.003) and B (p=0.037) (Table 3). In plot A (Table 4) the difference was consistent in the three subplots whereas in plot B (Table 5) only two adjacent subplots B2 and B3 appeared responsible for the significant difference observed. GOT and ACO showed significant X^2 values in some wholes plot, but not all subplots contribute equally to the differences. The MDH1 locus showed significant differences only within three subplots: A1, A3 and B4. These differences were masked when subplots were pooled within whole plots.

As in the analysis across plots and at the whole plot level, patterns of allele frequencies due to age class are not evident at the subplot level (Appendix B, Tables 5 - 14). In a few cases loci with a significant X^2 value one allele seemed to follow a pattern, such as alleles 5 and 7 at the MDH1 locus, subplot A1 (Appendix B, Table 5), and allele 6 at the PGM2 locus, subplot A2 (Appendix B, Table 6).

However, trends were not more noticeable or frequent than at other analysis levels.

Table 3a. Change in allele frequencies for two most common alleles due to age (S 0 1 2 3) effect.

Plot	Locus	df	X^2	p
A	SOD2	4	11.019	.026
A	PGI2	4	6.016	.198
A	GOT	4	8.508	.075
A	MDH1	4	3.956	.412
A	MDH2	4	3.314	.507
A	MDH5	4	3.022	.554
A	PGM2	4	2.378	.667
A	TPI1	4	1.991	.737
A	ACO	4	4.434	.350
B	CAT	4	31.488	.000
B	SOD2	4	7.323	.120
B	PGI2	4	1.576	.813
B	GOT	4	15.201	.004
B	MDH1	4	5.099	.277
B	MDH2	4	.622	.961
B	MDH5	4	2.459	.652
B	PGM2	4	4.488	.344
B	TPI1	4	4.906	.297
B	ACO	4	1.490	.828
C	CAT	4	6.658	.155
C	SOD2	4	2.797	.592
C	PGI2	4	1.152	.886
C	GOT	4	6.648	.156
C	MDH1	4	.346	.987
C	MDH2	4	.701	.951
C	MDH5	4	5.181	.269
C	PGM2	4	3.688	.450
C	TPI1	4	4.360	.359
C	ACO	4	7.520	.111

Table 4a. Change in allele frequencies for two most common alleles due to age (S 0 1 2) effect at subplot level for plot A.

Subplot	Locus	df	X^2	p
1	SOD2	3	2.613	.455
1	PGI2	3	3.925	.270
1	GOT	3	1.162	.762
1	MDH1	3	5.493	.139
1	MDH2	3	5.803	.122
1	MDH5	3	2.677	.444
1	PGM2	3	3.033	.387
1	TPI1	3	3.766	.288
1	ACO	3	8.632	.035
2	SOD2	3	4.780	.189
2	PGI2	3	5.844	.119
2	GOT	3	4.198	.241
2	MDH1	3	1.755	.625
2	MDH2	3	.107	.991
2	MDH5	3	2.770	.428
2	PGM2	3	3.117	.374
2	TPI1	3	6.935	.074
2	ACO	3	1.258	.739
3	SOD2	3	5.064	.167
3	PGI2	3	.101	.992
3	GOT	3	20.181	.000
3	MDH1	3	6.997	.072
3	MDH2	3	7.124	.068
3	PGM2	3	3.085	.379
3	TPI1	3	4.228	.238
3	ACO	3	6.003	.111

Table 5a. Change in allele frequencies for two most common alleles due to age (S 0 1 2) effect at subplot level for plot B.

Subplot	Locus	df	χ^2	p
1	SOD2	3	7.632	.054
1	PGI1	3	1.149	.765
1	GOT	3	8.750	.033
1	MDH1	3	5.537	.136
1	MDH2	3	.703	.873
1	MDH5	3	2.577	.462
1	PGM2	3	5.539	.136
1	TPI1	3	6.763	.080
1	ACO	3	8.764	.033
2	SOD2	3	12.178	.007
2	PGI2	3	2.794	.424
2	GOT	3	5.588	.133
2	MDH1	3	3.931	.269
2	MDH2	3	7.324	.062
2	MDH5	3	3.026	.388
2	PGM2	3	9.523	.023
2	TPI1	3	6.165	.104
2	ACO	3	2.735	.434
3	SOD2	3	3.195	.362
3	PGI2	3	2.941	.401
3	GOT	3	11.858	.008
3	MDH1	3	.354	.950
3	MDH2	3	4.111	.250
3	MDH5	3	1.893	.595
3	PGM2	3	.940	.816
3	TPI2	3	1.374	.712
3	ACO	3	3.140	.371
4	CAT	3	30.976	.000
4	SOD2	3	2.889	.409
4	PGI2	3	1.728	.631
4	GOT	3	8.417	.038
4	MDH1	3	7.112	.068
4	MDH2	3	4.107	.250
4	MDH5	3	2.811	.422
4	PGM2	3	1.778	.620
4	TPI1	3	.529	.912
4	ACO	3	1.333	.721

Table 6a. Change in allele frequencies for two most common alleles due to age (S 0 1 2) effect at subplot level for plot C.

Subplot	Locus	df	χ^2	p
1	CAT	3	6.272	.099
1	SOD2	3	7.454	.059
1	PGI2	3	.403	.940
1	GOT	3	6.634	.085
1	MDH1	3	1.504	.681
1	MDH2	3	1.779	.620
1	MDH5	3	2.677	.444
1	PGM2	3	3.484	.323
1	TPI1	3	3.182	.364
1	ACO	3	1.671	.643
2	SOD2	3	1.514	.679
2	PGI2	3	2.882	.410
2	GOT	3	10.642	.014
2	MDH1	3	3.573	.311
2	MDH2	3	1.083	.781
2	MDH5	3	2.667	.446
2	PGM2	3	6.191	.103
2	TPI1	3	7.529	.057
2	ACO	3	3.330	.343
3	SOD2	3	3.045	.385
3	PGI2	3	1.422	.700
3	GOT	3	5.168	.160
3	MDH1	3	1.999	.753
3	MDH2	3	1.573	.666
3	MDH5	3	3.251	.354
3	PGM2	3	1.506	.681
3	TPI1	3	3.391	.335
3	ACO	3	13.345	.004

When X^2 tests were performed on the two most common alleles only, the results were similar to what has already been discussed for the whole plot analysis (*i.e.*, significant differences decrease considerably). At subplot level, there were four cases in which inclusion of all alleles did not permit detection of allele frequency differences due to age class, however, when using only the two most common alleles significant differences were detected: GOT and ACO in subplot B1 (Table 5a), PGM2 in subplot B2 (Table 5a), GOT in subplot C2 (Table 6a).

Although significant changes in allele frequency by age class have been detected, inability to identify consistent allozyme patterns associated with age class at different spatial levels, suggests that if selection is present it is weak and inconsistent. Presence of rare alleles, found at all loci except GOT and MDH2, make the analysis more sensitive, but the results show that this sensitivity still reveals no consistent pattern. Because of environmental variation in time and the environmental influence on some life history characteristics (*i.e.*, reproduction), the different age classes studied, may actually represent different genetic samples (*i.e.* different gene pool).

3.3. Spatial structure

Allele frequencies for whole plots, combined across age classes, are shown in table 7. All isozymes except MDH5 and TPI1 exhibited significant differences in frequency (Table

Table 7. Allele frequencies by plot combined across age classes.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2		.010	.002
	2	1.00	.990	.998
SOD2	1	.031	.021	.051
	2	.045	.030	.038
	4	.907	.930	.845
	8	.017	.019	.065
PGI2	2	.470	.467	.405
	4	.525	.524	.582
	8	.005	.008	.013
GOT	2	.278	.297	.238
	4	.515	.486	.523
	9	.207	.216	.239
MDH1	3	.759	.856	.857
	5	.113	.078	.068
	7	.128	.065	.074
MDH2	2	.167	.127	.128
	8	.833	.873	.872
MDH5	2	.002	.005	.002
	4	.998	.995	.998
PGM1	4	1.00	1.00	1.00
PGM2	4	.449	.453	.393
	6	.072	.123	.197
	8	.389	.367	.400
	9	.090	.055	.010
TPI1	2	.055	.049	.049
	4	.944	.951	.951
TPI2	4	1.00	1.00	1.00
ACO	2	.124	.145	.106
	4	.791	.778	.874
	6	.085	.077	.020

Table 8. Change in allele frequencies due to plot effect combined across age classes.

Locus	df	X^2	p
CAT	2	20.454	.000
SOD2	6	78.944	.000
PGI2	4	18.101	.001
GOT	4	13.233	.010
MDH1	4	58.772	.000
MDH2	2	10.901	.004
MDH5	2	2.816	.245
PGM2	6	173.903	.000
TPI1	2	.687	.709
ACO	4	76.120	.000

8) indicating that location is an important variable in the study of population structure.

Allozyme frequencies are more discrete by location than by age class. When examining a given locus one plot will differ in terms of allele frequency more than the other two (Table 7). Plot A and B have a similar pattern for PGI2, PGM2, and ACO, but plots B and C are more similar for MDH1 and MDH2. The allele frequencies for TPI1 and MDH5 are very homogeneous across locations.

Differences between plots are less pronounced when data are analyzed within each age class as compared to the analysis over all age classes, but the pattern of significant X^2 values is consistent (Table 9). The locus

Table 9. Change in allele frequencies due to plot effect within age classes.

Age	Locus	df	X^2	p
S	CAT	2	3.295	.193
S	SOD2	6	26.731	.000
S	PGI2	4	1.586	.811
S	GOT	4	3.558	.469
S	MDH1	4	7.198	.126
S	MDH2	2	.194	.908
S	MDH5	2	3.623	.163
S	PGM2	6	27.126	.000
S	TPI1	2	1.230	.541
S	ACO	4	22.126	.000
0	CAT	2	24.081	.000
0	SOD2	6	27.328	.000
0	PGI2	4	9.001	.061
0	GOT	4	9.822	.044
0	MDH1	4	30.603	.000
0	MDH2	2	2.168	.338
0	MDH5	2	2.258	.323
0	PGM2	6	59.827	.000
0	TPI1	2	1.339	.512
0	ACO	4	22.188	.000
1	SOD2	6	35.051	.000
1	PGI2	4	5.495	.240
1	GOT	4	2.954	.566
1	MDH1	4	17.004	.002
1	MDH2	2	3.107	.211
1	MDH5	2	1.834	.400
1	PGM2	6	70.702	.000
1	TPI1	2	1.627	.443
1	ACO	4	8.297	.081
2	SOD2	6	9.426	.151
2	PGI2	4	6.197	.185
2	GOT	4	11.703	.020
2	MDH1	4	7.011	.135
2	MDH2	2	6.051	.049
2	MDH5	2	.341	.843
2	PGM2	6	24.675	.000
2	TPI1	2	.979	.613
2	ACO	4	21.664	.000

cont.

cont.

3	SOD2	6	15.157	.019
3	PGI2	4	4.656	.324
3	GOT	4	4.618	.329
3	MDH1	4	14.029	.007
3	MDH2	2	2.610	.271
3	PGM2	6	31.058	.000
3	TPI1	2	4.174	.124
3	ACO	4	12.823	.012

PGM2 shows significant differences within all age classes. MDH1 does not reveal significant differences for age classes S and 2, yet the same trend in frequency is present over all age classes (plot A is distinct from B and C).

The frequency of allele 9 at the PGM2 locus is highest in plot A and lowest in plot C for all age classes (Appendix B, Tables 15 - 19). The allele 6 has the reverse trend.

Although the level of significance changes for some loci within a given age class, the allele frequency patterns shown by plots do not change (Table 7).

Similar to the age effect, the location effect was also considered cases where only the two most common alleles were included (Table 9a).

For location, most of the significant differences are masked when only the two most common alleles are included. There are cases that the probability levels are the same. For PGI2 at age class 0 the difference due to plot effect is not expressed for all alleles. It is expressed, however, when the two most common alleles are considered (allele 2

Table 9a. Change in allele frequencies for two most common alleles due to plot effect within each age class.

Age	Locus	df	X^2	p
S	CAT	2	3.295	.193
S	SOD2	2	9.926	.007
S	PGI2	2	1.380	.502
S	GOT	2	.741	.690
S	MDH1	2	4.989	.083
S	MDH2	2	.194	.908
S	MDH5	2	3.623	.163
S	PGM2	2	1.384	.501
S	TPI1	2	1.230	.541
S	ACO	2	4.730	.094
0	CAT	2	24.081	.000
0	SOD2	2	4.353	.113
0	PGI2	2	6.646	.036
0	GOT	2	2.242	.326
0	MDH1	2	7.671	.022
0	MDH2	2	2.168	.338
0	MDH5	2	2.258	.323
0	PGM2	2	6.359	.042
0	TPI1	2	1.339	.512
0	ACO	2	4.061	.131
1	SOD2	2	3.504	.173
1	PGI2	2	1.753	.416
1	GOT	2	2.105	.349
1	MDH1	2	6.530	.038
1	MDH2	2	3.107	.211
1	MDH5	2	1.834	.400
1	PGM2	2	5.333	.069
1	TPI1	2	1.627	.443
1	ACO	2	.440	.803
2	SOD2	2	2.490	.288
2	PGI2	2	4.233	.120
2	GOT	2	10.709	.005
2	MDH1	2	4.185	.123
2	MDH2	2	6.051	.049
2	MDH5	2	.341	.843
2	PGM2	2	4.341	.114
2	TPI2	2	.979	.613
2	ACO	2	5.559	.062

cont.

cont.

3	SOD2	2	.196	.907
3	PGI2	2	2.452	.293
3	GOT	2	3.705	.157
3	MDH1	2	6.371	.041
3	MDH2	2	2.610	.271
3	PGM2	2	.368	.832
3	TPI2	2	4.174	.124
3	ACO	2	1.941	.379

and 4). SOD2, MDH1, PGM2, and ACO are the most representative plot effects for all age classes. MDH1 shows significant difference in allele frequency at age classes 0, 1, and 3 for any level of alleles (including rare alleles) tested. MDH2 is barely significant, and the significance is expressed only for age class 2. The highest number of loci showing significant differences associated with spatial effect is found at age class 0. Considering that this age class, compared to 1, 2, and 3 has been subjected to less environmental pressure, the highest number of loci showing significant X^2 values due to plot effect indicates different population structure at early ages.

3.4. Spatial fine-structure and age effect

When the allele frequencies for each age class are considered within plots the trend of loci which allele frequencies are significantly different due to subplot effect is different than observed for whole plots (Tables 10, 11, 12, and 13).

Table 10. Change in allele frequencies due to subplot effect within plot for age class S.

Plot	Locus	df	χ^2	p
A	SOD2	6	4.893	.558
A	PGI2	4	5.800	.215
A	GOT	4	16.660	.002
A	MDH1	4	30.262	.000
A	MDH2	2	4.943	.084
A	PGM2	6	7.482	.279
A	TPI1	2	.358	.836
A	ACO	4	.795	.939
B	CAT	3	4.982	.173
B	SOD2	9	11.668	.233
B	PGI2	6	13.264	.039
B	GOT	6	26.825	.000
B	MDH1	6	5.693	.458
B	MDH2	3	11.026	.012
B	MDH5	3	1.672	.643
B	PGM2	9	24.844	.003
B	TPI1	3	.864	.834
B	ACO	6	3.702	.717
C	CAT	2	4.326	.115
C	SOD2	6	3.736	.712
C	PGI2	4	5.948	.203
C	GOT	4	4.954	.292
C	MDH1	4	6.262	.180
C	MDH2	2	1.886	.389
C	MDH5	2	2.295	.317
C	PGM2	6	14.643	.023
C	TPI1	2	8.614	.013
C	ACO	4	11.822	.019

Table 10a. Change in allele frequencies for two most common alleles due to subplot effect within plot for age class S.

Plot	Locus	df	χ^2	p
A	SOD2	2	.791	.673
A	PGI2	2	3.388	.184
A	GOT	2	9.170	.010
A	MDH1	2	18.874	.000
A	MDH2	2	4.943	.084
A	PGM2	2	.124	.940
A	TPI2	2	.358	.836
A	ACO	2	.376	.828
B	CAT	3	4.982	.173
B	SOD2	3	3.008	.390
B	PGI2	3	9.222	.026
B	GOT	3	23.610	.000
B	MDH1	3	3.462	.326
B	MDH2	3	11.026	.012
B	MDH5	3	1.672	.643
B	PGM2	3	7.601	.055
B	TPI1	3	.864	.834
B	ACO	3	1.429	.699
C	CAT	2	4.326	.115
C	SOD2	2	1.809	.405
C	PGI2	2	3.303	.192
C	GOT	2	2.980	.225
C	MDH1	2	2.085	.353
C	MDH2	2	1.886	.389
C	MDH5	2	2.295	.317
C	PGM2	2	6.041	.049
C	TPI1	2	8.614	.013
C	ACO	2	.154	.926

Table 11. Change in allele frequencies due to subplot effect within plot for age class 0.

Plot	Locus	df	χ^2	p
A	SOD2	6	7.839	.250
A	PGI2	4	6.269	.180
A	GOT	4	11.364	.023
A	MDH1	4	12.050	.017
A	MDH2	2	3.164	.206
A	MDH5	2	2.204	.332
A	PGM2	6	10.143	.119
A	TPI1	2	6.335	.042
A	ACO	4	14.168	.007
B	CAT	3	37.385	.000
B	SOD2	9	20.635	.014
B	PGI2	6	8.779	.186
B	GOT	6	12.161	.058
B	MDH1	6	11.177	.083
B	MDH2	3	5.435	.143
B	MDH5	3	2.783	.426
B	PGM2	9	16.712	.053
B	TPI1	3	3.380	.337
B	ACO	6	23.969	.001
C	SOD2	6	2.206	.900
C	PGI2	4	10.398	.034
C	GOT	4	10.125	.038
C	MDH1	4	6.864	.143
C	MDH2	2	.826	.662
C	PGM2	4	14.843	.005
C	TPI1	2	8.916	.012
C	ACO	4	3.229	.520

Table 11a. Change in allele frequencies for two most common alleles due to subplot effect within plot for age class 0.

Plot	Locus	df	X^2	p
A	SOD2	2	.970	.616
A	PGI2	2	4.065	.131
A	GOT	2	6.157	.046
A	MDH1	2	3.827	.148
A	MDH2	2	3.164	.206
A	MDH5	2	2.204	.332
A	PGM2	2	3.628	.163
A	TPI2	2	6.335	.042
A	ACO	2	8.083	.018
B	CAT	3	37.385	.000
B	SOD2	3	10.003	.019
B	PGI2	3	1.577	.665
B	GOT	3	7.469	.058
B	MDH1	3	2.290	.514
B	MDH2	3	5.435	.143
B	MDH5	3	2.783	.426
B	PGM2	3	8.309	.040
B	TPI1	3	3.380	.337
B	ACO	3	9.051	.029
C	SOD2	2	1.099	.577
C	PGI2	2	1.500	.472
C	GOT	2	5.128	.077
C	MDH1	2	.168	.919
C	MDH2	2	.826	.662
C	PGM2	2	13.721	.001
C	TPI1	2	8.916	.012
C	ACO	2	1.025	.599

Table 12. Change in allele frequencies due to subplot effect within plot for age class 1.

Plot	Locus	df	X^2	p
A	SOD2	6	20.667	.002
A	PGI2	4	2.212	.697
A	GOT	4	3.502	.478
A	MDH1	4	9.600	.048
A	MDH2	2	2.451	.294
A	PGM2	6	9.903	.129
A	TPI1	2	2.818	.244
A	ACO	4	4.999	.287
B	SOD2	9	9.509	.392
B	PGI2	6	3.600	.731
B	GOT	6	12.283	.056
B	MDH1	6	15.606	.016
B	MDH2	3	2.466	.482
B	MDH5	3	2.780	.427
B	PGM2	9	6.162	.724
B	TPI1	3	5.127	.163
B	ACO	6	17.152	.009
C	SOD2	6	3.045	.803
C	PGI2	4	5.555	.235
C	GOT	4	10.856	.028
C	MDH1	4	2.288	.683
C	MDH2	2	1.281	.527
C	PGM2	6	3.455	.750
C	TPI1	2	10.370	.006
C	ACO	4	14.655	.005

Table 12a. Change in allele frequencies for two most common alleles due to subplot effect within plot for age class 1.

Plot	Locus	df	χ^2	p
A	SOD2	2	3.476	.176
A	PGI2	2	.008	.996
A	GOT	2	.189	.910
A	MDH1	2	4.023	.134
A	MDH2	2	2.451	.294
A	PGM2	2	4.684	.096
A	TPI1	2	2.818	.244
A	ACO	2	4.467	.107
B	SOD2	3	.494	.920
B	PGI2	3	.817	.845
B	GOT	3	3.005	.391
B	MDH1	3	10.126	.018
B	MDH2	3	2.466	.482
B	MDH5	3	2.780	.427
B	PGM2	3	.583	.900
B	TPI1	3	5.127	.163
B	ACO	3	3.999	.262
C	SOD2	2	1.545	.462
C	PGI2	2	1.251	.535
C	GOT	2	8.970	.011
C	MDH1	2	.016	.992
C	MDH2	2	1.281	.527
C	PGM2	2	.917	.632
C	TPI1	2	10.370	.006
C	ACO	2	12.716	.002

Table 13. Change in allele frequencies due to subplot effect within plots for age class 2.

Plot	Locus	df	χ^2	p
A	SOD2	6	13.084	.042
A	PGI2	4	2.537	.638
A	GOT	4	6.633	.157
A	MDH1	4	7.595	.108
A	MDH2	2	3.359	.186
A	MDH5	2	2.204	.332
A	PGM2	6	3.227	.780
A	TPI1	2	4.728	.094
A	ACO	4	3.240	.518
B	SOD2	9	9.246	.415
B	PGI2	6	5.395	.494
B	GOT	6	3.641	.725
B	MDH1	6	3.318	.768
B	MDH2	3	3.898	.273
B	MDH5	3	2.783	.426
B	PGM2	9	15.994	.067
B	TPI1	3	5.547	.136
B	ACO	6	14.507	.024
C	SOD2	6	10.102	.120
C	PGI2	4	6.828	.145
C	GOT	4	6.433	.169
C	MDH1	4	8.835	.065
C	MDH2	2	.080	.961
C	MDH5	2	1.629	.443
C	PGM2	6	5.316	.504
C	TPI1	2	1.730	.421
C	ACO	4	7.424	.115

Table 13a. Change in allele frequencies for two most common alleles due to subplot effect within plot for age class 2.

Plot	Locus	df	χ^2	p
A	SOD2	2	1.330	.514
A	PGI2	2	.333	.846
A	GOT	2	1.556	.459
A	MDH1	2	1.664	.435
A	MDH2	2	3.359	.186
A	MDH5	2	2.204	.332
A	PGM2	2	.240	.887
A	TPI1	2	4.728	.094
A	ACO	2	1.897	.387
B	SOD2	3	6.303	.098
B	PGI2	3	.868	.833
B	GOT	3	1.009	.799
B	MDH1	3	.559	.906
B	MDH2	3	3.898	.273
B	MDH5	3	2.783	.426
B	PGM2	3	2.340	.505
B	TPI1	3	5.547	.136
B	ACO	3	4.076	.253
C	SOD2	2	6.449	.040
C	PGI2	2	2.490	.288
C	GOT	2	4.939	.085
C	MDH1	2	7.221	.027
C	MDH2	2	.080	.961
C	MDH5	2	1.629	.443
C	PGM2	2	1.845	.398
C	TPI1	2	1.730	.421
C	ACO	2	7.155	.028

During the early stage of development, mainly age class 0, the loci express more significant differences in allele frequency, and are similar to those at the plot level (Table 11). At later stages (age class 2), the subplot effect is reduced (Table 13). This trend is different when compared to the plot level where even at the adult stage, the significant differences in allele frequencies are expressed at the SOD2, MDH1, PGM2, and ACO locus (Table 9). The decrease of spatial heterogeneity at the subplot level at age class 2 could be interpreted as population subdivision in time.

TPI1 locus has not revealed significant differences in allele frequency either due to age and/or location effect at the plot level. The lowest *p* value observed due to age influence in allele frequency was at subplot C2 (Table 6, p=.057). However, within age classes S, 0, and 1, mainly in plot C, this isozyme has shown significant allozymic variation among subplots (Tables 10, 11, and 12).

Among age classes S, 0, and 1, the MDH1 shows significant X^2 values for changes in allele frequencies in plot A (Tables 10, 11, and 12), possibly due to subplot A1 and A3 (Table 4).

For plots, the SOD2 locus lacks evidence for significant differences in allozymic variation at age class 2. However, the subplot influence revealed significant X^2 values for age class 0 in plot B (Table 11), age class 1 in plot A (Table 12), and age class 2 in plot A (Table 13).

While significant differences due to plot influences are

observed in all age classes for the PGM2 locus, subplots display significant changes in allele frequency for age class S in plot B and C, and age class 0 in plot C.

The ACO locus showed significant X^2 values for age class S, 0, 1, and 2 within plots, but mainly in B and C.

The X^2 tests for the two most common alleles are consistent with what has been observed, *i.e.*, loci showed higher probability levels than when all alleles are included (Tables 10a, 11a, 12a, and 13a). However, there are cases such as ACO, MDH1, and SOD2 at age class 2 that the inclusion of only two most common alleles the differences became significant which were not otherwise.

The results do not provide evidence that the alleles are associated to plot or subplot within a given age class. The variant allele 2 observed in CAT was found only at age classes S and 0; Thus, its presence was considered as a sampling error.

The TPI1 locus shows significant differences at the subplot level. The difference is mainly in plot C for age class S, 0, and 1, where subplots C2 and C3 have lower frequencies for allele 2 than C1 (See appendix B, Tables 22, 25, and 28).

For PGM2, the age and location (plot and subplot level) effects are important. Across plots, especially for alleles 6 and 9, the difference among age classes for allele 6 is small and increase slightly with age [p=.10 (S), .11

(0), .14 (1), .15 (2), and .14 (3)], while allele 9 bounces around and does not show a pattern in frequency (Table 1). However, across space (Table 7) there is a considerable difference among plots A, B, and C for allele 6 (p=.072, .123, and .197 respectively), while for allele 9 there is a big decrease in frequency from plot A to C (p=.090, .055, and .011).

For whole plots, PGM2 shows significant differences in allele frequency mainly at plot C. At the subplot level significant differences are revealed for specific locations such as A3, B1, and C1, when the analyses are conducted including all alleles (Tables 4, 5, and 6). Within age classes (S, 0, 1, 2, and 3), this locus showed a significant location effect (Plot level, Table 9) in all of them. At the subplot level, significant differences were mainly at the early stages of the life cycle (S and 0) in plots B and C (Tables 10 and 11). Although, age is an important variable, the location has a stronger effect in showing significant differences in allele frequency.

There is no evidence to conclude that selection is present because specific alleles cannot be related with life cycle stages (age classes), and/or a strong pattern in allele frequency cannot be associated with location (plot or subplot), yet there is a strong location effect.

The location effect shows that the population is sub-structured, and is more likely to be related to the species' biological reproductive features (partial selfing, limited

gene flow, etc.) which contribute to a given amount of allelic variation to be confined in space, even within a very short distance (<100 m).

The results reported in this study agree with findings that show temporal differentiation in gene frequency to be less pronounced than spatial differentiation. Schaal and Levin (1976) found no systematic change of gene frequency with age for *Liatris cylindracea* Michx. in a demographic genetic study, but found the allele frequencies highly structured spatially (Schaal 1975). Linhart *et al.* (1981b) reported similar results in *Pinus ponderosa*, stating that "age-related differentiation is very subtle, and consists of few cases of deviations of genotypic distributions from Hardy-Weinberg expectations". Two populations of *Camellia japonica* have shown to be very stable through time in spite of a moderate to high degree in spatial differentiation (Caddell 1989).

Population subdivision either temporal or spatial is suggested by Gregorius *et al.* (1986) to be associated with high genetic diversity of loci (few alleles with relatively high frequency, Brown and Weir 1983). The association between population subdivision and genetic diversity of alleles refers to, if evolutionary forces are present, the genetic subdivision is enhanced at loci with high diversity (rare alleles more sensitive to sample size).

Of eight polymorphic loci considered in this study, all

but GOT and MDH2 have at least one rare allele. It was mentioned that rare alleles are important in showing differences either at temporal or spatial subdivisions, because when the two common alleles are included in the statistical analysis, the probability levels ($p \leq .05$) have increased or significant differences have disappeared. Few loci which were not expressing significant changes in allele frequency, gained significance. This pattern of change in levels of probability is consistent for both the age and location effects. Thus, the increase in probability levels for cases of the two most common alleles may suggest that there are no selective forces acting upon those loci under consideration.

The lack of observable patterns was possibly due to the presence of rare alleles. Provided that there is a close association of high diversity at the locus level and evolutionary forces involved be easier to be detected, a strong pattern for GOT should be detected if selection were to be present. The GOT locus shares its polymorphism among three alleles with similar frequency. PGM2 and PGI2 when considered for the two most common alleles, have their respective allele frequencies relatively high, however, in any case a pattern is observed.

Other loci are characterized by the presence of one predominant allele associated with one or two rare ones. Whether the presence of rare alleles prevents selection forces from being detected, or selection forces were not

strong enough for the statistical analyses to detect them. The results reported do not show evidence of strong selection. The non-consistent differences detected at the plot and subplot levels show that there is a spatial structure. However, the differences detected due to age classes also imply temporal structure. This does not appear to be caused by selection, but by age classes being represented by different genetic sampling (Namkoong 1984). Although outcrossing rates are clearly under control, the mating structure of plant species is also plastic and subject to environmental influences, mainly species dependent on biotic pollination agents such as insects (Clegg 1980). Furthermore, tree populations are characterized by overlapping populations, therefore data of this sort have to be analyzed with caution (Caddell 1989).

Selection at the spatial level has been reported by Mitton *et al.* (1977) in *Pinus ponderosa* where variation in gene frequency is detected over distances of several hundred of meters and is found to be associated with slopes of different aspects. Another study with the same species (Linhart *et al.* 1981b) reports genetically heterogeneous subpopulations associated with cone production, aphid distribution and deer browsing. Recent studies have revealed differences in genetic structure due to air pollutants for *Picea abies* (Bergmann and Scholz 1987) and *Fagus sylvatica* (Müller-Starch 1985) .

The stronger location effect in this study is consistent with reports found throughout the literature. The subpopulation is subdivided at a fine spatial level, but as in temporal subdivision there is no evidence of a consistent pattern of selection. Inconsistent changes in loci, which present significant changes in allele frequency due to space within age class, suggest that differences might be caused by partial selfing and/or restricted gene flow. Tulip tree is an insect-pollinated species, and it is expected that insects visit flowers and trees that are closer to each other increasing the chance for spatial heterogeneity. The best example from this study is the locus PGM2 which has a very low frequency of allele 9 at plot C and this allele is more likely to be rare at this plot due to limited gene flow rather than to microhabitat selection. The species was reported by Brotschol (1983) as self-compatible with a mixed mating system. Although the extent of outcrossing varies among and within populations the average estimate reported was t=0.934.

Kim (1985) presents one case of viability selection for *Fagus sylvatica*, where he was able to detect different performances of seedlings of different provenances growing in two different environmental conditions. He observed that a given genotype would perform better in a particular condition based on the allele present in either the homozygous or heterozygous state.

Gregorius *et al.* (1986) found that when two-year seed

production of *Fagus sylvatica* were bulked the spatial heterogeneity decreased; the results with *L. tulipifera* have shown the opposite trend. The results herein show that all age classes, when combined, show much stronger spatial subdivisions than within each age class. One possible explanation is that, in this study, the life cycle stages are very distinct as opposed to the two subsequent years. This shows that different years can produce seed lots that represent different genetic samplings even though they are from the same area.

3.5. Heterozygosity as a measure of population dynamics

Average heterozygosity follows an increasing trend with age (Table 14)at which populations were sampled. The differences among age classes are on the order of 10^{-2} with standard errors on the order of 10^{-3}.

The variance components among plots were estimated separately for each age class (Table 15). The estimates were considered at the plot level age class 3 (adult trees) has not been considered at the subplot level. Since relatively large sample sizes were used, estimates at the subplot level for age classes S, 0, 1, and 2 for the mean and standard error are of the same order of magnitude (10^{-3}).

Average heterozygosities were tested under the null hypothesis ($H_{\cdot}$) that the average heterozygosity of the two age classes were the same with 95% of probability.

Table 14. Average heterozygosity, by age class and its respective standard error (std. error).

Age	Heterozygosity	Std. error
S	.2000	.0050
0	.1990	.0045
1	.2228	.0046
2	.2394	.0047
3	.2508	.0086

Table 15. Estimates of variance component (order 10^{-3}) for for different age classes.

Var. component	S	0	1	2	3
Var(Plot)	-.1354	-.0984	-.1033	.0149	-.3622
Var(Ind.(Plot)	.7449	.2494	.2675	.4839	-.4734
Var(Locus*Plot)	.9594	.7525	.6277	.6374	2.778
Var(error)	.0250	.0206	.0215	.0222	.0735

There was no evidence to reject H· when average heterozygosities were compared between age class S and 0, as well as for the comparison between age class 2 and 3. However, the differences between age class 0 and 1, as well as 1 and 2 are significantly different.

Results of goodness of fit χ^2 tests to evaluate the genotypic frequency distribution are shown in table 16.

Deviations showing significant excesses of homozygosity

Table 16. Deviation from Hardy-Weinberg expectations for subpopulations at different loci.

SUBPOP.	SOD2	PGI2	GOT	MDH1
A 1 S	-.0133	-.0164	.0211	.0229
A 2 S	.0690**	.0433	.1099**	.0108
A 3 S	.0052	.0169	.1475**	.0482*
B 1 S	-.0056	.0123	.0000	.0344*
B 2 S	-.0016	.0194	-.0002	.0088
B 3 S	-.0026	.0998*	.0494	-.0209
B 4 S	-.0019	.0129	.0677*	.0232
C 1 S	-.0056	.0650	.0998**	-.0197
C 2 S	-.0199	.0321	.1083**	-.0069
C 3 S	.0049	.0190	.0736	-.0038
A 1 0	-.0010	-.0209	-.0113	-.0084
A 2 0	.0178	.0436	.0095	.0602
A 3 0	-.0011	.0375	.0095	.0516
B 1 0	-.0013	-.0001	.0307	.0000
B 2 0	-.0007	-.0716*	.2400*	.0031
B 3 0	-.0001	.0100	.0237	-.0156
B 4 0	-.0027	.0264	-.0413	-.0049
C 1 0	-.0156	.0479	.0412	.0054
C 2 0	.0232	.0600	.0291	.0276
C 3 0	-.0144	-.0025	.0319	-.0126
A 1 1	-.0039	.0375	.0102	.0891**
A 2 1	.0111	-.0025	.0353	.0824**
A 3 1	-.0027	.0375	.0751*	.0071
B 1 1	.0077	.0096	.0520	-.0100
B 2 1	-.0036	.0175	.0153	.0231
B 3 1	-.0020	.0096	-.0227	-.0056
B 4 1	-.0055	.0436	.0036	.0175
C 1 1	-.0150	-.0121	.0761	-.0100
C 2 1	.0039	-.0316	.0296	-.0204
C 3 1	.0059	.0400	.0519	.0204
A 1 2	-.0087	-.0164	-.0105	-.0176
A 2 2	-.0037	-.0204	.0050	-.0500
A 3 2	.0028	.0284	.0031	.0004
B 1 2	.0056	-.0136	.0086	-.0172
B 2 2	.0031	-.0100	.0458	.0076
B 3 2	-.0043	.0500	.0131	.0032
B 4 2	-.0100	.0191	.0236	-.0289
C 1 2	.0078	.0104	.0662	-.0124
C 2 2	-.0121	-.0600	-.0113	-.0104
C 3 2	.0131	-.0025	.0254	.0011
A 0 3	.0164*	-.0136	.0077	.0044
B 0 3	-.0121	.0100	.0213	-.0256
C 0 3	-.0241	-.0625	-.0417	-.0196

cont.

SUBPOP.	MDH2	PGM2	TPI1	ACO
A 1 S	.0143	-.0414	.0215*	.0625*
A 2 S	.0346	.0545	.0153	.0553
A 3 S	.0139	.0829*	.0181*	.0299
B 1 S	-.0127	-.0627	.0444**	.0423
B 2 S	-.0309	.0350	-.0066	.0798**
B 3 S	-.0016	.1494**	-.0025	.0798**
B 4 S	.0479*	.0816*	-.0026	.0721**
C 1 S	-.0249	.0572	.0211*	.0373*
C 2 S	-.0089	.0447	--	.0703**
C 3 S	.0278	.0606	-.0046	.0153
A 1 0	0000	-.0009	-.0081	.0119
A 2 0	.0142	.0296	.0319**	.0604**
A 3 0	.0079	.0556	-.0004	.0671*
B 1 0	.0175	.0175	-.0049	.0311
B 2 0	.0119	.0199	-.0004	.0239
B 3 0	-.0104	.0636	.0164*	.0476*
B 4 0	.0039	-.0136	-.0025	.0925**
C 1 0	-.0100	.0416	.0100	-.0169
C 2 0	.0279*	.0356	-.0004	.0119
C 3 0	.0004	-.0201	-.0004	-.0064
A 1 1	-.0144	.0279	-.0064	.0871**
A 2 1	.0311	.0504	-.0009	.0674**
A 3 1	0000	-.0225	-.0016	.0346*
B 1 1	-.0225	.0199	-.0036	.0511*
B 2 1	-.0064	-.0001	-.0001	.0559
B 3 1	-.0144	.0200	-.0036	-.0129
B 4 1	-.0144	-.0409	.0175**	.0375
C 1 1	-.0025	.0175	-.0169	-.0064
C 2 1	-.0196	-.0044	-.0025	-.0144
C 3 1	-.0100	-.0800*	-.0004	.1879**
A 1 2	-.0425	.0599	-.0009	-.0025
A 2 2	-.0030	.0086	-.0004	.0153
A 3 2	-.0289	-.0516	-.0064	-.0041
B 1 2	-.0204	.0450	-.0001	.0471
B 2 2	-.0124	.0436	-.0009	.0239
B 3 2	-.0084	-.0116	-.0009	-.0169
B 4 2	.0079	.0304	-.0049	.0424
C 1 2	-.0144	-.0096	-.0049	.0404*
C 2 2	.0028	.0311	-.0025	-.0049
C 3 2	.0031	.0119	.0191**	-.0062
A 0 3	-.0200	-.0272	-.0036	-.0062
B 0 3	-.0144	-.0225	.0336**	-.0306
C 0 3	-.0196	-.0169	-.0004	-.0037

significant deviations: * α=.05 ** α=.01

are more frequent in the age class S than in any other class, especially for ACO, GOT, TPI1, and PGM2. For ACO at age class S all ten subpopulations show an excess of homozygous genotypes, and six of the ten have expressed significant deviation from Hardy-Weinberg expectation. For the other age classes, the number of significant deviations decreases until it reaches the class 3 (adult trees) where the deviations switch from positive to negative *i.e.*, there exists a slight excess of heterozygous genotypes. Although, this excess of heterozygosity increases gradually with age class (from S, 0, 1, 2, and 3), these deviations are not significant.

In the case of GOT, the number of significant deviations has dropped considerably from S to 0. Nevertheless, the change in sign for those deviations was not observed as in the ACO locus, and even the adult trees show a slight excess of homozygosity for the subpopulations sampled.

TPI1, at age class S shows four significant deviations out of five, with an excess of homozygosity. The other deviations are towards an excess of heterozygous genotypes, but none are significant. In the other age classes, the significant deviations with an excess of homozygosity have dropped to two cases in age class 0, 1 in age class 1, 1 in age class 2, and the deviation is still significant towards homozygosity for the adults in plot B . An excess of heterozygosity even though not significant, becomes more frequent with an increase in age classes 0, 1, 2, and 3.

The PGM2 locus shows three significant deviations out of eight observed with an excess of homozygosity at age S. These significant deviations declined in the following age class, increasing in proportion of values indicating an increase of heterozygous genotypes, though those deviations are not significant.

Very few significant deviations, scarcely spread in different age classes, but mainly at S, 0, and 1, are apparent at loci MDH1, MDH2, PGI2, and SOD2. The SOD2 locus shows a significant excess of homozygosity as adults. Thus, SOD2 and TPI1 are the only loci expressing an excess of homozygosity at the adult stage. The PGM2 and PGI2 loci, show one case each of a significant excess in heterozygosity. Such a low frequency of occurrence could well be due to chance (sampling error) rather than representing a case of heterozygous superiority.

At age class S (seeds), of all deviations estimated, about 70% represents deviations from the Hardy-Weinberg expectation (Table 16), which suggests excess homozygosity. Of the 70% with deviations towards homozygous genotypes, about 43% are statistically significant. Of the 30% with excess heterozygotes none are significant.

In age class 0 (newly germinated seedlings), the equivalent 70% has dropped to 57,5% with the remaining 40% of deviation indicating an excess of heterozygosity and only a few cases were observed deviations equal to zero.

The significant excess of homozygosity at this age class is 17% as opposed to 43% at age class S. The percentage of deviations towards an excess of homozygosity drops continually with age class: 48.75% (1), 46.25% (2), and 25% (3). However, the number of significant deviations, does not follow the same trend. In age class 1, the proportion of significant deviations was 23% as opposed to 17% at age class 0; it decreases again at age class 2 (5.4%), and at age class 3 this proportion is 33,33%. The reason for such a high proportion of the latter is associated with small sample sizes for this age class.

Given these changes in proportions of significant deviations, even in the absence of a clear pattern from age class S to 3 (43%, 17%, 23%, 2%, and 33%); there is a continual decrease of the deviations towards homozygosity (70%, 57.5%, 48.75%, 46.25%, and 25%). From these results, one cannot conclude that there is heterozygous genotypic selection, but one can infer that there is a directionality of the changing genotypic frequencies from an excess of homozygous genotypes to an equilibrium, and in a few cases, a possible excess of heterozygosity.

The change in sign for those deviations, may not represent an actual excess or deficiency of a given genotype, since most deviations are not statistically significant. The only statistically valid inference that can be drawn is that a significant excess of homozygous genotypes exists at the earliest life cycle stage (age S), and this occurs in about

30% (proportion of significant deviations) of the loci of the subpopulation sampled. The remaining 70% of the cases cannot be distinguished from the existing Hardy-Weinberg equilibrium since their deviation estimates (+ or -) are not statistically significant. As the age class increases, a higher proportion of the samples go to this equilibrium stage (*e.g.* about 92% of the subpopulation sampled - adult trees) for all considered loci. This change may not represent selection for heterozygous genotypes, but rather the elimination of homozygous individuals which may carry deleterious alleles in some of their loci, which seems to occur during the early stages of the life cycle.

The current results do not provide evidence to support a case of polymorphism maintained by heterozygous superiority since cases of significant deviation towards an excess of heterozygous genotypes are at very low levels.

Levels of heterozygosity of different age structure is another way to look at the genetic architecture of a population. One of most common statistics used in the presentation of population genetic data is the heterozygosity average over the loci scored (Weir and Cockerham 1989). The failure to observe a systematic change in gene frequency between age classes does not preclude changes in mean heterozygosity (Schaal and Levin, 1976). As previously mentioned, an increase in heterozygosity (*i.e.* the probability that a given individual taken from a population is heterozygous at a

particular locus) has been observed in plant populations as they get older.

Results reported in forest trees have shown changes in genotypic frequency distributions among different life cycle stages, even with no significant changes in allele frequency (Linhart *et al.* 1981b, Shaw and Allard 1982, Brotschol 1983, Farris and Mitton 1984, Cheliak *et al.* 1985, Yazdani *et al.* 1985, Muona *et al.* 1987, and Caddell 1989). Thus, the observed results of this study are not unusual.

The observed significant differences in genotypic distributions suggest that the major change occurs from the newly germinated seedlings to the time when plants are stablished in the population (*ca.* 10 years). Della-Bianca (1983) reported that at age of 13 years the tulip tree population in a regenerated stand after clearcutting has its maximum density and it continuously declines after this age. Although the population declines in density there was no significant change in heterozygosity between age class 2 (7 - 10 years old) and the adult trees. The shift in genotypic distribution seems to be related to community development where some individuals are established and other are eliminated, but results do not support evidence that the elimination is a directionally genetically determined process.

The results of the fixation index have shown a consistent decrease in the proportion of positive values which indicates a decrease in excess homozygosity. Since the proportion of significant deviation is more concentrated at

age S and O, the remaining estimates imply that the genotypic distribution is in Hardy-Weinberg equilibrium. Similar results for the same species were reported by Brotschol (1983).

The reduction of a significant excess at an early stage indicates that the elimination of homozygotes is less likely to represent the selection for heterozygotes, but the elimination of inbred individuals that carry deleterious alleles (Tigerstedt *et al.* 1982, Shaw and Allard 1982, Koski 1982, Muona *et al.* 1987, Namkoong and Bishir 1987, Namkoong *et al.* 1988). This finding is in agreement with the mixed mating system described by Brotschol (1983). Another explanation for the observed excess of homozygosity could be the so-called Wahlund effect (effect similar to inbreeding which shows an excess of homozygosity due to cluster subdivided populations). Since, the population is structured at a fine level of sampling subdivision, the Wahlund effect cannot be ruled out because of situations where a few families may be clustered in a very confined space.

There was no evidence for heterozygosity superiority as shown by Linhart *et al.* (1981a, b), and Farris and Mitton (1984). Of all estimates, only two, one case for PGI2 and another for PGM2, which indicates excess of heterozygous genotypes. This proportion is lower than should be expected by chance under the significance level tested.

Considering that the majority of the significant excess

of homozygosity was eliminated at early stages, and that a large proportion of subpopulations at several loci markers were close to Hardy-Weinberg frequency distributions, the observed data are in agreement with the outcrossing rate (t=.934) estimated by Brotschol (1983). A similar estimate (t=.97) was found by Sewell (personal communication) for tulip tree. Within the fraction of individuals produced by self-fertilization in predominantly outcrossed populations, a positive correlation is expected between the marker locus homozygosity and the inbreeding coefficient of an individual. The elimination of inbred individuals increases heterozygosity at the marker loci which, however, does not indicate selection (Ennos 1989).

3.6. Linkage disequilibrium.

Results of linkage disequilibrium are grouped by plot and age class (Table 17).

The percentage of significant coefficients for linkage disequilibrium varies among plots within a given age class as well as inside a plot for different age classes. The average percentage lies within a range of 6.4 to 10.8%. Although the range itself is higher than the level expected based on sampling error (95% of probability), there is no consistent association of particular alleles among age classes or subpopulations. Conversely, the presence of a high proportion of significant linkage disequilibrium does

Table 17. Percentage of significant linkage disequilibrium estimates at two loci, for individual plots and their average at different age classes.

Age	A	B	C	Average
S	16.7 (84)	8.39 (143)	7.53 (93)	10.87 (320)
0	5.43 (92)	8.33 (132)	12.9 (85)	8.90 (309)
1	7.14 (84)	8.33 (120)	10.7 (84)	8.73 (288)
2	6.52 (92)	7.81 (128)	5.0 (100)	6.44 (320)
3	17.85 (28)	3.57 (28)	7.14 (28)	9.52 (84)

The values in parenthesis represent numbers of pairs of loci tested.

not indicate the presence of selection because of the finely clustered distribution of families even if random mating can produce an apparent disequilibrium. Similarly, selfing causes the appearance of multilocus association as contrasted with outcrossing (Allard *et al. 1972*, Brown 1979, Weir and Cockerham 1989). Therefore, the detected linkage disequilibrium estimates can suggest presence of selection only if a consistent association of particular alleles is observed (Weir 1979).

Comparing these results to those obtained for *L. tulipifera*, by Roberds and Brotschol (1985), the percentages of significant allele associations are considered very low. They have reported about 76% of one or more non-allelic associations that were significantly different than expected just by chance for seedling population. The percentage found in current studies is 16.7%, 8.4%, and 7.5% for plots A, B,

and C respectively (subplots pooled for age classes S 0 1 2), which averaged out to 10.8%.

One possible explanation for such different proportions of linkage disequilibrium (76% vs 10.8%) found in those different studies for the same species may reflect the decrease in proportion of significant estimates eliminated by the sampling method, which reveals the actual population subdivision fairly accurately. This is important to observe because structure, if ignored, may lead to misinterpretation of genetic data, *e.g.* linkage disequilibrium caused by subdivision (Epperson, 1989).

The variation of significant estimates among plots as well as for different age classes within a plot may represent the structure of the population at the subplot level associated with different genetic sampling (*i.e.*, different trees contribute for seed production in different years) being surveyed by the statistical sampling at different life cycle stages.

For adult trees, the average percentage of significant locus combinations is 9.5%, but with a large variation among plots. This average percentage of significant pair combinations is also lower than reported by Roberds and Brotschol (1985).

Although the percentage of significant pair combinations is low, the sample size considered here is large (*ca.* 1000 pairs). It is fair to conclude, that in a few situations such as age class S (plot A), age 0 (plot C), and age 3

(plot A), the association of non-allelic loci seems to be relatively important. Provided that almost all substructure effects have been eliminated by estimating the measures of linkage disequilibrium at the subplot level, the low significant proportion found is in agreement with the possibility of existence of subdivision within subplots due to the species' biological reproductive features (*e.g.* limited gene flow). It can be also merely due to sampling error, since genetic studies reported by Parks *et al.* (1990) showed no evidence that these loci are linked.

Measures of linkage disequilibrium evaluate the amount of significant coefficients which in turn, translate into an assessment of which genes associate in gametes between a given pair of loci. These measures aid in identifying evolutionary forces involved in population structure. Weir and Cockerham (1989) recommended that disequilibrium coefficients be considered in all statistical analysis of genotypic data.

4. CONCLUSION

The genetic architecture of this natural tulip tree population can be described as follows;

1) The population is structured in time and space; however, neither one was recognized as a result of a natural selection process.

2) The lack of consistent patterns for differences in allele frequency for different age classes does not support evidence for selection. One possible constraint when trying to detect this evolutionary force, may be the presence of rare alleles. The best candidates to detect the presence of selection are the loci markers, which result in major polymorphisms where two or three alleles in high frequency may enhance selective effects (Lewontin 1985).

PGI2 and GOT fit the loci-marker description; however, they do not give a clearer pattern than any other loci. If major polymorphisms are maintained by balancing selection (Lewontin 1985, Bergmann and Scholz 1987), the allele frequencies would be in stable equilibrium, and not subject to change unless there is a major event to disrupt this equilibrium. The two loci PGI2 and GOT could be cases of polymorphisms maintained by balancing selection, but the lack of a pattern in changes of allele frequencies do not support such a conclusion.

Another explanation for the lack of pattern in allele frequency may be a divergence between genetic (different genotypic frequencies) and actual statistical sampling at

different life cycle stages (Namkoong 1984, Yazdani 1985, Caddell 1989). The changes in probability levels for loci markers at different spatial levels indicate spatial subdivision. The substructure in the population is at the subplot level.

3) Substructure in space is much stronger than in time. Significant differences in allele frequencies at different loci for plots within age classes, suggest that the population is substructured at the subplot level. There is no evidence that this substructure is due to microhabitat selection. The substructure is more likely to be related with the species' reproductive biology characteristics such as mixed mating systems, and/or a limited gene flow.

Among the age classes, the location effect is sufficiently consistent that the significance of the differences in allele frequencies is stronger than it is among plots within location. This contrasts with the age effect which, though also significantly different, are not consistent among locations.

No specific allele is associated with a specific age class. Therefore, there was no indication of viability selection at considered loci markers as found by Kim (1985) in *Fagus sylvatica*.

Statistical analyses, either in space or time, of the two most common alleles show the probability levels to be always higher than when all alleles are included. This can

be interpreted as added evidence for absence of selection. Although the rare alleles may mask the presence of selection, it is important to consider them because depending upon how they are grouped, their frequency can change considerably (see allele 6 and 9 at PGM2 locus, Table 7). Rare alleles have been thought to increase the chance of survival in *Fagus sylvatica* in areas of environmental stress due to air pollutants (Müller-Starck 1985). Rare alleles may be maintained in the population by purifying selection (Lewontin 1985), and therefore it is expected that most rare alleles in a population is "protected" by the heterozygous state.

4) There is a change in heterozygosity for different age classes. This change appears to be related to the elimination of homozygous genotypes originated by partial selfing or limited gene flow. The excess of homozygosity decreases as the population gets older.

The estimates (fixation index) do not indicate that the genotypic frequency distribution goes beyond the frequency expected by the Hardy-Weinberg expectation. The majority of homozygous elimination seems to occur at an early stage of the life cycle even though the population keeps decreasing in density as it matures.

The excess of homozygotes seems to be more related to selfing, however, it can not be ruled out that the samples could be substructured due a cluster of a few families. The majority of subpopulations are nearly in equilibrium with

respect to Hardy-Weinberg expectation, and furthermore, there is no evidence for heterozygous superiority. Due to decrease in population density as the population ages, even small heterozygous advantage would resolve in a large proportion of heterozygotes much greater, than expected under assumption of Hardy-Weinberg equilibrium.

5) There is no evidence that alleles of different loci are selectively pairing together more frequently than expected by random association. This result agrees with what was stated above, because if strong and consistent linkage disequilibrium were reported at the subpopulation level, it would be a good indication of selection (Weir 1979). However, linkage can also be expressed due to different causes (*e.g.* selfing, substructure). Therefore, if the reported low measures are not merely due to experimental error,they might be due to the effect of family structure in subplots.

Results in this study seemed to lead to one major conclusion; that there is no selection operating upon loci under consideration. The observed approach to Hardy-Weinberg equilibrium at an early stage (age class 0, or even at S with 70% showing no evidence of departure from random mating expectation) implies outcrossing, and this is in agreement with the outcrossing rates reported herein. The species is also described as self-compatible and having a mixed mating system. The current genetic findings associated with

reproduction and early growth of tulip tree, mainly seed production presents one divergent point which prevents a clear understanding of the species' reproductive biology.

As previously mentioned, the amount of samaras produced is in order of 10^6 seeds per hectare, but the seeds have very low viability (10%), and even lower the percentage of seed germination (Fowles 1965, Della-Bianca 1981). Considering that the outcrossing rate is high, one would expect a higher percentage of filled seeds, even with some partial selfing. Additionally, sound seeds seem not to have significant differences in percentage of germination, whether they have originated from cross pollination, open pollination or selfing (Taft 1965). The general statement is that if seeds are filled, they are capable of germinating when given proper conditions.

The antithesis proposed here is that the species is highly self-incompatible, and possibly due to restricted gene flow for its dependence on insects for pollination, the massive amount of seeds presents low viability due to frequent geitonogamy. The notion of self-incompatibility can be supported by results presented by Taft (1965) that selfing has produced on average 3.5% of filled samaras. Self-pollination has also been reported by Parks *et al.* (1983) with low percentage of sound seeds.

The majority of this low percentage of viable seeds may represent the product of cross-fertilization (xenogamy). This would agree with statement mentioned above (sound seeds

germinate). If this is so, the seeds that are formed have already gone through a major selection event and all the unsound samaras represent zygotes which had not been formed and/or are already eliminated.

Therefore, the outcrossing rate estimated by loci markers for tulip tree may not represent the actual degree of outcrossing. By this hypothesis the sound seeds represent a population that has survived intense pre- and/or pos-zygotic elimination. The remaining excess homozygosity due to the effect of inbreeding or population subdivision decreases in the following age classes so that the adult genotypic distribution is close to Hardy-Weinberg expectations.

An alternative explanation to the low seed viability may be resulted of the present phenomenon called dichogamy, here being the case of protogyny, *i.e.*, the stigmas mature prior to anthesis of the flower (Parks, personal communication). Thus, the low viability of the seeds whether caused by ineffective pollination, protogyny or combination of both, the weight of evidence still would not support evidence for tulip tree be considered self-compatible with mixed mating system. Nevertheless some individuals can produce seeds by selfing, I state that the great majority of sound seeds are originated from outcrossing.

The structure present in this tulip tree population is considered to be due to its mating system as opposed to

natural selection on the loci under consideration. This stresses the importance of estimates of mating systems in population analyses (Clegg 1980, Hamrick 1982). This study represents one piece of evidence that only an integrated biological study can provide important insights into the species' biology.

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Appendix:A

A1. Extraction and staining buffer solutions.

a) Extraction buffer

250 ml	Deionized wate
1.5 g	Sodium Phosphate (dibasic)
17.5 g	Sucrose
6.3 g	Polyvinylpyrrolidone
125 mg	Dithiothreitol
250 mg	Ascorbic aci
250 mg	Diethyldithiocarbmic acid
125 mg	Sodium Metabisulfate
125 mg	Sodium Borate (borax)
.5 ml	Mercaptoethanol
2.5 ml	Polyethylene Glycol
50 mg	Shikimic acid
50 mg	Aconitic acid
50 mg	6-Phosphogluconic acid

b) Staining buffers

.1M Tris-HCl pH 8.0 (.1M Trizma base, pH 8.0 with HCl)

.1M Tris-HCl pH 8.5 (.1M Trizma base, pH 8.5 with HCl)

.1M Na Acetate pH 5.0 (.1M Sodium Acetate, pH with glacial acetic acid)

GOT substrate solution

400 ml	Deionized water
146.1 mg	Ketoglutaric acid
532,4 mg	Aspartic acid
2 g	Polyvinylpyrrolidone
5.68 g	Sodium Phosphate (dibasic)

Appendix:A

A2. Gel system composition and eletrophoretic parameters

a) Gel systems

	Amount (g)
Lithium borate tricitrate (LBTC)	77.7 starch 26.0 sucrose
Morpholine citrate (MC)	77.7 starch 18.0 sucrose

b)

Gel system vol.(ml) in gel buffer	Electrode buffer (conc. in mol.)	Gel buffer (proportion)
LBTC - 600 ml	.192M Boric acid .038M Lithium hydroxide pH-8.3	Elec. buffer (1 part) .051M Trizma .007M C. ac. pH-8.3 (9 parts)
MC - 600 ml	.04M Citric ac. pH-6.5 with morpholine	Elec. buffer (1 part) Deio. H_2O (19 parts)

c) Wattage and running time

Gel system	Wattage	Time
LBTC	14.5 W	6.0 hours
MC	19.0 W	7.0 hours

d) Enzymes resolved per gel system

Slice sequence	LBTC	MC
bottom	CAT	PER
2nd	(double)	SAD
3rd	SOD	MDH
4th	PGI	PGM
5th	GOT	TPI
5th	IDH	ACO

Appendix:A

A3. Staining recipes

a) Catalase (CAT)

50 ml Deionized water
.5 g Potassium ferricyanide
.5 g Ferric chloride
(stain solution)

(H_2O_2 solution)*

200 ml Deionized water
.25 ml 30% H_2O_2

Add H_2O_2 solution to gel and store at room temperature in the dark for 8 minutes. Rinse twice with deionized water. Add stain solution and watch bands develop. Rinse twice with deionized water. Add fixative. Can be scored on the next day.

b) Superoxide Dismutase (SOD)

75 ml .05M Tris-HCl pH-8.5
25 mg EDTA*
16 mg Riboflavin*
1.5 ml NBT*

Add EDTA and Riboflavin to buffer. Vigorously swirl solution using a magnetic stirrer. Add NBT and pour onto gel slice. Expose under 40-60W light bulb, agitating frequently. Keep watching to make sure it does not over stain. Put deionized water and it **Must** be scored on the same day.

c) Phosphoglucose Isomerase (PGI)

50 mg Fructose-6-phosphate
.5 ml G-6-PDH (# 6378) or 1 drop of (# 5760)
.5 ml $MgCl_2$
.5 ml TPN
.5 ml MTT*
.2 ml PMS*

Incubate at 37 °C. Can be scored the next day. If use G-6-PDH (#5760), instead of TPN, use .5 ml of DPN. The drop is ± 40µl.

d) Glutamate Oxaloacetate Transaminase (GOT)

50 ml GOT substrate solution
50 mg Fast BB blue*

Incubate 1-2 hours. Can be scored next day.

e) Malate Dehydrogenase (MDH)

50 ml .1M Tris-HCl pH-8.5
.3 ml Malic acid
.5 ml DPN
.5 ml NBT*
.5 ml PMS*

Incubate for 3-4 hours. Can be scored the next day.

f) Phosphoglucomutase (PGM)

50 ml .1M Tris-HCl pH-8.5
50 mg EDTA
250 mg Glucose-1-phosphate
1 ml G-6-PDH (#6378) or one drop (#5760)
1 ml $MgCl_2$
.5 ml TPN
.75 ml MTT*
.2 ml PMS*

Incubate for 3-4 hours. Can be scored the next day. If used G-6-PDH #5760 as in PGI use .5ml DPN.

g) Triosephosphate Isomerase (TPI)

30 ml .1M Tris-HCl pH-8.0
200 mg α-glicerophosphate
200 mg Pyruvate
1 ml DPN
1 drop LDH
1 drop α-Gpdh
(solution A)

Incubate at 37 °C in dark for 2 hours. After incubation, bring substrate solution down to pH-2.0 with concentrated HCl, for 5 minutes. After this period bring the substrate solution up pH-7.0 with ≈ 4N NaOH. Then, add the following:

1 ml Arsenate
1 ml DPN
1 ml NBT*
.2 ml PMS*
4 drops of G-3-PDH

Incubate for 3-4 hours. Can be scored the next day.

h) Aconitase (ACO)

50 ml .1M Tris-HCl pH-8.0
25 mg cis-Aconitic acid
.5 ml IDH
.5 ml TPN
.5 ml $MgCl_2$
.3 ml PMS*
1 ml MTT*

Incubate for 3-4 hours. Can be scored the next day.

* Do not add until immediately before staining.

All the enzymes are incubate at 37 °C unless specified otherwise.

Appendix:A

A4. Stain reagents

Name	Composition	Conc.
DPN (NAD)	Beta-Nicotinamida adenine dinucleotide 1g/50ml (N-7004)	20 mg/ml
TPN (NADP)	Nicotinamida adenine dinucleotide phosphate 1g/100ml (N-0505)	10 mg/ml
MTT	3-(4,5-Dimethylthiasol-2-yl)-diphenyltetrazolium bromide 1g/100ml (M-2128)	10 mg/ml
NBT	Nitro Blue Tetrazolium 1g/100ml (N-6876)	10 mg/ml
PMS	Phenazine methosulfate .5g/100ml (P-9625)	5 mg/ml
Fast BB Blue	Fast BB Blue salt 10g/100ml (F-0250)	100 mg/ml
$MgCl_2$	Magnesium chloride 10g/100ml (M-0250)	100 mg/ml
$CaCl_2$	Calcium chloride 1.5g/100ml (C-3881)	.1 M
Arsenate	Sodium arsenate 10g/100ml (A-6756)	100 mg/ml
Malate	DL-Malic acid (neutralized) NaOH to pH-8.0 10g/100ml (M-0875)	100 mg/ml

** Prepare all the reagents with deionized water. Store all the reagents in cooler. Sigma Chemical are in parentheses.

Fixative solution: 1600 ml water
1600 ml methanol
350 ml glacial acetic acid

Appendix:A

A5. Other stain reagents

cis- Aconitic acid (A-7251)

(EDTA) Ethylenediaminetetraacetic acid (ED2SS)

Ferric chloride (F-2877)

Fructose-1,6-diphosphate (752-1)

D-Fructose-6-phosphate (F-3627)

(G-3-PDH) Glyceraldehyde-3-phosphate dehydrogenase (G-0763)

alpha-D-Glucose-1-phosphate (G-7000)

(G-6-PDH) Glucose-6-phosphate dehydrogenase(G-6378) 2000 units/50ml H_2O (This is divided into 1ml (40 units/ml) and kept frozen.

(α-GPDH) alpha-Glycerophosphate dehydrogenase (G-6751)

Isocitric acid (I-1252)

(IDH) Isocitric dehydrogenase (I-5882) 1000 units/15ml of 75% glycerol (G-5516). This is divided into .5ml volumes and kept frozen.

(LDH) L-Latic dehydrogenase (G-2500)

(NADH) Beta-Nicotinamide adenine dinucleotide, reduced form (N-8129)

Potassium ferricyanide (P-8131)

Pyruvic acid (P-2256)

(-)-Shikimic acid (S-5375)

*** The Appendix:A is all based on *Liriodendron* Eletrophoresis procedures. All the credit here goes to Dr. C. Parks and his students who helped preparing it.

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Appendix:B

Table 1. Allele frequencies by subpopulations. The subpopulations are identified as follows. In the identification capital letters A, B, and C are plots. The midle numbers refer to subplots, and the third identification to the age class.

LOCUS	A 0 3	A 1 2	A 2 2	A 3 2	B 0 3	B 1 2	B 2 2	B 3 2	B 4 2
CAT									
(N)	50	50	49	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SOD2									
(N)	50	43	49	49	50	50	50	46	50
1	0.020	0.012	0.010	0.092	0.030	0.020	0.020	0.043	0.030
2	0.030	0.035	0.031	0.010	0.040	0.060	0.080	0.011	0.040
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.940	0.907	0.939	0.867	0.890	0.880	0.870	0.935	0.900
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.010	0.047	0.020	0.031	0.040	0.040	0.030	0.011	0.030
PGI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.560	0.420	0.430	0.460	0.490	0.440	0.480	0.500	0.460
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.440	0.580	0.560	0.540	0.500	0.560	0.500	0.500	0.530
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.010	0.0	0.010	0.0	0.020	0.0	0.010
GOT									
(N)	48	48	50	50	41	49	49	47	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.250	0.271	0.270	0.240	0.280	0.347	0.306	0.330	0.300
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.448	0.510	0.480	0.630	0.354	0.418	0.510	0.447	0.420
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.302	0.219	0.250	0.130	0.366	0.235	0.184	0.223	0.280
MDH1									
(N)	50	50	49	50	50	49	50	49	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.660	0.760	0.735	0.860	0.840	0.806	0.820	0.806	0.830
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.150	0.080	0.122	0.080	0.070	0.102	0.100	0.082	0.120
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.190	0.160	0.143	0.060	0.090	0.092	0.080	0.112	0.050
MDH2									
(N)	50	50	49	50	50	49	50	49	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.200	0.250	0.153	0.170	0.120	0.143	0.180	0.092	0.110
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.800	0.750	0.847	0.830	0.880	0.857	0.820	0.908	0.890

Appendix:B
Table 1. cont.

MDH5									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.010	0.0	0.0	0.0	0.010	0.010	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	0.990	1.000	1.000	1.000	0.990	0.990	1.000
PGM1									
(N)	50	50	48	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM2									
(N)	49	50	49	50	50	49	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.459	0.490	0.418	0.460	0.450	0.531	0.420	0.460	0.360
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.071	0.090	0.143	0.090	0.090	0.082	0.110	0.170	0.210
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.367	0.360	0.357	0.350	0.400	0.337	0.380	0.350	0.360
9	0.102	0.060	0.082	0.100	0.060	0.051	0.090	0.020	0.070
TPI1									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.060	0.210	0.020	0.080	0.080	0.010	0.030	0.030	0.070
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.940	0.790	0.980	0.920	0.920	0.990	0.970	0.970	0.930
TPI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACO									
(N)	49	50	50	50	40	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.092	0.080	0.140	0.100	0.112	0.160	0.190	0.110	0.130
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.837	0.850	0.790	0.790	0.825	0.730	0.690	0.870	0.760
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.071	0.070	0.070	0.110	0.063	0.110	0.120	0.020	0.110

Appendix:B
Table 1. cont.

LOCUS	C 0 3	C 1 2	C 2 2	C 3 2	A 1 1	A 2 1	A 3 1	B 1 1	B 2 1
CAT									
(N)	50	50	50	49	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SOD2									
(N)	50	49	50	46	48	50	48	48	50
1	0.070	0.061	0.050	0.065	0.0	0.060	0.031	0.063	0.030
2	0.030	0.010	0.030	0.076	0.063	0.070	0.021	0.031	0.020
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.790	0.888	0.890	0.772	0.938	0.830	0.948	0.885	0.940
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.110	0.041	0.030	0.087	0.0	0.040	0.0	0.021	0.010
PGI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.450	0.330	0.390	0.450	0.450	0.440	0.450	0.470	0.450
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.550	0.640	0.600	0.550	0.550	0.550	0.550	0.520	0.550
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.030	0.010	0.0	0.0	0.010	0.0	0.010	0.0
GOT									
(N)	48	49	49	49	44	49	50	48	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.188	0.255	0.163	0.255	0.250	0.214	0.270	0.323	0.316
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.500	0.510	0.663	0.510	0.534	0.520	0.570	0.490	0.500
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.313	0.235	0.173	0.235	0.216	0.265	0.160	0.188	0.184
MDH1									
(N)	50	50	49	50	47	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.860	0.820	0.898	0.830	0.755	0.760	0.770	0.900	0.870
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.070	0.110	0.020	0.050	0.096	0.180	0.080	0.030	0.040
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.070	0.070	0.082	0.120	0.149	0.060	0.150	0.070	0.090
MDH2									
(N)	50	50	49	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.140	0.120	0.133	0.130	0.120	0.170	0.200	0.150	0.080
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.860	0.880	0.867	0.870	0.880	0.830	0.800	0.850	0.920

Appendix:B
Table 1. cont.

MDH5									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.010	0.010	0.0	0.0	0.0	0.0	0.010	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	0.990	0.990	1.000	1.000	1.000	1.000	0.990	1.000
PGM1									
(N)	50	50	50	50	50	49	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.370	0.360	0.330	0.410	0.390	0.360	0.450	0.510	0.490
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.270	0.150	0.250	0.220	0.050	0.080	0.090	0.080	0.110
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.360	0.460	0.400	0.350	0.420	0.490	0.310	0.350	0.340
9	0.0	0.030	0.020	0.020	0.140	0.070	0.150	0.060	0.060
TPI1									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.020	0.070	0.050	0.030	0.080	0.030	0.040	0.180	0.010
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.980	0.930	0.950	0.970	0.920	0.970	0.960	0.820	0.990
TPI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACO									
(N)	49	50	50	49	50	49	49	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.061	0.120	0.040	0.143	0.170	0.163	0.082	0.110	0.120
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.939	0.860	0.930	0.837	0.730	0.765	0.837	0.830	0.710
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.020	0.030	0.020	0.100	0.071	0.082	0.060	0.170

Appendix:B
Table 1. cont.

LOCUS	B 3 1	B 4 1	C 1 1	C 2 1	C 3 1	A 1 0	A 2 0	A 3 0	B 1 0
CAT									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SOD2									
(N)	45	47	49	50	47	48	45	30	41
1	0.0	0.021	0.041	0.050	0.064	0.0	0.0	0.017	0.024
2	0.022	0.032	0.010	0.030	0.032	0.031	0.044	0.017	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.956	0.926	0.878	0.810	0.809	0.969	0.933	0.967	0.963
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.022	0.021	0.071	0.110	0.096	0.0	0.022	0.0	0.012
PGI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.470	0.420	0.360	0.440	0.400	0.460	0.580	0.450	0.490
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.520	0.580	0.610	0.540	0.600	0.530	0.420	0.550	0.490
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.010	0.0	0.030	0.020	0.0	0.010	0.0	0.0	0.020
GOT									
(N)	46	50	46	50	50	46	32	46	46
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.239	0.260	0.304	0.280	0.130	0.261	0.359	0.217	0.293
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.609	0.420	0.500	0.480	0.590	0.478	0.422	0.652	0.543
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.152	0.320	0.196	0.240	0.280	0.261	0.219	0.130	0.163
MDH1									
(N)	50	50	50	49	50	50	47	48	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.840	0.850	0.900	0.857	0.860	0.780	0.702	0.729	0.857
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.110	0.130	0.070	0.071	0.070	0.150	0.149	0.063	0.051
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.050	0.020	0.030	0.071	0.070	0.070	0.149	0.208	0.092
MDH2									
(N)	50	50	50	50	50	50	49	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.120	0.120	0.150	0.140	0.100	0.200	0.163	0.110	0.150
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.880	0.880	0.850	0.860	0.900	0.800	0.837	0.890	0.850

Appendix:B
Table 1. cont.

MDH5									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.010	0.0	0.0	0.010
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	0.990
PGM1									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.400	0.470	0.350	0.380	0.400	0.530	0.480	0.380	0.550
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.180	0.130	0.250	0.200	0.250	0.030	0.060	0.090	0.100
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.340	0.330	0.400	0.410	0.340	0.340	0.420	0.440	0.240
9	0.080	0.070	0.0	0.010	0.010	0.100	0.040	0.090	0.110
TPI1									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.060	0.050	0.130	0.050	0.020	0.090	0.090	0.020	0.070
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.940	0.950	0.870	0.950	0.980	0.910	0.910	0.980	0.930
TPI2									
(N)	50	50	50	50	50	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACO									
(N)	50	50	25	50	47	50	50	50	50
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.190	0.200	0.060	0.100	0.245	0.060	0.120	0.180	0.050
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.770	0.750	0.920	0.880	0.702	0.910	0.860	0.730	0.830
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.040	0.050	0.020	0.020	0.053	0.030	0.020	0.090	0.120

Appendix:B
Table 1. cont.

LOCUS	B 2 0	B 3 0	B 4 0	C 1 0	C 2 0	C 3 0	A 1 S	A 2 S	A 3 S
CAT									
(N)	50	50	50	50	50	50	40	49	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.130	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	0.870	1.000	1.000	1.000	1.000	1.000	1.000
SOD2									
(N)	39	41	48	36	49	50	39	48	48
1	0.0	0.0	0.0	0.042	0.020	0.040	0.038	0.073	0.042
2	0.0	0.012	0.052	0.028	0.061	0.040	0.077	0.094	0.063
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.974	0.988	0.948	0.875	0.867	0.880	0.885	0.813	0.875
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.026	0.0	0.0	0.056	0.051	0.040	0.0	0.021	0.021
PGI2									
(N)	50	50	50	50	50	50	40	49	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.510	0.500	0.440	0.350	0.400	0.450	0.563	0.439	0.429
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.460	0.500	0.560	0.610	0.600	0.550	0.438	0.551	0.551
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.030	0.0	0.0	0.040	0.0	0.0	0.0	0.010	0.020
GOT									
(N)	5	26	33	48	43	50	29	47	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.400	0.135	0.197	0.167	0.326	0.190	0.310	0.245	0.490
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.600	0.769	0.636	0.521	0.500	0.590	0.431	0.543	0.408
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.096	0.167	0.313	0.174	0.220	0.259	0.213	0.102
MDH1									
(N)	50	48	50	49	50	49	38	49	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.870	0.875	0.930	0.878	0.820	0.888	0.763	0.773	0.813
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.100	0.094	0.060	0.071	0.060	0.082	0.211	0.112	0.010
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.030	0.031	0.010	0.051	0.120	0.031	0.026	0.112	0.177
MDH2									
(N)	50	49	50	50	50	50	39	49	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.090	0.102	0.190	0.100	0.110	0.140	0.192	0.163	0.083
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.910	0.898	0.810	0.900	0.890	0.860	0.808	0.837	0.917

Appendix:B
Table 1. cont.

MDH5									
(N)	50	50	50	50	50	50	40	49	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM1									
(N)	50	50	50	50	50	50	40	49	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM2									
(N)	50	50	50	50	50	50	40	49	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.490	0.420	0.440	0.280	0.380	0.490	0.438	0.459	0.521
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.100	0.130	0.090	0.160	0.160	0.210	0.075	0.041	0.031
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.350	0.390	0.450	0.560	0.460	0.300	0.375	0.408	0.417
9	0.060	0.060	0.020	0.0	0.0	0.0	0.112	0.092	0.031
TPI1									
(N)	50	50	50	50	50	50	40	49	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.020	0.060	0.050	0.100	0.020	0.020	0.063	0.071	0.051
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.980	0.940	0.950	0.900	0.980	0.980	0.938	0.929	0.949
TPI2									
(N)	50	50	50	50	50	50	40	49	49
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACO									
(N)	50	50	49	50	50	50	40	49	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.170	0.170	0.173	0.120	0.090	0.080	0.125	0.143	0.156
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.810	0.820	0.776	0.870	0.910	0.920	0.750	0.704	0.719
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.020	0.010	0.051	0.010	0.0	0.0	0.125	0.153	0.125

Appendix:B
Table 1. cont.

LOCUS	B 1 S	B 2 S	B 3 S	B 4 S	C 1 S	C 2 S	C 3 S
CAT							
(N)	40	40	40	49	40	40	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.020	0.025	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	0.980	0.975	1.000	1.000
SOD2							
(N)	40	37	39	46	40	39	37
1	0.025	0.027	0.0	0.011	0.037	0.051	0.081
2	0.025	0.014	0.051	0.011	0.075	0.051	0.027
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.925	0.959	0.949	0.957	0.825	0.859	0.851
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.025	0.0	0.0	0.022	0.063	0.038	0.041
PGI2							
(N)	40	40	40	49	40	40	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.512	0.563	0.387	0.367	0.375	0.500	0.378
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.487	0.425	0.612	0.612	0.600	0.487	0.622
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.012	0.0	0.020	0.025	0.012	0.0
GOT							
(N)	36	39	40	48	40	39	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.486	0.410	0.275	0.198	0.325	0.333	0.230
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.333	0.321	0.475	0.625	0.387	0.474	0.527
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.181	0.269	0.250	0.177	0.287	0.192	0.243
MDH1							
(N)	40	37	38	49	37	36	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.875	0.865	0.855	0.867	0.784	0.917	0.824
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.037	0.054	0.105	0.041	0.081	0.042	0.095
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.087	0.081	0.039	0.092	0.135	0.042	0.081
MDH2							
(N)	40	37	38	49	38	37	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.112	0.176	0.039	0.184	0.158	0.095	0.162
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.887	0.824	0.961	0.816	0.842	0.905	0.838

Appendix:B
Table 1. cont.

MDH5							
(N)	40	40	39	49	40	39	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.012	0.013	0.010	0.0	0.0	0.014
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	0.987	0.987	0.990	1.000	1.000	0.986

PGM1							
(N)	40	39	40	48	40	39	36
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000

PGM2							
(N)	40	40	40	49	39	39	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.487	0.300	0.475	0.429	0.385	0.513	0.514
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.050	0.112	0.188	0.153	0.077	0.192	0.122
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.462	0.525	0.325	0.378	0.500	0.295	0.351
9	0.0	0.063	0.012	0.041	0.038	0.0	0.014

TPI1							
(N)	40	40	40	49	40	40	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.075	0.075	0.050	0.051	0.063	0.0	0.068
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.925	0.925	0.950	0.949	0.938	1.000	0.932

TPI2							
(N)	40	40	40	49	40	40	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.000	1.000	1.000	1.000	1.000	1.000	1.000

ACO							
(N)	40	40	40	49	40	40	37
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.162	0.112	0.125	0.163	0.112	0.087	0.095
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.712	0.787	0.787	0.776	0.887	0.825	0.892
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.125	0.100	0.087	0.061	0.0	0.087	0.014

Appendix:B
Table 2. Allele frequencies by age class for plot A.

Locus	Allele	Age Class S	0	1	2	3
CAT	2					
	4	1.00	1.00	1.00	1.00	1.00
SOD2	1	.052	.004	.031	.039	.020
	2	.078	.033	.051	.025	.030
	4	.856	.955	.904	.904	.940
	8	.015	.008	.014	.032	.010
PGI2	2	.471	.497	.447	.437	.560
	4	.518	.500	.550	.560	.440
	8	.011	.003	.003	.003	
GOT	2	.356	.270	.245	.260	.250
	4	.464	.528	.542	.541	.448
	9	.180	.202	.213	.199	.302
MDH1	3	.785	.738	.762	.785	.660
	5	.104	.121	.119	.094	.150
	7	.111	.141	.119	.121	.190
MDH2	2	.143	.158	.163	.191	.200
	8	.857	.842	.837	.809	.800
MDH5	2		.003		.003	
	4	1.00	.997	1.00	.997	1.00
PGM1	4	1.00	1.00	1.00	1.00	1.00
PGM2	4	.475	.463	.400	.456	.459
	6	.047	.060	.073	.107	.071
	8	.402	.400	.407	.356	.367
	9	.077	.077	.120	.081	.102
TPI1	2	.062	.067	.050	.043	.060
	4	.938	.933	.950	.957	.940
TPI2	4	1.00	1.00	1.00	1.00	1.00
ACO	2	.142	.120	.139	.107	.092
	4	.723	.833	.777	.810	.837
	6	.135	.047	.084	.083	.071

Appendix:B
Table 3. Allele frequencies by age class for plot B.

Locus	Allele	Age Class S	0	1	2	3
CAT	2	.006	.032			
	4	.994	.967	1.00	1.00	1.00
SOD2	1	.015	.006	.029	.028	.030
	2	.025	.018	.026	.048	.040
	4	.947	.967	.926	.895	.890
	8	.012	.009	.018	.028	.040
PGI2	2	.453	.485	.453	.470	.490
	4	.538	.502	.542	.523	.500
	8	.009	.012	.005	.007	.010
GOT	2	.331	.232	.285	.321	.281
	4	.451	.627	.503	.449	.354
	9	.218	.141	.212	.231	.366
MDH1	3	.866	.883	.865	.816	.840
	5	.058	.076	.077	.101	.070
	7	.009	.012	.005	.007	.010
MDH2	2	.131	.133	.117	.131	.120
	8	.869	.867	.883	.869	.880
MDH5	2	.009	.005	.002	.005	
	4	.991	.995	.998	.995	1.00
PGM1	4	1.00	1.00	1.00	1.00	1.00
PGM2	4	.423	.475	.467	.442	.450
	6	.127	.105	.125	.143	.090
	8	.420	.357	.340	.357	.400
	9	.030	.063	.068	.058	.060
TPI1	2	.062	.050	.045	.035	.080
	4	.938	.950	.955	.965	.920
TPI2	4	1.00	1.00	1.00	1.00	1.00
ACO	2	.142	.141	.155	.147	.112
	4	.766	.809	.765	.763	.825
	6	.092	.050	.080	.090	.063

Appendix:B
Table 4. Allele frequencies by age class for plot C.

Locus	Allele	Age Class S	0	1	2	3
CAT	2	.008				
	4	.992	1.00	1.00	1.00	1.00
SOD2	1	.056	.033	.051	.058	.070
	2	.052	.044	.024	.038	.030
	4	.845	.874	.832	.852	.790
	8	.047	.048	.093	.052	.110
PGI2	2	.419	.400	.400	.390	.450
	4	.568	.587	.583	.597	.550
	8	.013	.013	.017	.013	
GOT	2	.297	.223	.236	.225	.187
	4	.461	.539	.524	.561	.500
	9	.241	.238	.240	.214	.313
MDH1	3	.841	.862	.872	.849	.860
	5	.073	.071	.071	.060	.070
	7	.086	.067	.057	.091	.070
MDH2	2	.138	.117	.130	.127	.140
	8	.862	.883	.870	.873	.860
MDH5	2	.004			.007	
	4	.996	1.00	1.00	.993	1.00
PGM1	4	1.00	1.00	1.00	1.00	1.00
PGM2	4	.469	.383	.377	.367	.370
	6	.130	.177	.233	.207	.270
	8	.383	.440	.383	.403	.360
	9	.017		.007	.023	
TPI1	2	.043	.047	.067	.050	.020
	4	.957	.953	.933	.950	.980
TPI2	4	1.00	1.00	1.00	1.00	1.00
ACO	2	.098	.097	.147	.101	.061
	4	.867	.900	.820	.876	.938
	6	.034	.003	.033	.023	

Appendix:B
Table 5. Allele frequencies by age class for subplot A1.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.038			.012
	2	.077	.031	.063	.035
	4	.885	.969	.937	.907
	8				.046
PGI2	2	.563	.460	.450	.420
	4	.437	.530	.550	.580
	8		.010		
GOT	2	.310	.261	.250	.271
	4	.431	.478	.534	.510
	9	.258	.261	.216	.219
MDH1	3	.763	.780	.755	.760
	5	.211	.150	.096	.080
	7	.026	.070	.149	.160
MDH2	2	.192	.200	.120	.250
	8	.808	.800	.880	.750
MDH5	2		.01		
	4	1.00	.99	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.437	.530	.390	.490
	6	.075	.030	.050	.090
	8	.375	.340	.420	.360
	9	.113	.100	.140	.060
TPI1	2	.063	.090	.080	.030
	4	.937	.910	.920	.970
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.125	.060	.170	.080
	4	.750	.910	.730	.850
	6	.125	.030	.100	.070

Appendix:B
Table 6. Allele frequencies by age class for subplot A2.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.073		.060	.010
	2	.094	.044	.070	.030
	4	.812	.933	.830	.940
	8	.021	.022	.040	.020
PGI2	2	.440	.580	.440	.430
	4	.550	.420	.550	.560
	8	.010		.010	.010
GOT	2	.245	.359	.214	.270
	4	.543	.422	.520	.480
	9	.213	.219	.265	.250
MDH1	3	.775	.702	.760	.735
	5	.112	.149	.180	.122
	7	.112	.149	.060	.143
MDH2	2	.163	.163	.170	.153
	8	.837	.837	.830	.847
MDH5	2				.010
	4	1.00	1.00	1.00	.990
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.459	.480	.360	.420
	6	.041	.060	.080	.143
	8	.410	.420	.490	.357
	9	.092	.040	.070	.082
TPI1	2	.071	.090	.030	.020
	4	.929	.910	.970	.980
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.143	.120	.163	.140
	4	.704	.860	.765	.790
	6	.153	.020	.071	.070

Appendix:B
Table 7. Allele frequencies by age class for subplot A3.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.042	.017	.031	.092
	2	.063	.017	.021	.010
	4	.875	.967	.948	.867
	8	.021			.031
PGI2	2	.429	.450	.450	.460
	4	.551	.550	.550	.540
	8	.020			
GOT	2	.490	.217	.270	.240
	4	.408	.652	.570	.630
	9	.102	.130	.160	.130
MDH1	3	.813	.729	.770	.860
	5	.010	.063	.080	.080
	7	.177	.208	.150	.060
MDH2	2	.083	.110	.200	.170
	8	.917	.890	.800	.830
MDH5	2				
	4	1.00	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.521	.380	.450	.460
	6	.031	.090	.090	.090
	8	.417	.440	.310	.350
	9	.031	.090	.150	.100
TPI1	2	.051	.020	.040	.080
	4	.949	.980	.960	.920
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.156	.180	.082	.100
	4	.719	.730	.837	.790
	6	.125	.090	.082	.110

Appendix:B
Table 8. Allele frequencies by age class for subplot B1.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.025	.024	.062	.020
	2	.025		.031	.060
	4	.925	.963	.885	.880
	8	.025	.012	.021	.040
PGI2	2	.512	.490	.470	.440
	4	.488	.490	.520	.560
	8		.020	.010	
GOT	2	.486	.293	.323	.347
	4	.333	.543	.490	.418
	9	.181	.163	.187	.235
MDH1	3	.875	.857	.900	.806
	5	.037	.051	.030	.102
	7	.088	.092	.070	.092
MDH2	2	.113	.150	.150	.143
	8	.887	.850	.850	.857
MDH5	2		.01	.01	
	4	1.00	.99	.99	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.487	.550	.510	.530
	6	.050	.100	.080	.082
	8	.463	.240	.350	.337
	9		.110	.060	.051
TPI1	2	.075	.070	.060	.010
	4	.925	.930	.940	.990
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.163	.050	.110	.160
	4	.712	.830	.830	.730
	6	.125	.120	.060	.110

Appendix:B
Table 9. Allele frequencies by age class for subplot B2.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.027		.030	.020
	2	.013		.020	.080
	4	.959	.974	.920	.870
	8		.026	.010	.030
PGI2	2	.562	.510	.450	.480
	4	.425	.460	.550	.500
	8	.013	.030		.020
GOT	2	.410	.400	.316	.306
	4	.320	.600	.500	.510
	9	.270		.184	.184
MDH1	3	.865	.870	.870	.820
	5	.054	.100	.040	.100
	7	.081	.030	.090	.080
MDH2	2	.176	.090	.080	.180
	8	.824	.910	.920	.820
MDH5	2	.012			.010
	4	.988	1.00	1.00	.990
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.300	.490	.490	.420
	6	.113	.100	.110	.110
	8	.525	.350	.340	.380
	9	.062	.060	.060	.090
TPI1	2	.075	.020	.010	.030
	4	.925	.980	.990	.970
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.112	.170	.120	.190
	4	.790	.810	.710	.690
	6	.100	.020	.170	.120

Appendix:B
Table 10. Allele frequencies by age class for subplot B3.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1				.043
	2	.051	.012	.022	.011
	4	.949	.988	.955	.935
	8			.022	.011
PGI2	2	.387	.500	.470	.500
	4	.613	.500	.520	.500
	8			.010	
GOT	2	.275	.135	.240	.330
	4	.475	.769	.609	.447
	9	.250	.096	.152	.223
MDH1	3	.855	.875	.840	.806
	5	.105	.094	.110	.081
	7	.034	.031	.050	.112
MDH2	2	.040	.102	.120	.092
	8	.960	.898	.880	.908
MDH5	2	.013	.010		.010
	4	.987	.990	1.00	.990
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.475	.420	.400	.460
	6	.187	.130	.180	.170
	8	.325	.390	.340	.350
	9	.013	.060	.080	.020
TPI1	2	.050	.060	.060	.030
	4	.950	.940	.940	.970
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.125	.170	.190	.110
	4	.787	.820	.770	.870
	6	.087	.010	.040	.020

Appendix:B
Table 11. Allele frequencies by age class for subplot B4.

Locus	Allele	Age Class S	0	1	2
CAT	2	.020	.130		
	4	.980	.870	1.00	1.00
SOD2	1	.011		.021	.030
	2	.011	.052	.032	.040
	4	.960	.950	.925	.900
	8	.022		.021	.030
PGI2	2	.367	.440	.420	.460
	4	.612	.560	.580	.530
	8	.020		.021	.030
GOT	2	.198	.197	.260	.300
	4	.625	.634	.420	.420
	9	.177	.167	.320	.280
MDH1	3	.867	.930	.850	.830
	5	.041	.060	.130	.120
	7	.092	.010	.020	.050
MDH2	2	.184	.190	.120	.110
	8	.816	.810	.880	.890
MDH5	2	.010			
	4	.989	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.428	.440	.470	.360
	6	.153	.090	.130	.210
	8	.378	.450	.330	.360
	9	.041	.020	.070	.070
TPI1	2	.051	.050	.050	.070
	4	.949	.950	.950	.930
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.163	.173	.200	.130
	4	.775	.775	.750	.760
	6	.061	.051	.050	.110

Appendix:B
Table 12. Allele frequencies by age class for subplot C1.

Locus	Allele	Age Class S	0	1	2
CAT	2	.025			
	4	.975	1.00	1.00	1.00
SOD2	1	.037	.042	.041	.061
	2	.075	.028	.010	.010
	4	.825	.875	.877	.888
	8	.065	.056	.071	.041
PGI2	2	.375	.350	.360	.330
	4	.600	.610	.610	.640
	8	.025	.040	.030	.030
GOT	2	.325	.167	.304	.255
	4	.388	.521	.500	.510
	9	.287	.312	.196	.235
MDH1	3	.784	.877	.900	.820
	5	.081	.071	.070	.110
	7	.135	.051	.030	.070
MDH2	2	.158	.100	.150	.120
	8	.842	.900	.850	.880
MDH5	2				
	4	1.00	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.385	.280	.350	.360
	6	.077	.160	.250	.150
	8	.500	.560	.400	.460
	9	.038	.000	.000	.030
TPI1	2	.063	.100	.130	.070
	4	.937	.900	.870	.930
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.112	.120	.060	.120
	4	.888	.870	.920	.860
	6		.011	.020	.020

Appendix:B
Table 13. Allele frequencies by age class for subplot C2.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.051	.020	.050	.050
	2	.051	.061	.030	.030
	4	.859	.867	.810	.890
	8	.038	.051	.110	.030
PGI2	2	.500	.400	.440	.390
	4	.487	.600	.540	.600
	8	.012		.020	.010
GOT	2	.333	.325	.280	.163
	4	.474	.500	.480	.663
	9	.192	.174	.240	.173
MDH1	3	.917	.820	.850	.898
	5	.042	.060	.070	.020
	7	.042	.012	.070	.082
MDH2	2	.095	.110	.140	.133
	8	.905	.890	.860	.867
MDH5	2				.010
	4	1.00	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.513	.380	.380	.330
	6	.192	.160	.200	.250
	8	.295	.460	.410	.400
	9			.010	.020
TPI1	2		.020	.050	.050
	4	1.00	.980	.950	.950
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.088	.090	.100	.040
	4	.825	.910	.880	.930
	6	.087		.020	.030

Appendix:B
Table 14. Allele frequencies by age class for subplot C3.

Locus	Allele	Age Class S	0	1	2
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.081	.040	.064	.065
	2	.027	.040	.032	.076
	4	.851	.880	.808	.772
	8	.040	.040	.096	.087
PGI2	2	.378	.450	.400	.450
	4	.622	.550	.600	.550
	8				
GOT	2	.229	.190	.130	.255
	4	.527	.590	.590	.510
	9	.243	.220	.280	.235
MDH1	3	.824	.888	.860	.830
	5	.095	.082	.070	.050
	7	.081	.031	.070	.120
MDH2	2	.162	.140	.100	.130
	8	.838	.860	.900	.870
MDH5	2	.014			
	4	.986	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.513	.490	.400	.410
	6	.126	.210	.250	.220
	8	.351	.300	.340	.350
	9	.010		.010	.020
TPI1	2	.067	.020	.020	.030
	4	.932	.980	.980	.970
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.095	.080	.245	.143
	4	.892	.920	.702	.837
	6	.014		.053	.020

Appendix:B
Table 15. Allele frequencies by plot within age class S.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2		.006	.009
	4	1.00	.994	.991
SOD2	1	.052	.015	.056
	2	.078	.025	.052
	4	.855	.947	.845
	8	.015	.012	.047
PGI2	2	.471	.453	.419
	4	.518	.538	.568
	8	.011	.009	.013
GOT	2	.356	.331	.297
	4	.464	.451	.461
	9	.180	.218	.241
MDH1	3	.785	.866	.841
	5	.104	.058	.073
	7	.111	.076	.086
MDH2	2	.143	.131	.138
	8	.857	.869	.862
MDH5	2		.009	.004
	4	1.00	.991	.996
PGM1	4	1.00	1.00	1.00
PGM2	4	.475	.423	.470
	6	.047	.127	.130
	8	.401	.420	.383
	9	.077	.030	.017
TPI1	2	.062	.062	.043
	4	.938	.938	.957
TPI2	4	1.00	1.00	1.00
ACO	2	.142	.142	.098
	4	.723	.766	.867
	6	.135	.092	.034

Appendix:B
Table 16. Allele frequencies by plot within age class 0.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2		.033	
	4	1.00	.967	1.00
SOD2	1	.004	.006	.033
	2	.032	.018	.044
	4	.955	.967	.874
	8	.008	.009	.048
PGI2	2	.497	.485	.400
	4	.500	.503	.587
	8	.003	.012	.013
GOT	2	.270	.232	.223
	4	.528	.623	.539
	9	.202	.141	.237
MDH1	3	.738	.883	.861
	5	.121	.076	.071
	7	.141	.041	.067
MDH2	2	.158	.133	.117
	8	.842	.867	.883
MDH5	2	.003	.005	
	4	.997	.995	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.463	.475	.383
	6	.060	.105	.177
	8	.400	.357	.440
	9	.077	.063	
TPI1	2	.067	.050	.047
	4	.933	.950	.953
TPI2	4	1.00	1.00	1.00
ACO	2	.120	.140	.097
	4	.833	.810	.900
	6	.047	.050	.003

Appendix:B
Table 17. Allele frequencies by plot within age class 1.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.031	.029	.051
	2	.051	.026	.024
	4	.904	.926	.832
	8	.014	.018	.093
PGI2	2	.447	.453	.400
	4	.550	.542	.583
	8	.033	.005	.017
GOT	2	.245	.285	.236
	4	.542	.503	.524
	9	.213	.212	.240
MDH1	3	.762	.865	.873
	5	.119	.077	.070
	7	.119	.057	.057
MDH2	2	.163	.117	.130
	8	.837	.883	.870
MDH5	2		.003	
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.400	.467	.377
	6	.073	.125	.233
	8	.407	.340	.383
	9	.120	.068	.007
TPI1	2	.050	.045	.067
	4	.950	.955	.993
TPI2	4	1.00	1.00	1.00
ACO	2	.138	.155	.147
	4	.777	.765	.819
	6	.085	.080	.033

Appendix:B
Table 18. Allele frequencies by plot within age class 2.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.039	.028	.058
	2	.025	.048	.038
	4	.904	.895	.852
	8	.032	.028	.052
PGI2	2	.437	.470	.390
	4	.560	.522	.597
	8	.003	.007	.013
GOT	2	.260	.321	.224
	4	.540	.448	.561
	9	.199	.231	.214
MDH1	3	.785	.816	.849
	5	.094	.101	.060
	7	.121	.083	.091
MDH2	2	.191	.131	.127
	8	.809	.869	.872
MDH5	2	.003	.005	.007
	4	.997	.995	.993
PGM1	4	1.00	1.00	1.00
PGM2	4	.456	.442	.367
	6	.107	.143	.207
	8	.356	.357	.403
	9	.081	.058	.023
TPI1	2	.043	.035	.050
	4	.957	.965	.950
TPI2	4	1.00	1.00	1.00
ACO	2	.107	.147	.101
	4	.810	.763	.876
	6	.083	.090	.025

Appendix:B
Table 19. Allele frequencies by plot within age class 3.

Locus	Allele	Plot A	Plot B	Plot C
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.020	.030	.070
	2	.030	.040	.030
	4	.940	.890	.790
	8	.010	.040	.110
PGI2	2	.560	.490	.450
	4	.440	.500	.550
	8		.010	
GOT	2	.250	.280	.187
	4	.448	.354	.500
	9	.302	.366	.313
MDH1	3	.660	.840	.860
	5	.150	.070	.070
	7	.190	.090	.070
MDH2	2	.200	.120	.140
	8	.800	.880	.860
MDH5	2			
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.459	.450	.370
	6	.071	.090	.270
	8	.367	.400	.360
	9	.102	.060	
TPI1	2	.060	.080	.020
	4	.940	.920	.980
TPI2	4	1.00	1.00	1.00
ACO	2	.092	.113	.061
	4	.837	.825	.939
	6	.071	.062	

Appendix:B
Table 20. Allele frequencies by subplot for plot A, within age class S.

Locus	Allele	Subplot A1	Subplot A2	Subplot A3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.038	.073	.042
	2	.077	.094	.063
	4	.885	.813	.875
	8		.021	.021
PGI2	2	.563	.439	.428
	4	.437	.551	.551
	8		.010	.020
GOT	2	.310	.245	.490
	4	.431	.543	.408
	9	.259	.213	.102
MDH1	3	.763	.775	.812
	5	.210	.112	.011
	7	.026	.112	.177
MDH2	2	.192	.163	.083
	8	.807	.837	.917
MDH5	2			
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.437	.459	.521
	6	.075	.041	.031
	8	.375	.408	.417
	9	.113	.092	.031
TPI1	2	.063	.071	.051
	4	.937	.929	.949
TPI2	4	1.00	1.00	1.00
ACO	2	.125	.143	.156
	4	.750	.704	.719
	6	.125	.153	.125

Appendix:B
Table 21. Allele frequencies by suplot for plot B, within age class S.

Locus	Allele	Subplot B1	B2	B3	B4
CAT	2				.020
	4	1.00	1.00	1.00	.980
SOD2	1	.025	.027		.011
	2	.025	.013	.051	.011
	4	.925	.959	.949	.956
	8	.025			.022
PGI2	2	.513	.563	.387	.367
	4	.487	.425	.613	.612
	8		.012		.021
GOT	2	.486	.410	.275	.198
	4	.333	.320	.475	.625
	9	.181	.269	.250	.177
MDH1	3	.875	.865	.855	.867
	5	.037	.054	.105	.041
	7	.087	.081	.039	.092
MDH2	2	.113	.176	.039	.184
	8	.887	.824	.961	.816
MDH5	2		.013	.013	.010
	4	1.00	.987	.987	.990
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.487	.300	.475	.429
	6	.050	.113	.187	.153
	8	.463	.525	.325	.377
	9		.062	.013	.041
TPI1	2	.075	.075	.050	.051
	4	.925	.925	.950	.949
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.163	.113	.125	.163
	4	.712	.787	.787	.775
	6	.125	.100	.087	.061

Appendix:B

Table 22. Allele frequencies by subplot for plot C, within age class S.

Locus	Allele	Subplot C1	C2	C3
CAT	2	.025		
	4	.975	1.00	1.00
SOD2	1	.037	.050	.081
	2	.075	.050	.027
	4	.825	.859	.851
	8	.063	.040	.041
PGI2	2	.375	.500	.378
	4	.600	.487	.622
	8	.025	.013	
GOT	2	.325	.333	.230
	4	.387	.474	.527
	9	.288	.193	.243
MDH1	3	.784	.917	.824
	5	.081	.042	.095
	7	.135	.041	.081
MDH2	2	.158	.095	.162
	8	.842	.905	.838
MDH5	2			.130
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.385	.513	.513
	6	.077	.192	.122
	8	.500	.295	.351
	9	.038		.013
TPI1	2	.063		.067
	4	.937	1.00	.932
TPI2	4	1.00	1.00	1.00
ACO	2	.112	.087	.095
	4	.887	.825	.892
	6		.087	.013

Appendix:B

Table 23. Allele frequencies by subplot for plot A, within age class 0.

Locus	Allele	Subplot A1	Subplot A2	Subplot A3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1			.017
	2	.031	.044	.017
	4	.969	.933	.967
	8		.022	
PGI2	2	.460	.580	.450
	4	.530	.420	.550
	8	.010		
GOT	2	.261	.359	.217
	4	.478	.422	.652
	9	.261	.219	.130
MDH1	3	.780	.702	.729
	5	.150	.149	.062
	7	.070	.149	.208
MDH2	2	.200	.163	.110
	8	.800	.837	.890
MDH5	2	.010		
	4	.990	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.530	.480	.380
	6	.030	.060	.090
	8	.340	.420	.440
	9	.100	.040	.090
TPI1	2	.090	.090	.020
	4	.910	.910	.980
TPI2	4	1.00	1.00	1.00
ACO	2	.060	.120	.180
	4	.910	.860	.730
	6	.030	.020	.090

Appendix:B

Table 24. Allele frequencies by subplot for plot B, within age class 0.

Locus	Allele	Subplot B1	B2	B3	B4
CAT	2				.130
	4	1.00	1.00	1.00	.870
SOD2	1	.024			
	2			.012	.052
	4	.963	.974	.988	.948
	8	.012	.056		
PGI2	2	.490	.510	.500	.440
	4	.490	.460	.500	.560
	8	.020	.030		
GOT	2	.293	.400	.135	.197
	4	.543	.600	.769	.636
	9	.163		.096	.167
MDH1	3	.857	.870	.875	.930
	5	.051	.100	.094	.060
	7	.092	.030	.031	.010
MDH2	2	.150	.090	.102	.190
	8	.850	.910	.898	.810
MDH5	2	.010		.010	
	4	.990	1.00	.990	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.550	.490	.420	.440
	6	.100	.100	.130	.090
	8	.240	.350	.390	.450
	9	.110	.060	.060	.020
TPI1	2	.070	.020	.060	.050
	4	.930	.980	.940	.950
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.050	.170	.170	.173
	4	.830	.810	.820	.776
	6	.120	.020	.010	.051

Appendix:B

Table 25. Allele frequencies by subplot for plot C, within age class 0.

Locus	Allele	Subplot C1	Subplot C2	Subplot C3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.042	.020	.040
	2	.028	.061	.040
	4	.875	.867	.880
	8	.056	.051	.040
PGI2	2	.350	.400	.450
	4	.610	.600	.550
	8	.040		
GOT	2	.167	.325	.190
	4	.521	.500	.590
	9	.312	.174	.220
MDH1	3	.877	.820	.888
	5	.071	.060	.082
	7	.051	.120	.030
MDH2	2	.100	.110	.140
	8	.900	.890	.860
MDH5	2			
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.280	.380	.490
	6	.160	.160	.210
	8	.560	.460	.300
	9			
TPI1	2	.100	.020	.020
	4	.900	.980	.980
TPI2	4	1.00	1.00	1.00
ACO	2	.120	.090	.080
	4	.870	.910	.920
	6	.010		

Appendix:B

Table 26. Allele frequencies by subplot for plot A, within age class 1.

Locus	Allele	Subplot A1	Subplot A2	Subplot A3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1		.060	.031
	2	.063	.070	.021
	4	.937	.830	.948
	8		.040	
PGI2	2	.450	.440	.450
	4	.550	.550	.550
	8		.010	
GOT	2	.250	.214	.270
	4	.534	.520	.570
	9	.216	.265	.160
MDH1	3	.755	.760	.770
	5	.096	.180	.080
	7	.149	.060	.150
MDH2	2	.120	.170	.200
	8	.880	.830	.800
MDH5	2			
	4	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.390	.360	.450
	6	.050	.080	.090
	8	.420	.490	.310
	9	.140	.070	.150
TPI1	2	.080	.030	.040
	4	.920	.970	.960
TPI2	4	1.00	1.00	1.00
ACO	2	.170	.163	.082
	4	.730	.765	.837
	6	.100	.071	.081

Appendix:B

Table 27. Allele frequencies by subplot for plot B, within age class 1.

Locus	Allele	Subplot B1	B2	B3	B4
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.063	.030		.021
	2	.031	.020	.022	.032
	4	.885	.940	.956	.925
	8	.021	.010	.022	.021
PGI2	2	.470	.450	.470	.420
	4	.520	.550	.520	.580
	8	.010		.010	
GOT	2	.323	.316	.239	.260
	4	.490	.500	.609	.420
	9	.187	.184	.152	.320
MDH1	3	.900	.870	.840	.850
	5	.030	.040	.110	.130
	7	.070	.090	.050	.020
MDH2	2	.150	.080	.120	.120
	8	.850	.920	.880	.880
MDH5	2	.010			
	4	.990	1.00	1.00	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.510	.490	.400	.470
	6	.080	.110	.180	.130
	8	.350	.340	.340	.330
	9	.060	.060	.080	.070
TPI1	2	.060	.010	.060	.050
	4	.940	.990	.940	.950
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.110	.120	.190	.200
	4	.830	.710	.770	.750
	6	.060	.170	.040	.050

Appendix:B

Table 28. Allele frequencies by subplot for plot C, within age class 1.

Locus	Allele	Subplot C1	Subplot C2	Subplot C3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.041	.050	.064
	2	.010	.030	.032
	4	.877	.810	.808
	8	.071	.110	.096
PGI2	2	.360	.440	.400
	4	.610	.540	.600
	8	.030	.020	
GOT	2	.304	.280	.130
	4	.500	.480	.590
	9	.196	.240	.280
MDH1	3	.900	.857	.860
	5	.070	.071	.070
	7	.030	.071	.070
MDH2	2	.150	.140	.100
	8	.850	.860	.900
MDH5	2			
	4	1.00	1.00	1.00
PGM2	4	.350	.380	.400
	6	.250	.200	.250
	8	.400	.410	.340
	9		.010	.010
TPI1	2	.130	.050	.020
	4	.870	.950	.980
TPI2	4	1.00	1.00	1.00
ACO	2	.060	.010	.245
	4	.920	.880	.702
	6	.020	.020	.053

Appendix:B
Table 29. Allele frequencies by subplot for plot A, within age class 2.

Locus	Allele	Sbplots A1	A2	A3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.012	.010	.092
	2	.035	.031	.010
	4	.907	.939	.867
	8	.046	.020	.031
PGI2	2	.420	.430	.460
	4	.580	.560	.540
	8		.010	
GOT	2	.271	.270	.240
	4	.510	.480	.630
	9	.219	.250	.130
MDH1	3	.760	.735	.860
	5	.080	.122	.080
	7	.160	.143	.060
MDH2	2	.250	.153	.170
	8	.750	.847	.830
MDH5	2		.010	
	4	1.00	.990	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.490	.418	.460
	6	.090	.143	.090
	8	.360	.357	.350
	9	.060	.082	.100
TPI1	2	.030	.020	.080
	4	.970	.980	.920
TPI2	4	1.00	1.00	1.00
ACO	2	.080	.140	.100
	4	.850	.790	.790
	6	.070	.070	.110

Appendix:B

Table 30. Allele frequencies by subplot for plot B, within age class 2.

Locus	Allele	Subplot B1	B2	B3	B4
CAT	2				
	4	1.00	1.00	1.00	1.00
SOD2	1	.020	.020	.044	.030
	2	.060	.080	.011	.040
	4	.880	.870	.935	.900
	8	.040	.030	.011	.030
PGI2	2	.440	.480	.500	.460
	4	.560	.500	.500	.530
	8		.020		.010
GOT	2	.347	.306	.330	.300
	4	.418	.510	.447	.420
	9	.235	.184	.223	.280
MDH1	3	.806	.820	.806	.830
	5	.102	.100	.082	.120
	7	.092	.080	.112	.050
MDH2	2	.143	.180	.092	.110
	8	.857	.820	.908	.890
MDH5	2		.010	.010	
	4	1.00	.990	.990	1.00
PGM1	4	1.00	1.00	1.00	1.00
PGM2	4	.531	.420	.460	.360
	6	.081	.110	.170	.210
	8	.337	.380	.350	.360
	9	.051	.090	.020	.070
TPI1	2	.010	.030	.030	.070
	4	.990	.970	.970	.930
TPI2	4	1.00	1.00	1.00	1.00
ACO	2	.160	.190	.110	.130
	4	.730	.690	.870	.760
	6	.110	.120	.020	.110

Appendix:B
Table 31. Allele frequencies by subplot for plot C, within age class 2.

Locus	Allele	Subplots C1	C2	C3
CAT	2			
	4	1.00	1.00	1.00
SOD2	1	.061	.050	.065
	2	.010	.030	.076
	4	.888	.890	.772
	8	.041	.030	.087
PGI2	2	.330	.390	.450
	4	.640	.600	.550
	8	.030	.010	
GOT	2	.255	.163	.255
	4	.510	.663	.510
	9	.235	.174	.235
MDH1	3	.820	.898	.830
	5	.110	.020	.050
	7	.070	.082	.120
MDH2	2	.120	.133	.130
	8	.880	.867	.870
MDH5	2	.010	.010	
	4	.990	.990	1.00
PGM1	4	1.00	1.00	1.00
PGM2	4	.360	.330	.410
	6	.150	.250	.220
	8	.460	.400	.350
	9	.030	.020	.020
TPI1	2	.070	.050	.030
	4	.930	.950	.970
TPI2	4	1.00	1.00	1.00
ACO	2	.120	.040	.143
	4	.860	.930	.837
	6	.020	.030	.020