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Demographic genetics of the American beech (*Fagus grandifolia* Ehrh.) III. Genetic substructuring of coastal plain population in Maryland

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Abstract

Spatiotemporal genetic substructurings were investigated in the American beech population of the east-central coastal plain in Maryland. All trees including seedlings, various sizes of juveniles, and mature trees within the study site (10 × 100 m) were mapped, diameters measured, and leaves collected for allozyme analyses. Eleven polymorphic loci in eight enzyme systems were examined: *6Pgdh2*, *6Pgdh3*, *Acp2*, *Adh1*, *Adh2*, *Fum*, *Got1*, *Got3*, *Lap*, *Pgi*, and *Pgm2*. A total of 1945 trees were analyzed and 595 multilocus genotypes were detected. Six size-classes and 10 spatial blocks were discriminated for spatiotemporal analyses. Parameters for genetic variations (heterozygosity, Simpson's index, Shannon-Weaver's index, and inbreeding coefficient) decreased in larger size-classes. These genetic parameters fluctuated in spatial blocks of 10 m intervals, in which certain alleles were characteristic of specific blocks. The spatial autocorrelation by Moran's *I* and coancestry revealed the ranges of genetic relatedness to be only 20–30 m. Multilocus genotype analyses showed that higher genetic variations occur in larger size-classes and at gap openings where seed shadows for mother trees are overlapped. The relationships among reproductive trees, seedlings and juveniles suggested that the seed dispersal range of the American beech is normally in the range of 30–40 m. The mechanisms of a remarkably high genetic polymorphism maintained in this once artificially disturbed and grazed forest are discussed as related to conservation biology.

Keywords: allozyme, coastal plain, *Fagus grandifolia*, *F*-statistics, size-class discrimination, spatial substructuring.

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Introduction

The American beech (*Fagus grandifolia* Ehrh.) is one of the representative dominant elements in the north-eastern hardwood forests of North America, spreading over once heavily glaciated territories in the Great Lakes region and central to northern Quebec, with its further southern range extension over the montane zones and coastal plains in the east-central USA (Brown 1922; Shelford 1963; Delcourt & Delcourt 1987; Rohrig & Ulrich 1991). The American beech has two characteristic growth forms related to the absence or presence of vegetative offshoot formation; one regenerates only through sexual reproduction, and the other reproduces sexually as well as asexually, forming root sucker off-shoots (Camp 1950; Ward 1961; Houston & Houston 1994; Peters 1997; Kitamura *et al*. 2000). The former type spreads widely in the Coastal Plain in the eastern to south-eastern USA as well as in the Mississippi basin and the Ozark mountains (Kitamura & Kawano 2001).

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Our previous demographic genetic studies of the American beech have revealed that there occur unique but diverse spatiotemporal genetic substructures related to its reproductive systems as noted (Kitamura *et al*. 2000, 2001). In the present series of studies, we have consistently used multilocus genotypes to demonstrate clonal substructures in the patch or local populations, and also size-class discrimination was introduced to show chronological genetic differentiation among different size-classes, i.e. different generations (Kitamura *et al*. 2000, 2001).

The present study, as one in a series of demographic genetic analyses of the American beech populations, attempts to examine the demographic genetic structures of the coastal plain populations in Maryland, focusing on the aspects of recovery, and maintenance mechanisms of genetic variations within a local population during the process of secondary succession (Kitamura *et al*. 2000, 2001; Kitamura & Kawano 2001). This specific site covered by a typical northern mixed hardwood-pine forest is located at the estuary of the Rhode River, running into Chesapeak Bay. The American beech populations typical of the east-central coastal plain are mainly regenerated by seed reproduction and show no root-sucker formation (Kitamura & Kawano 2001). The site had once been a pasture, was left abandoned, and recovered as a forest stand during the past 150 years. Like other *Fagus* species, the American beech also shows mast-flowering and fruiting with several-year intervals (Peters 1997). As the American beech is a typical outbreeder (Kitamura *et al*. 1998), genetic variations could have rapidly recovered within a local population with overlapping generations from a limited number of founder trees (Kawano & Kitamura 1997; Kitamura *et al*. 1997a,b). In view of conservation biology it is also interesting to ascertain the effect of artificial disturbances and the recovery of genetic variation during the regeneration processes of a local population (Frankel *et al*. 1995).

Materials and methods

Study site

A transect (10×100 m) was established in the American beech population within the property of the Smithsonian Environmental Research Center (SERC), Edgewater, Maryland.

In view of the geographic range of the species, this population is located at the east-central coastal plain, which belongs to the Oak-Pine forest region (Rohrig & Ulrich 1991). The American beech is dominant there and mixed with *Liriodendron tulipifera*, *Quercus ocoteaefolia*, and *Liquidambar styraciflua*. The American beech in this region seldom shows root sucker off-shoots. Large patches of May-apple (*Podophyllum peltatum*), Jack-in-the Pulpit (*Arisaema triphyllum*), and *Uvularia perfoliata* develop on the forest floor.

Following the mast-flowering and fruiting of the American beech in 1994, a number of seedlings were observed in the following year. The establishment of the seedlings was successful, and seedlings were abundant within and around the transect.

Sample collection and enzyme electrophoreses

All beech trees within the transect and mature trees surrounding the transect were mapped, their diameters at the ground height (DGH) and/or the breast height (d.b.h) were measured, and leaves were collected for enzyme electrophoreses. Samplings of juveniles (y-axis >0 m) and mature trees were carried out from May to June 1994. All the seedlings, juveniles (y-axis <0 m), and additional mature trees were sampled in May 1995.

Enzyme extractions, electrophoreses and detections of allozymes have been described in earlier published works (Davis 1964; Orstein 1964; Shiraishi 1988; Kitamura & Kawano 2001). Eleven polymorphic loci were used for the analyses: *6Pgdh2*, *6Pgdh3*, *Acp2*, *Adh1*, *Adh2*, *Fum*, *Got1*, *Got3*, *Lap*, *Pgi*, and *Pgm2*.

Data analyses

For analyzing the spatiotemporal genetic differentiations, all the sampled trees were discriminated into both size-classes and spatial blocks. Six size-classes were distinguished: Class 0 stands for seedlings (in 1995), Classes 1–4 stands for juveniles according to the main stem diameter (DGH) differences, and Class 5 stands for mature trees (Table 1). All the sampled trees were discriminated in 10 spatial blocks (b1 to b10) by the x-coordinate of the location (Table 1). Spatial blocks were at 10 m-intervals except for b1 and b10, which included trees beyond the margin of 0 m and 100 m, respectively.

Genotypic and allelic differentiations in size-classes and spatial blocks were examined by χ^2 tests of independence (Workman & Niswander 1970; Sokal & Rohlf 1995). Genetic parameters used for analyses were: heterozygosity (*Hs*) (Nei & Roychoudhury 1974), Simpson's index (*D*) (Peet 1974), Shannon-Weaver's diversity index (*J′*) (Peet 1974), and the number of alleles in common (*NAC*) (Surles *et al*. 1990; Hamrick *et al*. 1993; Berg & Hamrick 1995). The Wright's *F*-statistics (Wright 1922, 1965) were also used. Changes in genetic parameters, such as *Hs*, *D*, *J′*, and F_{IS} along size-classes and/or spatial blocks were tested by Kendall's τ (Sokal & Rohlf 1995). Leveraged residuals were calculated for standardized residuals (Sokal & Rohlf 1995) divided by standard deviations ($d_{ij} = e_{ij}/V_{ij}^{-1/2}$).

Spatial autocorrelations (Sokal & Oden 1978a,b) were calculated for overall population by the Moran's *I* and the

Table 1 Number of trees for each size-class and each spatial block.

	Size-class	Spatial block b1 x < 10 m	b2 10 < x < 20 m	b3 20 < x < 30 m	b4 30 < x < 40 m	b5 40 < x < 50 m	b6 50 < x < 60 m	b7 60 < x < 70 m	b8 70 < x < 80 m	b9 80 < x < 90 m	b10 90 m < x	Total
0	seedlings in 1995	5	11	54	117	101	84	107	174	129	91	873
1	juveniles DGH < 5 mm	23	24	45	163	132	24	22	49	12	13	507
2	5 mm < juveniles (DGH) < 10 mm	18	61	24	79	21	12	20	29	28	9	301
3	10 mm < juveniles (DGH) < 25 mm	11	10	0	22	23	7	28	27	35	9	172
4	25 mm < juveniles (DGH) < 300 mm	5	1	7	3	12	12	17	6	6	6	75
5	300 mm < mature trees (d.b.h)	2	3	2	1	0	2	1	2	1	3	17
Total		64	110	132	385	289	141	195	287	211	131	1945

coancestry (r_{ij}) (Cockerham 1969; Loiselle *et al*. 1995). The genetic relatedness around the reproductive trees was separately analyzed by r_{ij} for 14 mother trees. For all of the spatial autocorrelation analyses, distance classes of 5 m-intervals were applied.

Results

A total of 1945 trees were sampled in the 10×100 m transect, and 595 multilocus genotypes were discriminated by 11 loci. The number of trees for each size-class and each spatial block is summarized in Table 1.

Genetic substructurings among size-classes

The number of trees for the size-class was largest in Class 0 (seedlings); the number sharply decreased with increase in size-classes, and was smallest in number in Class 5 (mature trees) (Kitamura *et al*. 1998) (Table 1). The total number of trees included in each size-class showed the typical L-shaped distribution.

The genotypic, allelic, and zygotic frequencies for each size-class in each locus are shown in Appendix I-1, 2 and 3, while three typical loci are shown in fan diagrams (Fig. 1). Significant differences among size-classes were observed in *6Pgdh2*, *Acp2*, *Fum*, *Lap*, and *Pgm2* for genotypes, and *Acp2*, *Fum*, *Lap*, and *Pgm2* for alleles.

High polymorphisms were maintained in *Fum*, *Lap* and *6Pgdh3* (Fig. 1c). It is notable that rare genotypes and alleles were only observed in the smaller size-classes for *Acp2*, *Adh2*, and *Got1* (Fig. 1b). Specific genotypes and alleles were observed in specific size-classes for *Got3*, *Pgi*, and *Pgm2*. The frequencies of homozygotes increased with an increase in size-classes 0–5 for *6Pgdh3*, and *Lap*. (Appendix II). The low frequency of homozygotes in size-class 5 may be biased because of low numbers of mature individuals available for genetic analysis in this study plot. Leveraged residuals were calculated for the alleles of four significant loci by the χ^2 test for independence (Fig. 2). The highest variation in allele frequencies existed in size-class 0 and 1, which suggested that these small size-classes include higher genetic variation than larger size-classes.

Changes in genetic parameters among size-classes such as *Hs*, *D*, *J′*, and F_{IS} are demonstrated in Fig. 3. These parameters, especially for *J′* and F_{IS}, showed a decrease with increase in size-classes. The changes in the genetic parameters in each locus affected the average values, which were all significant, except for *NAC* (Appendix II). The results of *Hs*, *D*, and *J′* showed a decrease of genetic variations with increase in size-classes. Notable evidence is that the F_{IS} decreased in larger size-classes, and the parameter shifted from positive to negative values.

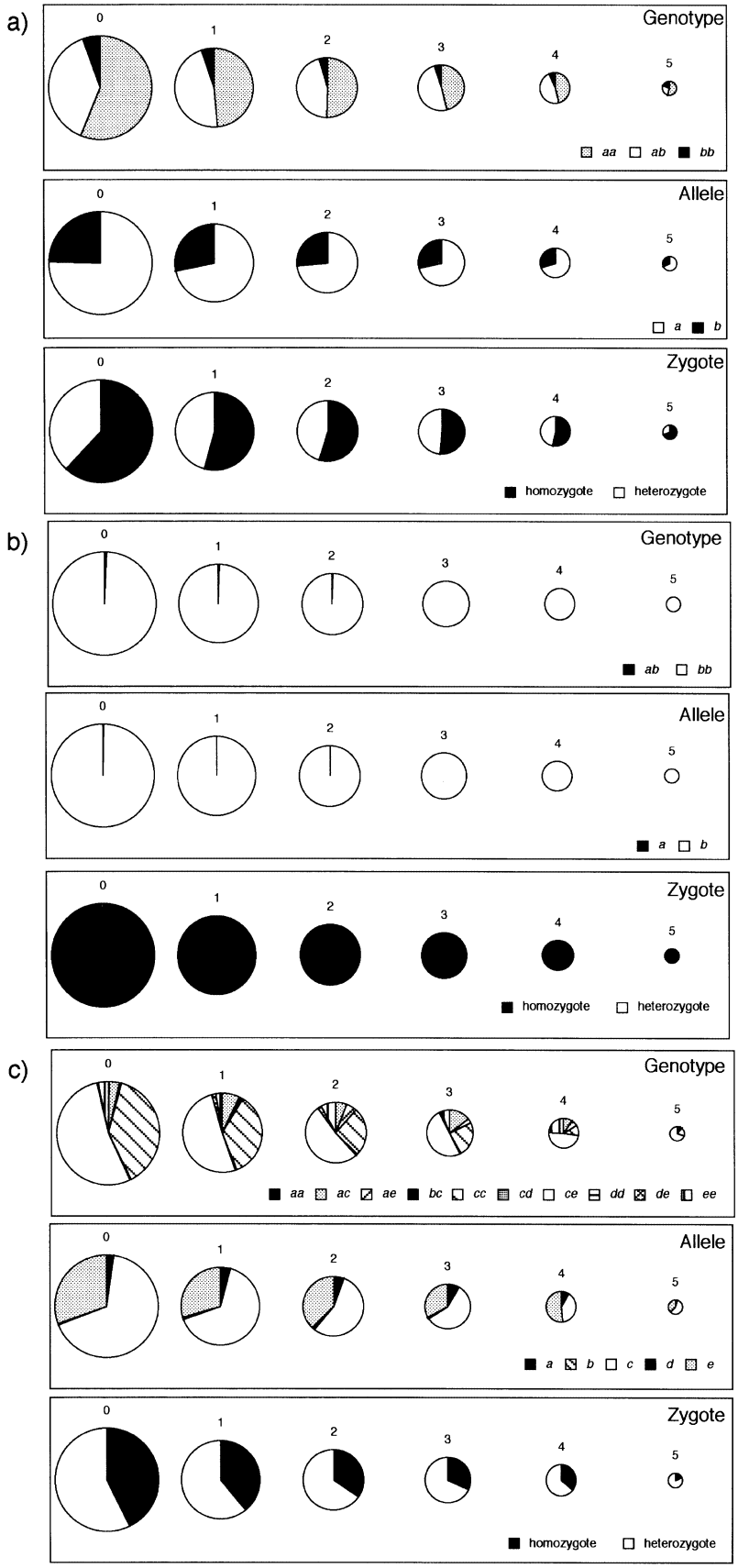

Fig. 1 Genotypic, allelic, and zygotic frequencies for each size-class. Size of the diagram represents the number of trees included in the size-class. (a) *6Pgdh2*; (b) *Got1*; (c) *Lap*.

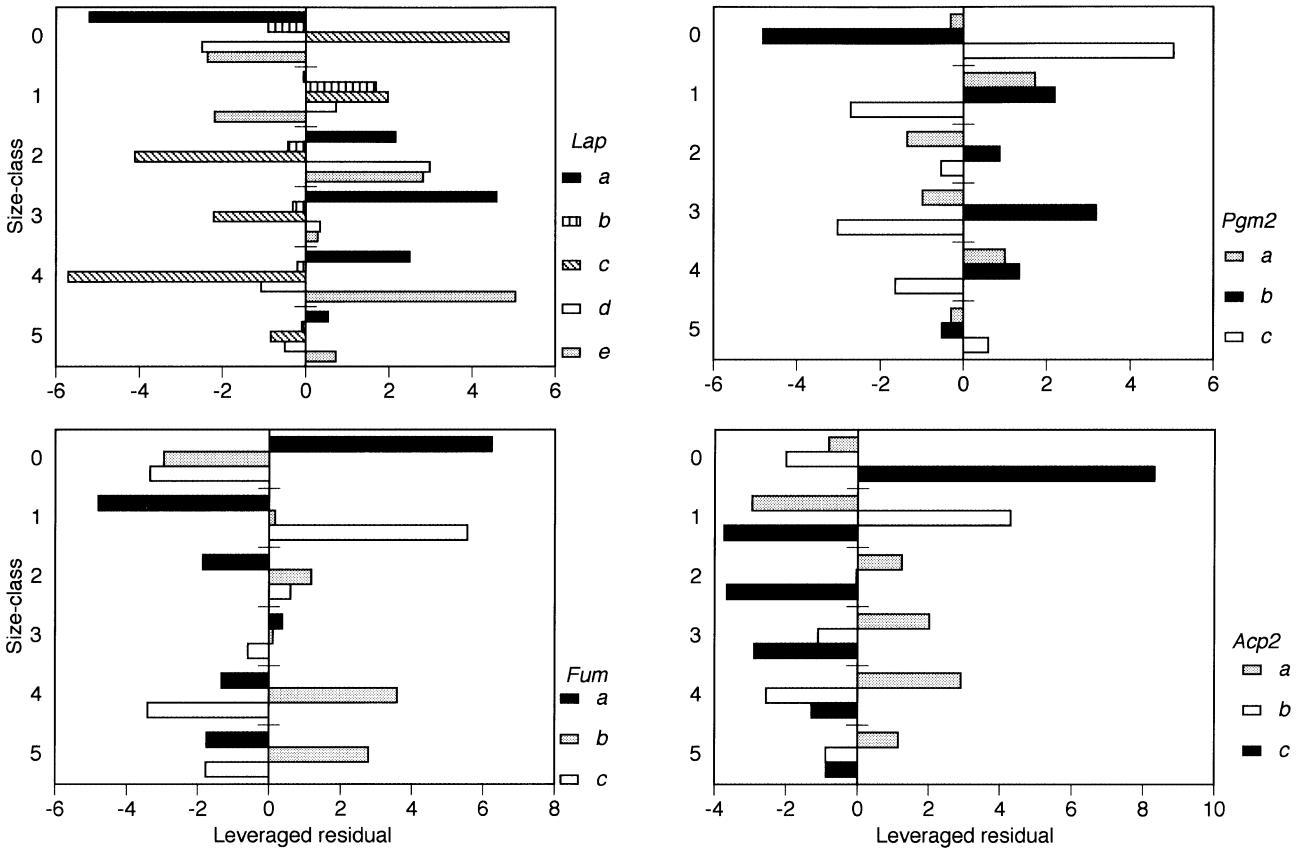

Fig. 2 Leveraged risiduals of alleles for significant loci among size-classes.

Spatial genetic substructurings in fine-scale

Distributions of genotypes for 11 polymorphic loci were investigated and typical three loci are shown in Fig. 4. Conspicuous localizations of specific genetic variations were observed in *Acp2*, *Adh1* (Fig. 4a), and *Fum* (Fig. 4b). These genetic localizations were characteristic of large-sized trees surrounded by small trees with identical genetic variations. Rare genotypes were observed in scattered small-sized trees in *Adh2*, *Got1*, *Got3*, *Pgi* (Fig. 4c), and *Pgm2*.

Significant spatial genetic heterogeneities were observed in *6Pgdh2*, *Acp2*, *Adh1*, *Fum*, *Lap*, and *Pgm2* for both genotype and allele frequencies (Appendix III-1, 2 and 3). Leveraged residuals were calculated for the alleles of significant six loci by the χ^2 test for independence (Fig. 5). The differences in these six loci in spatial blocks were distinct, as most of the leveraged residuals showed opposite trends in different alleles within a locus.

The changes in genetic parameters (*Hs*, *D*, *J′*, and F_{IS}) for spatial blocks are shown in Fig. 6. These parameters fluctuated among spatial blocks. Significant changes in spatial blocks were observed in *Adh2* for H_S, *6Pgdh2*, *Adh1*, *Adh2*, and *Got3* for *D*, *Adh2* and *Got3* for *J′*, and *6Pgdh3* and *Pgm2* for F_{IS} (Appendix IV).

Spatial autocorrelation analysis

Clear features of distrograms were demonstrated by spatial autocorrelations for the overall population (Fig. 7; Appendix V). Both calculations of spatial autocorrelation, Moran's *I* and coancestry, showed identical changes along distance classes; the highest positive estimations in the smallest distance class (0–5 m) smoothly decreased with an increase in distance. The distance classes at which the autocorrelation shifted from positive to negative were 20–30 m. Correlograms indicated negative or slightly positive values for farther distance classes than this shifting point.

Coancestry estimations were separately calculated for 14 mother trees. All 14 mother trees showed similar patterns of coancestry in every distance class. Distrograms of six typical mother trees are shown in Fig. 8. A positive autocorrelation in small distance classes was observed for all mother trees. However, the calculated values fluctuated in some of the loci. Correlograms for each mother tree converged from positive to negative on certain values at distance classes of 10–25 m. This distance indicates the range of genetic contribution by each reproductive individual.

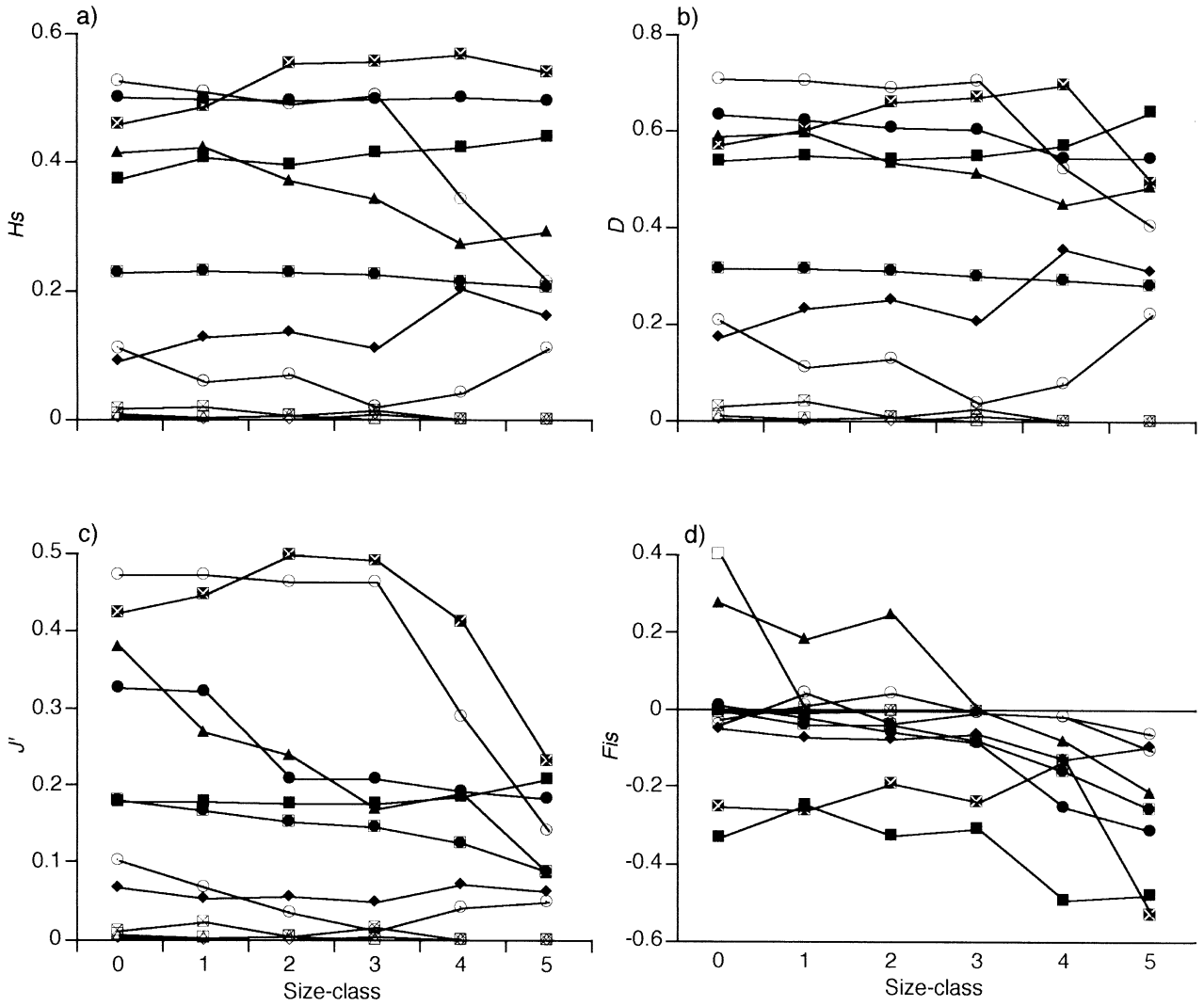

Fig. 3 Changes in genetic parameters for size-classes. (a) Heterozygosity, *Hs*; (b) Simpson's index, *D*; (c) Shannon-Weaver's index, *J'*; (d) inbreeding coefficient, F_{IS}. *6Pgdh2*; *Acp2*; *Adh2*; *Got1*; *Lap*; *Pgm*; *6Pgdh3*; *Adh1*; *Fum*; *Got3*; *Pgi*; *Average*.

Spatial and temporal substructurings for multilocus genotype

In this study plot, a total of 1945 trees were discriminated into 595 multilocus genotypes based on the 11 loci. The number of trees and multilocus genotypes for different size-classes and spatial blocks are demonstrated in Figs 9 and 10, respectively.

For size-classes, the number of trees showed a typical L-shaped histogram (Fig. 9). The number of trees decreased with an increase in size-class, as did the number of multilocus genotypes. However, the number of multilocus genotypes per tree, which is an indicator of genetic uniqueness of individuals, increased with larger size-classes.

The spatial distribution of trees is shown in Fig. 10. The same trend was observed, which indicates that the number of trees and the number of multilocus genotypes are the same, but the number of genotypes per tree was converse. The number of genotypes was highest in the spatial blocks that included mother trees and their progenies (b4, b5, b8, and b9), but it was lowest in spatial blocks that did not contain mother trees (b6, b7, and b10).

Discussion

Conspicuous features of spatiotemporal genetic substructurings were recognized in this study site in the east-central coastal plain of Maryland. The consequences of spatial and temporal interactions resulted in fine-scale genetic substructures within a local population. One remarkable piece of evidence obtained concerning the genetic differentiation among size-classes is that rare genotypes and alleles tended to be very rapidly extinguished from the

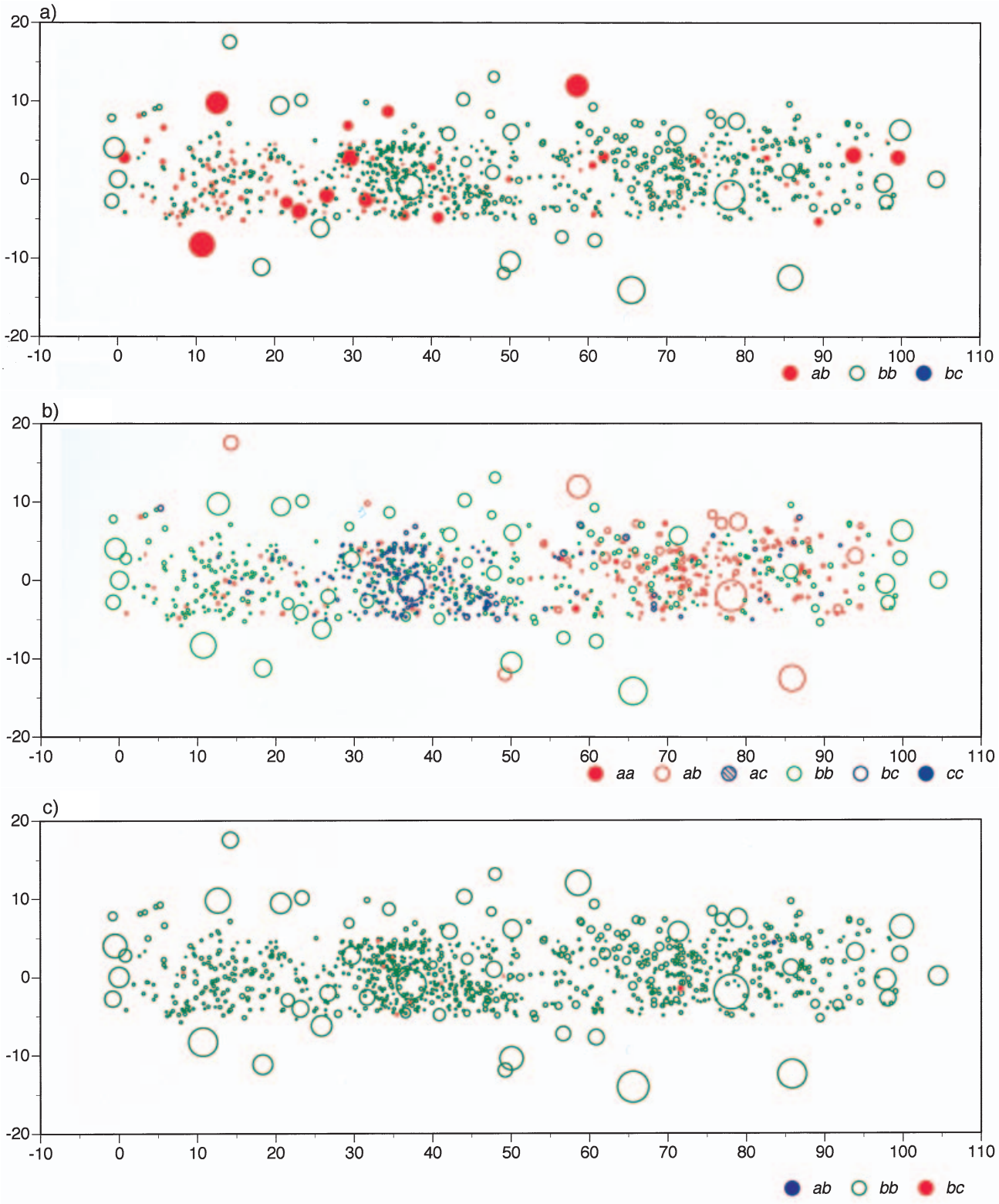

Fig. 4 Distributions of genotypes. The size of the circles is relative to the DGH of each tree. (a) *Adh1*; (b) *Fum*; (c) *Pgi*; (d) map of mature trees.

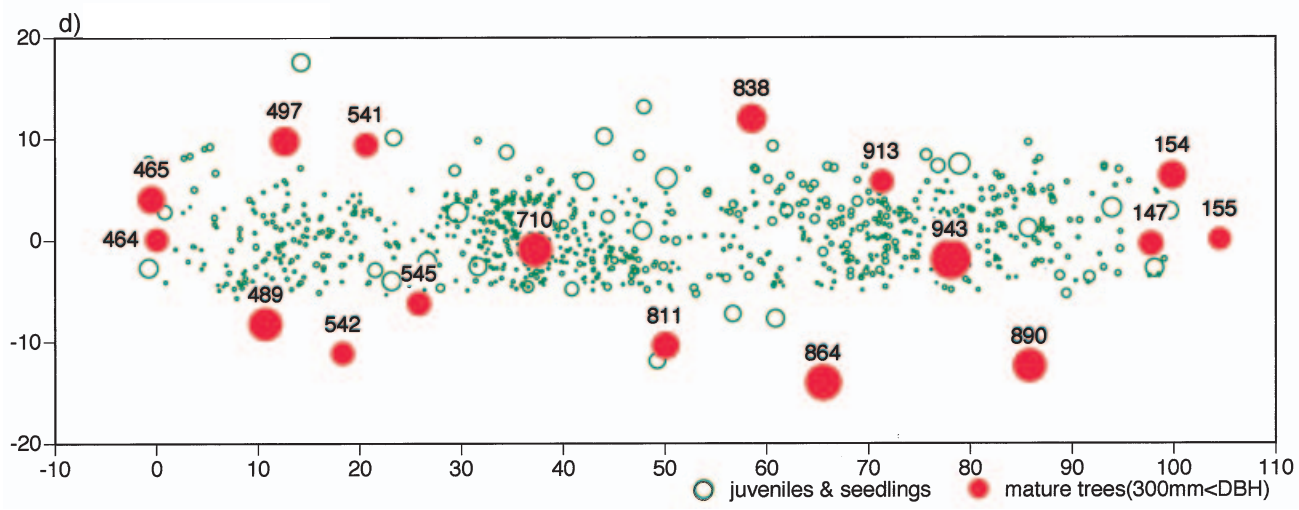

Fig. 4 *Continued*

sampled plot and, thus, such unique genotypes no longer exist in larger size-classes (Fig. 1, Appendix I–1, 2). The consequences are also reflected in the changes in the genetic parameters (Fig. 3). Decreases in *Hs*, *D*, and *J′* mean that the genetic variabilities become smaller in larger size-classes. The presence of rare genotypes and alleles in this study plot indicates that they may have been derived from those which occur in somewhat remote sites outside the study plot because the American beech is wind-pollinated and wind may occasionally carry pollens over a wider range. The occasional gene (pollen) flows from distant sites has a potential to introduce new genetic variations into neighboring patch populations within a local population (Kawano & Kitamura 1997), although such lower frequency genetic variations are exposed to risks of demographic stochasticity (Holsinger 2000).

In a long-lived woody species like the American beech, populations normally consist of overlapping generations (Kitamura *et al*. 1997b). It is notable that specific genetic variations were observed in a certain frequency in specific intermediate size-classes (Fig. 1, Appendix I). As the American beech is barochorous, individuals with specific rare genotypes may possibly be remnants of past reproductive events, and their mother trees may have already died and/or been removed from the present population. This suggests that the present-day existing population is composed of individuals, unique genotypes of which were brought into the population in the remote past and regenerated in a number of past mast-fruiting years, and thus, each mast-year may have had different heterogeneous gene pools.

The F_{IS} values showed a decrease, but shifted from positive to negative with increase in size-classes. This information indirectly suggests that heterozygotes have advantages in surviving and developing to the next size-class. A mixed mating followed by natural selection favoring heterozygotes (Brown 1979) was reported in conifers, such as Douglas-fir (Shaw & Allard 1985), *Pinus sylvestris* (Muona *et al*. 1987; Muona *et al*. 1988), and *Chamaecyparis obtusa* (Tang & Ide 1998).

The spatiotemporal genetic substructures were obviously formed by the interactions of various factors such as genetic heterogeneity among different size-classes and genetically heterogenous patches of established individuals belonging to different size-classes. However, the limited degree of gene dispersal in past reproductive seasons seems to be one of the major factors for the spatial genetic substructurings in this study site.

Genotype maps (Fig. 4) showed interesting features of localization in genetic variations (see also Fig. 5); notably, *Fum* demonstrated clear pictures of genetic relationships between reproductive individuals and seedlings and juveniles in the vicinity (Fig. 4b). The range of localization for a certain genetic variation suggests the consequences of seed dispersal by barochory, rather than the pollen dispersion by anemochore. According to the genotype maps (Fig. 4), the seed dispersal area seems to be rather limited and within a range of approximately 20–30 m in radius.

The degree of genetic differentiation can be measured by F_{ST}. The values of F_{ST} were larger among spatial blocks (0.041 ± 0.013) than size-classes (0.008 ± 0.003). This information suggests that the spatial genetic differentiation is much more significant due to the localized gene dispersal through seed dispersion. It is indeed laborious to analyze accurately the effectiveness of temporal genetic heterogeneity, namely to demonstrate differences among generations in spatial scales in relation to the intermittent reproductive events with several-year intervals. It is, however, worthwhile conducting in order to clarify the demographic genetic changes within a local population of

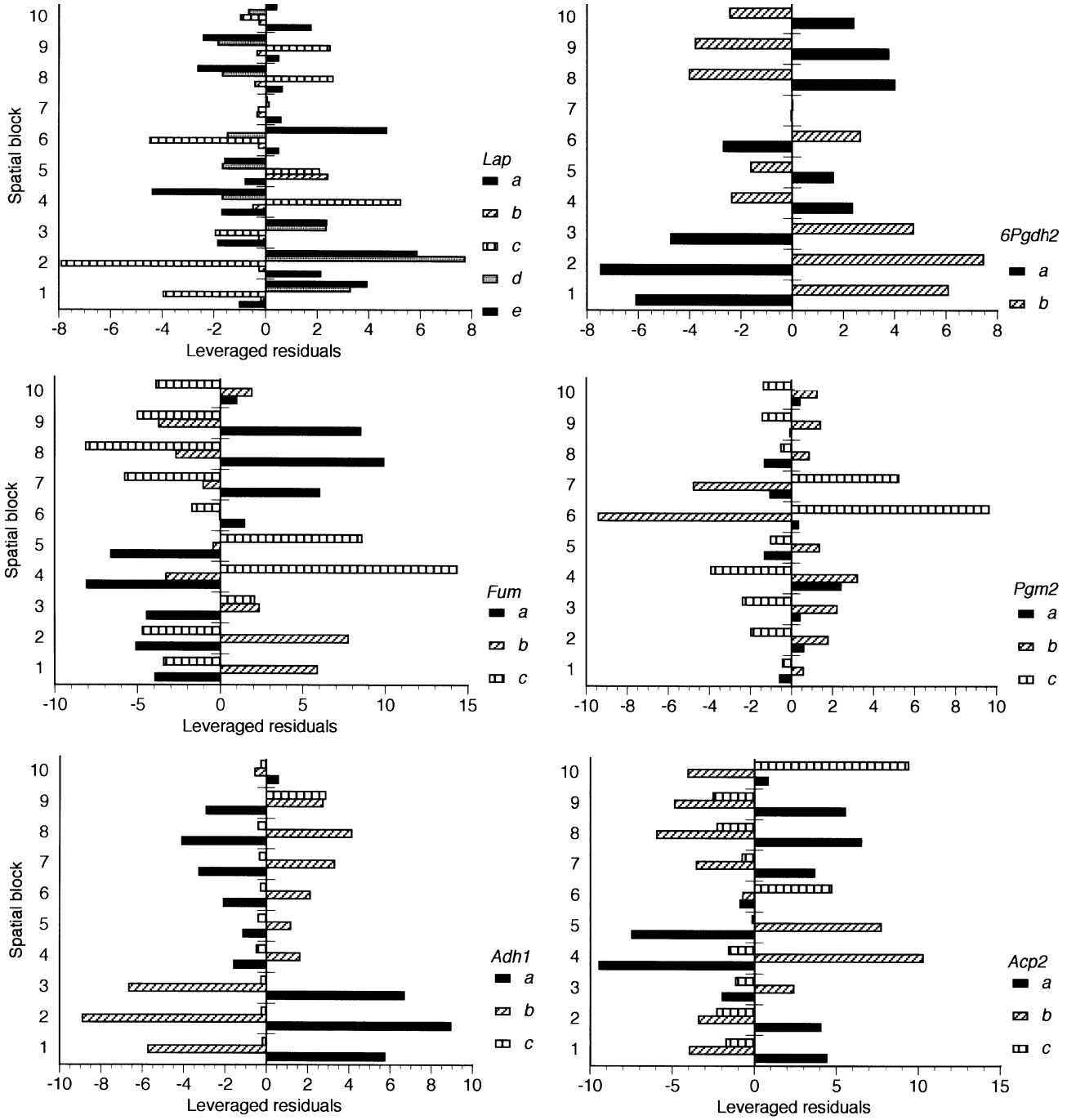

Fig. 5 Leveraged risiduals of alleles for significant loci among spatial blocks.

the American beech in the successional process of a forest community.

The spatial autocorrelation analyses revealed the quantitative ranges of genetic relatedness. The ranges of genetic relatedness indicated by the correlograms were 20–30 m in distance, which more or less corresponds to the ranges of seed dispersal (compare Fig. 7 with Fig. 4).

Flat correlograms of Moran's *I* (Fig. 7a) compared to root suckering populations (Kitamura *et al*. 2000) were more or less identical to the Piedmont population (Kitamura *et al*. 2001), which also does not show root sucker formation. This indicates that because pollen dispersion is normally leptokurtic (Colwell 1951), considerable overlaps in gene dispersal ranges for neighboring reproductive individuals occur in typical wind-pollinated species such as the American beech.

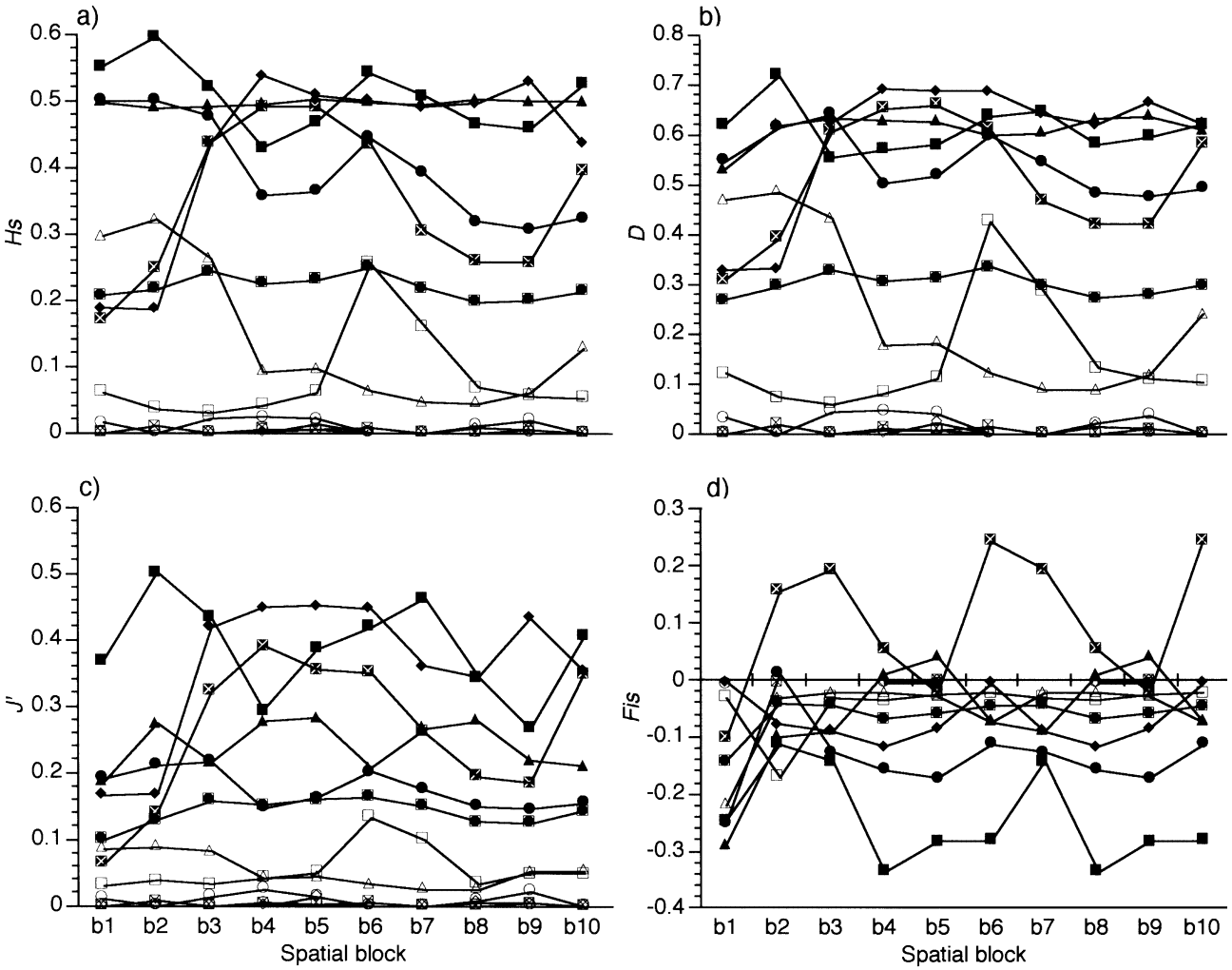

Fig. 6 Changes in genetic parameters for spatial blocks. (a) Heterozygosity, Hs; (b) Simpson's index, *D*; (c) Shannon-Weaver's index, *J'*; (d) inbreeding coefficient, F_{IS}. *Lap*; *6Pgdh3*; *Pgm*; *Adh1*; *Acp2*; *Got3*; *6Pgdh2*; *Fum*; *Pgi*; *Adh2*; *Got1*; *Average*.

The ranges of genetic contribution from a mother tree to progenies are demonstrated in distrograms (Fig. 8). The correlograms demonstrated uni-directional relationships from reproductive trees to progenies. The range of genetic relatedness was within a distance of 15–25 m during the period from reproductive events to establishment. Relatively intensive gene dispersions in the distance class of 0–5 m and sharp declines in the second distance class (5–10 m) were observed in mother trees no. 541 (Fig. 8c), 811 (Fig. 8f), 838, and 155. These mother trees were not located inside the transect, but up to 5 m outside, and the data set for the first distance class was composed of relatively small numbers of juveniles and seedlings. The estimated values might have been affected, and fluctuated by these small numbers of samples in the distance class of 0–5 m, but this may represent a normal trend in gene dispersal.

It is also interesting to see that the number of multilocus genotypes per tree increases in larger size-classes despite a sharp decline in tree number (Fig. 9), which is a background of the genetic uniqueness of this population. In terms of size-classes, larger size-class trees have more unusual genotypes than those of the smaller size-classes. This information suggests that the larger size-classes have maintained a variety of genetically different individuals, and those individuals have no doubt contributed to the production of genetically novel progenies for the next generations through sexual reproduction. This evidence is also confirmed in our large-scale sampling of all mother trees larger than 40 cm in diameter within a total area of the American beech population in the SERC forest (Kitamura *et al*. unpubl. data).

Critical data on the spatial differentiation of individuals in different blocks of the study plot have exhibited a sig-

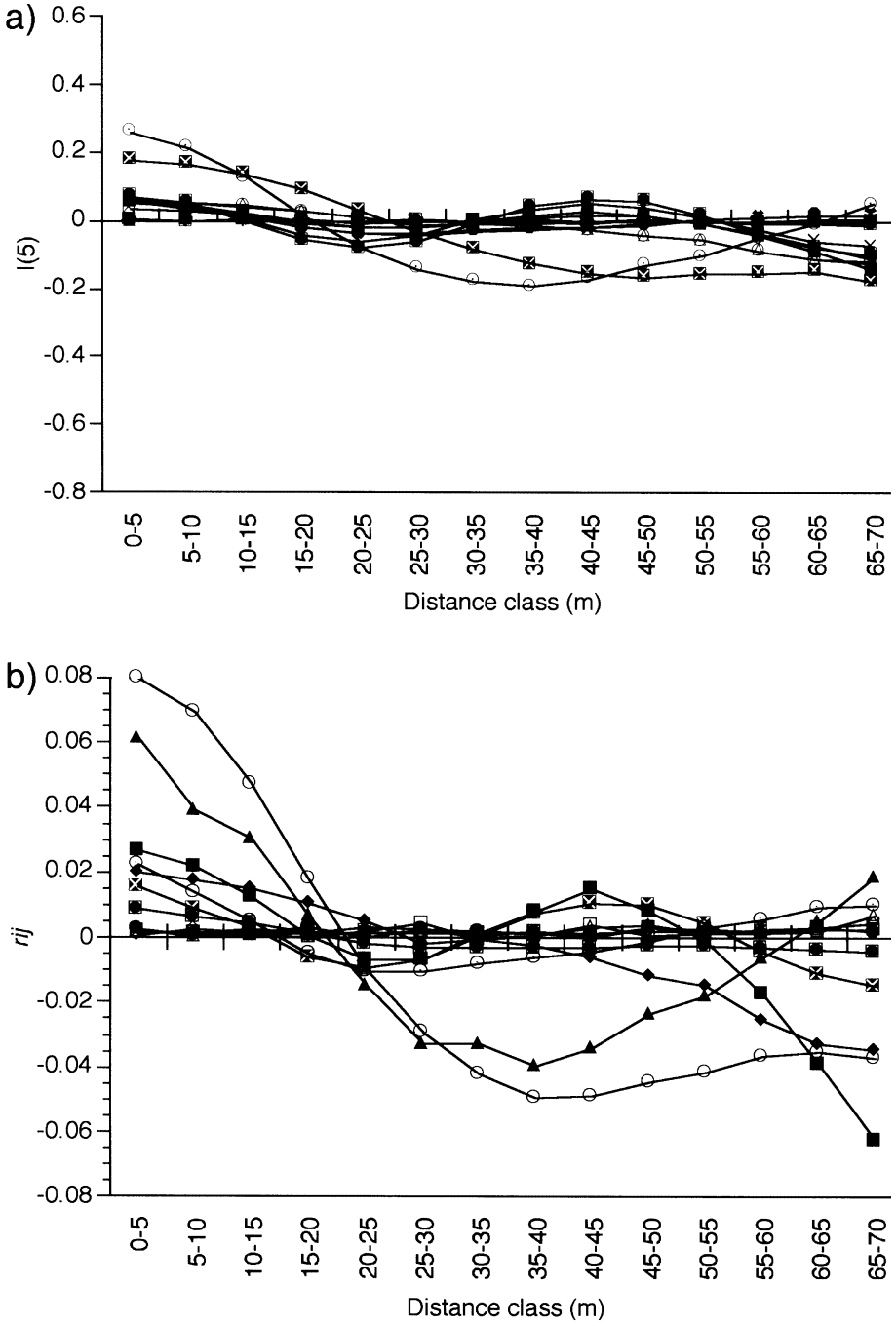

Fig. 7 Moran's *I* and coancestry for over all populations. (a) Moran's *I*, *6Pgdh2-a*; *6Pgdh3-c*; *Fum-a*; *Lap-c*; *Pgi-c*; *6Pgdh2-b*; *Adh1-a*; *Fum-b*; *Lap-e*; *Pgm-a*; *6Pgdh3-a*; *Adh1-b*; *Fum-c*; *Pgi-a*; *Pgm-b*; *6Pgdh3-b*; *Adh1-c*; *Lap-a*; *Pgi-b*; *Pgm-c*; (b) coancestry, *6Pgdh2*; *Acp2*; *Adh2*; *Got1*; *Lap*; *Pgm*; *6Pgdh3*; *Adh1*; *Fum*; *Got3*; *Pgi*; *Mean*.

nificant aspect of demographic genetic substructures in this study site. The number of different multilocus genotypes per tree was much higher among the spatial blocks where seed rains of different mother trees overlapped each other (Fig. 10). Such unique spatial assemblies of different genotypes in juvenile stages may be typical in trees with barochorous seeds (Fig. 4).

In addition to the afore-mentioned unique spatiotemporal genetic substructures, the levels of genetic variation in the SERC population turned out to be considerably higher compared to those of other woody species previously studied (Hamrick & Godt 1989). This forest is known to have once been a pasture that was heavily grazed approximately 150 years ago. The greater part of the population of American beech trees was obviously disturbed by farming and grazing at that time, and the trees that remained were left abandoned afterwards. Considering such a background of population history, the

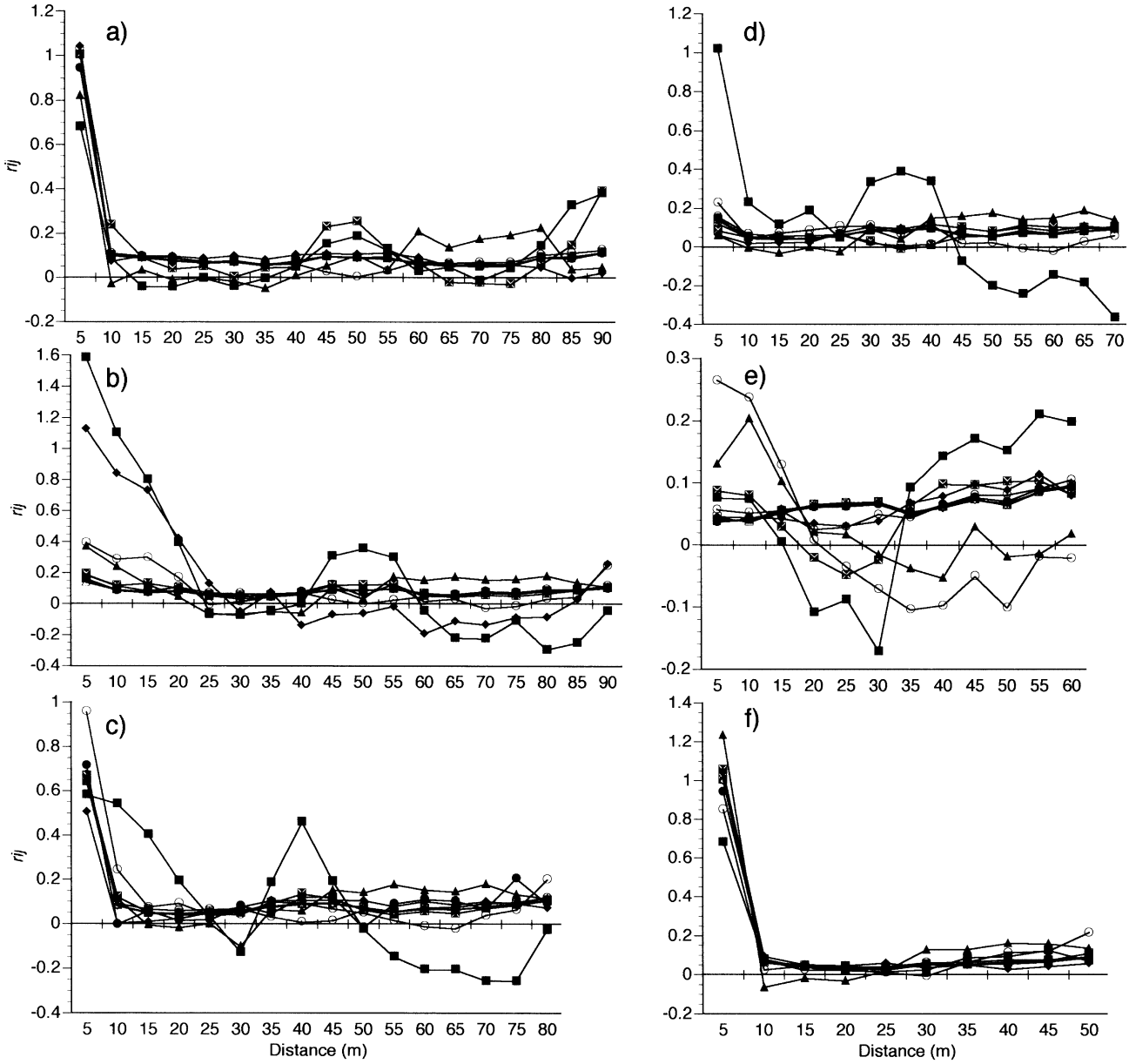

Fig. 8 Coancestries for six representative mother trees. (a) mt 115; (b) mt 489; (c) mt 541; (d) mt 545; (e) mt 710; (f) mt 811. —■— *6Pgdh2*; —●— *6Pgdh3*; —▲— *Acp2*; —◆— *Adh1*; —□— *Adh2*; —○— *Fum*; —△— *Got1*; —◇— *Got3*; —⊠— *Lap*; —⊠— *Pgi*; —○— *Pgm*.

levels of genetic variation maintained within the mature tree population may have been nearly lost when the forest was almost completely cleared. However, the considerably high genetic polymorphism found in the present study indicates a rapid recovery of genetic variations within this stand during the past 150 years. This information suggests that during the management of this land as pasturage, mature trees were lumbered extensively and became limited in numbers; however, the beech forest stand must have recovered due to the juveniles that remained, which possibly preserved a considerable amount of genetic variation. Successful offspring recruitment by means of seed production and seedling establishment in subsequent years would also have been a possible means for such a rapid recovery of genetic variation by introducing novel genetic variations through genetic recombination and reshuffling by sexual reproduction.

In view of conservation biology, it is remarkable to find that genetic variations have recovered so quickly within this small forest stand during a time span of only 150 years. We will further report and discuss the background of genetic recovery in a forthcoming paper based on the evidence concerning genetic variations of mature trees in the total SERC forest (Kitamura *et al.* unpubl. data).

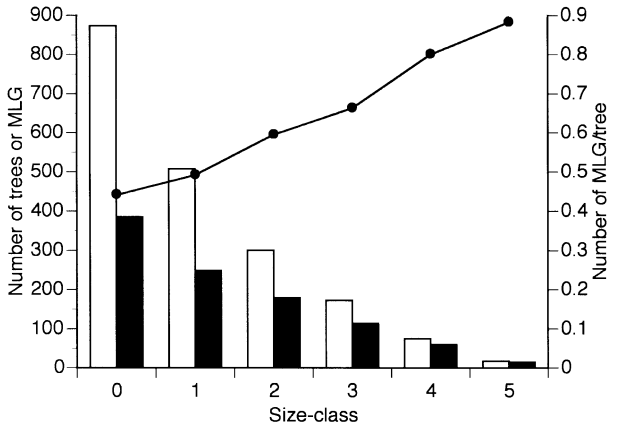

Fig. 9 The number of trees, multilocus genotypes (MLG), and multilocus genotypes per tree for size-classes. □ No. trees; ■ No. MLG; —●— No. MLG/tree.

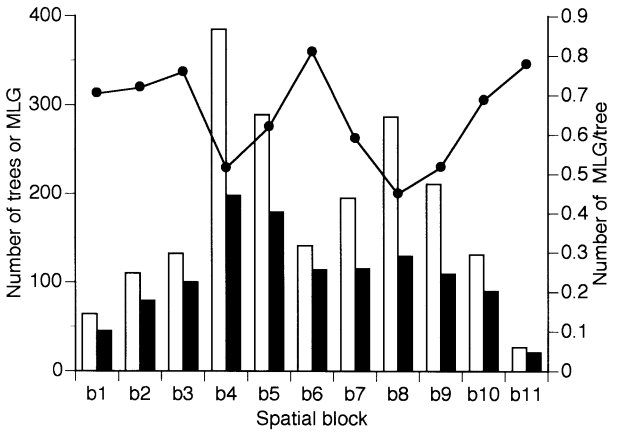

Fig. 10 The number of trees, multilocus genotypes (MLG), and multilocus genotypes per tree for spatial blocks. □ No. trees; ■ No. MLG; —●— No. MLG/tree.

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Appendix I-1. Genotype frequencies of size-classes for each locus.

Locus	Genotype	Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Total
*Lap**	*aa*	0.002	0.000	0.000	0.000	0.000	0.000	0.001
	ac	0.030	0.069	0.060	0.145	0.093	0.059	0.058
	ae	0.010	0.012	0.053	0.029	0.067	0.059	0.022
	bc	0.000	0.002	0.000	0.000	0.000	0.000	0.001
	cc	0.388	0.361	0.262	0.250	0.120	0.176	0.337
	cd	0.003	0.002	0.010	0.006	0.000	0.000	0.004
	ce	0.529	0.511	0.512	0.494	0.480	0.706	0.518
	dd	0.001	0.000	0.000	0.000	0.000	0.000	0.001
	de	0.001	0.016	0.023	0.012	0.000	0.000	0.009
	ee	0.034	0.028	0.080	0.064	0.240	0.000	0.050
*6Pgdh2**	*aa*	0.562	0.486	0.505	0.465	0.467	0.529	0.521
	ab	0.383	0.462	0.452	0.488	0.467	0.294	0.426
	bb	0.055	0.051	0.043	0.047	0.067	0.176	0.053
6Pgdh3	*ab*	0.006	0.004	0.000	0.000	0.000	0.000	0.004
	ac	0.001	0.002	0.000	0.000	0.000	0.000	0.001
	bb	0.297	0.306	0.302	0.267	0.173	0.235	0.292
	bc	0.488	0.500	0.522	0.541	0.627	0.647	0.508
	cc	0.208	0.188	0.176	0.192	0.200	0.118	0.195
*Fum***	*aa*	0.055	0.026	0.047	0.052	0.013	0.000	0.044
	ab	0.337	0.184	0.213	0.297	0.267	0.176	0.270
	ac	0.064	0.081	0.057	0.035	0.040	0.000	0.063
	bb	0.396	0.447	0.473	0.424	0.640	0.765	0.436
	bc	0.135	0.239	0.197	0.174	0.040	0.059	0.171
	cc	0.013	0.024	0.013	0.017	0.000	0.000	0.015
*Pgm2***	*ab*	0.005	0.010	0.000	0.000	0.013	0.000	0.005
	bb	0.884	0.943	0.930	0.983	0.960	0.882	0.918
	bc	0.110	0.045	0.070	0.017	0.027	0.118	0.076
	cc	0.001	0.002	0.000	0.000	0.000	0.000	0.001
Pgi	*ab*	0.000	0.002	0.000	0.006	0.000	0.000	0.001
	bb	0.985	0.980	0.997	0.988	1.000	1.000	0.987
	bc	0.015	0.018	0.003	0.006	0.000	0.000	0.012
Adh1	*ab*	0.095	0.135	0.146	0.117	0.227	0.176	0.121
	bb	0.904	0.865	0.854	0.883	0.773	0.824	0.878
	bc	0.001	0.000	0.000	0.000	0.000	0.000	0.001
Adh2	*aa*	0.001	0.000	0.000	0.000	0.000	0.000	0.001
	ab	0.003	0.002	0.003	0.000	0.000	0.000	0.003
	bb	0.995	0.998	0.997	1.000	1.000	1.000	0.997
*Acp2***	*aa*	0.583	0.531	0.620	0.614	0.693	0.647	0.583
	ab	0.250	0.331	0.273	0.339	0.280	0.353	0.285
	ac	0.047	0.014	0.003	0.000	0.013	0.000	0.026
	bb	0.099	0.125	0.103	0.047	0.013	0.000	0.097
	bc	0.002	0.000	0.000	0.000	0.000	0.000	0.001
	cc	0.019	0.000	0.000	0.000	0.000	0.000	0.008
Got1	*ab*	0.005	0.002	0.003	0.000	0.000	0.000	0.003
	bb	0.995	0.998	0.997	1.000	1.000	1.000	0.997
Got3	*ab*	0.001	0.000	0.000	0.006	0.000	0.000	0.001
	bb	0.999	1.000	1.000	0.994	1.000	1.000	0.999

Chi-squared test of independence: *significant ($P < 0.05$); **highly significant ($P < 0.01$)

Appendix I-2. Allele frequencies of size-classes for each locus.

Locus	Allele	Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Total
*Lap***	*a*	0.022	0.040	0.056	0.087	0.080	0.059	0.041
	b	0.000	0.001	0.000	0.000	0.000	0.000	0.000
	c	0.670	0.653	0.553	0.573	0.407	0.559	0.628
	d	0.003	0.009	0.017	0.009	0.000	0.000	0.007
	e	0.305	0.297	0.374	0.331	0.513	0.382	0.324
6Pgdh2	*a*	0.754	0.717	0.731	0.709	0.700	0.676	0.734
	b	0.246	0.283	0.269	0.291	0.300	0.324	0.266
6Pgdh3	*a*	0.003	0.003	0.000	0.000	0.000	0.000	0.002
	b	0.544	0.558	0.563	0.538	0.487	0.559	0.548
	c	0.453	0.439	0.437	0.462	0.513	0.441	0.450
*Fum***	*a*	0.255	0.158	0.182	0.218	0.167	0.088	0.210
	b	0.632	0.658	0.678	0.660	0.793	0.882	0.657
	c	0.112	0.184	0.140	0.122	0.040	0.029	0.133
*Pgm2***	*a*	0.002	0.005	0.000	0.000	0.007	0.000	0.003
	b	0.942	0.970	0.965	0.991	0.980	0.941	0.959
	c	0.056	0.025	0.035	0.009	0.013	0.059	0.039
Pgi	*a*	0.000	0.001	0.000	0.003	0.000	0.000	0.001
	b	0.993	0.990	0.998	0.994	1.000	1.000	0.993
	c	0.007	0.009	0.002	0.003	0.000	0.000	0.006
Adh1	*a*	0.048	0.067	0.073	0.058	0.113	0.088	0.061
	b	0.952	0.933	0.927	0.942	0.887	0.912	0.939
	c	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Adh2	*a*	0.003	0.001	0.002	0.000	0.000	0.000	0.002
	b	0.997	0.999	0.998	1.000	1.000	1.000	0.998
*Acp2***	*a*	0.731	0.703	0.758	0.784	0.840	0.824	0.738
	b	0.225	0.290	0.240	0.216	0.153	0.176	0.240
	c	0.043	0.007	0.002	0.000	0.007	0.000	0.022
Got1	*a*	0.002	0.001	0.002	0.000	0.000	0.000	0.002
	b	0.998	0.999	0.998	1.000	1.000	1.000	0.998
Got3	*a*	0.001	0.000	0.000	0.003	0.000	0.000	0.001
	b	0.999	1.000	1.000	0.997	1.000	1.000	0.999

Chi-squared test of independence: *significant ($P < 0.05$); **highly significant ($P < 0.01$)

Appendix I-3. Homozygote and heterozygote frequencies of size-classes for each locus.

Locus		Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Total
Lap	Homozygote	0.426	0.389	0.342	0.314	0.360	0.176	0.389
	Heterozygote	0.574	0.611	0.658	0.686	0.640	0.824	0.611
6Pgdh2	Homozygote	0.617	0.538	0.548	0.512	0.533	0.706	0.574
	Heterozygote	0.383	0.462	0.452	0.488	0.467	0.294	0.426
6Pgdh3	Homozygote	0.505	0.494	0.478	0.459	0.373	0.353	0.488
	Heterozygote	0.495	0.506	0.522	0.541	0.627	0.647	0.512
Fum	Homozygote	0.464	0.496	0.533	0.494	0.653	0.765	0.496
	Heterozygote	0.536	0.504	0.467	0.506	0.347	0.235	0.504
Pgm2	Homozygote	0.885	0.945	0.930	0.983	0.960	0.882	0.919
	Heterozygote	0.115	0.055	0.070	0.017	0.040	0.118	0.081
Pgi	Homozygote	0.985	0.980	0.997	0.988	1.000	1.000	0.987
	Heterozygote	0.015	0.020	0.003	0.012	0.000	0.000	0.013
Adh1	Homozygote	0.904	0.865	0.854	0.883	0.773	0.824	0.878
	Heterozygote	0.096	0.135	0.146	0.117	0.227	0.176	0.122
Adh2	Homozygote	0.997	0.998	0.997	1.000	1.000	1.000	0.997
	Heterozygote	0.003	0.002	0.003	0.000	0.000	0.000	0.003
Acp2	Homozygote	0.700	0.655	0.723	0.661	0.707	0.647	0.688
	Heterozygote	0.300	0.345	0.277	0.339	0.293	0.353	0.312
Got1	Homozygote	0.995	0.998	0.997	1.000	1.000	1.000	0.997
	Heterozygote	0.005	0.002	0.003	0.000	0.000	0.000	0.003
Got3	Homozygote	0.999	1.000	1.000	0.994	1.000	1.000	0.999
	Heterozygote	0.001	0.000	0.000	0.006	0.000	0.000	0.001

Appendix II. Kendall's coefficient for rank correlation (τ) for size-classes.

		Average Parameters				
Locus	Freq. homo[1]	*Hs*	*D*	*J′*	*Fis*	*NAC*
Lap	0.773*	– 0.600	– 0.330	– 0.333	– 0.070	–
6Pgdh2	0.067	– 0.870	– 0.730	– 0.470	0.333	–
6Pgdh3	1.000**	0.067	1.000**	1.000**	1.000**	–
Fum	– 0.730*	0.867*	0.867*	0.733*	0.333	–
Pgm2	– 0.070	0.067	0.067	0.333	0.200	–
Pgi	– 0.670	0.667	0.667	0.533	–	–
Adh1	0.600	– 0.600	– 0.600	– 0.070	0.600	–
Adh2	– 0.670	0.667	0.667	0.667	(1.000)	–
Acp2	0.200	0.733**	0.733**	0.867*	0.867*	–
Got1	– 0.670	0.667	0.667	0.667	–	–
Got3	– 0.200	0.200	0.200	0.200	–	–
Total	–	0.773*	0.867*	1.000*	1.000**	– 0.070

[1] Frequency of homozygotes.
Null hypothesis (Ho): the parameter increases with increase in size-class.
*significant ($P < 0.05$); **highly significant ($P < 0.01$).
Data lacking one or more classes are not calculated.

Appendix III-1. Genotype frequencies of spatial blocks for each locus.

Locus	Genotype	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10
*Lap***	*aa*	0.000	0.000	0.000	0.000	0.000	0.014	0.051	0.000	0.000	0.000
	ac	0.000	0.027	0.030	0.060	0.048	0.028	0.051	0.084	0.090	0.084
	ae	0.047	0.109	0.008	0.000	0.021	0.035	0.041	0.007	0.000	0.038
	bc	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
	cc	0.172	0.127	0.242	0.431	0.374	0.220	0.354	0.369	0.389	0.282
	cd	0.000	0.000	0.008	0.005	0.003	0.000	0.010	0.003	0.000	0.008
	ce	0.578	0.473	0.621	0.491	0.529	0.539	0.472	0.526	0.498	0.542
	dd	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	de	0.063	0.100	0.015	0.000	0.000	0.000	0.005	0.000	0.000	0.000
	ee	0.141	0.164	0.068	0.013	0.021	0.163	0.067	0.010	0.024	0.046
*6Pgdh2***	*aa*	0.188	0.255	0.409	0.538	0.547	0.447	0.513	0.619	0.630	0.618
	ab	0.625	0.527	0.402	0.460	0.429	0.440	0.441	0.367	0.360	0.359
	bb	0.188	0.218	0.189	0.003	0.024	0.113	0.046	0.014	0.009	0.023
6Pgdh3	*ab*	0.000	0.000	0.000	0.005	0.014	0.000	0.000	0.003	0.000	0.000
	ac	0.000	0.009	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
	bb	0.219	0.336	0.326	0.317	0.284	0.270	0.297	0.273	0.289	0.267
	bc	0.641	0.500	0.477	0.488	0.509	0.546	0.533	0.493	0.479	0.534
	cc	0.141	0.155	0.197	0.190	0.194	0.184	0.164	0.231	0.232	0.198
*Fum***	*aa*	0.000	0.009	0.008	0.003	0.003	0.043	0.087	0.101	0.109	0.046
	ab	0.125	0.128	0.114	0.086	0.100	0.355	0.456	0.502	0.474	0.331
	ac	0.016	0.000	0.076	0.117	0.107	0.050	0.026	0.028	0.047	0.046
	bb	0.813	0.807	0.561	0.366	0.446	0.411	0.379	0.345	0.313	0.515
	bc	0.047	0.055	0.212	0.395	0.304	0.135	0.051	0.024	0.052	0.062
	cc	0.000	0.000	0.030	0.034	0.038	0.007	0.000	0.000	0.005	0.000
*Pgm2***	*ab*	0.000	0.009	0.008	0.013	0.000	0.007	0.000	0.000	0.005	0.008
	bb	0.938	0.964	0.970	0.958	0.941	0.702	0.831	0.930	0.943	0.947
	bc	0.063	0.027	0.023	0.029	0.055	0.291	0.164	0.070	0.052	0.046
	cc	0.000	0.000	0.000	0.000	0.003	0.000	0.005	0.000	0.000	0.000
Pgi	*ab*	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.005	0.000
	bb	0.984	1.000	0.977	0.977	0.979	1.000	1.000	0.990	0.981	1.000
	bc	0.016	0.000	0.023	0.021	0.021	0.000	0.000	0.010	0.014	0.000
*Adh1***	*ab*	0.359	0.400	0.311	0.097	0.100	0.064	0.046	0.045	0.057	0.137
	bb	0.641	0.600	0.689	0.903	0.900	0.936	0.954	0.955	0.938	0.863
	bc	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
Adh2	*aa*	0.000	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
	ab	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.007	0.005	0.000
	bb	1.000	1.000	1.000	1.000	0.989	1.000	1.000	0.993	0.995	1.000
*Acp2***	*aa*	0.813	0.755	0.535	0.392	0.432	0.547	0.696	0.727	0.719	0.611
	ab	0.188	0.200	0.295	0.405	0.345	0.292	0.223	0.227	0.252	0.168
	ac	0.000	0.000	0.008	0.018	0.014	0.066	0.022	0.018	0.010	0.130
	bb	0.000	0.045	0.155	0.177	0.195	0.066	0.054	0.028	0.019	0.053
	bc	0.000	0.000	0.000	0.005	0.000	0.029	0.000	0.000	0.000	0.000
	cc	0.000	0.000	0.008	0.003	0.014	0.029	0.005	0.000	0.000	0.038
Got1	*ab*	0.000	0.009	0.000	0.005	0.003	0.007	0.000	0.000	0.005	0.000
	bb	1.000	0.991	1.000	0.995	0.997	0.993	1.000	1.000	0.995	1.000
Got3	*ab*	0.000	0.000	0.000	0.003	0.003	0.000	0.000	0.000	0.000	0.000
	bb	1.000	1.000	1.000	0.997	0.997	1.000	1.000	1.000	1.000	1.000

Chi-squared test of independence: *significant ($P < 0.05$); **highly significant ($P < 0.01$)

Appendix III-2. Allele frequencies of spatial blocks for each locus.

Locus	Allele	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10
*Lap***	*a*	0.023	0.068	0.019	0.030	0.035	0.046	0.046	0.045	0.045	0.061
	b	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000
	c	0.461	0.377	0.572	0.709	0.666	0.504	0.621	0.676	0.682	0.599
	d	0.031	0.050	0.019	0.003	0.002	0.000	0.008	0.002	0.000	0.004
	e	0.484	0.505	0.390	0.258	0.296	0.450	0.326	0.277	0.273	0.336
*6Pgdh2***	*a*	0.500	0.518	0.610	0.768	0.761	0.667	0.733	0.802	0.810	0.798
	b	0.500	0.482	0.390	0.232	0.239	0.333	0.267	0.198	0.190	0.202
6Pgdh3	*a*	0.000	0.005	0.000	0.003	0.007	0.000	0.003	0.002	0.000	0.000
	b	0.539	0.586	0.564	0.564	0.545	0.543	0.564	0.521	0.528	0.534
	c	0.461	0.409	0.436	0.434	0.448	0.457	0.433	0.477	0.472	0.466
*Fum***	*a*	0.070	0.073	0.102	0.104	0.107	0.245	0.328	0.366	0.370	0.235
	b	0.898	0.899	0.723	0.606	0.649	0.656	0.633	0.608	0.576	0.712
	c	0.031	0.028	0.174	0.290	0.244	0.099	0.038	0.026	0.055	0.054
*Pgm2***	*a*	0.000	0.005	0.004	0.006	0.000	0.004	0.000	0.000	0.002	0.004
	b	0.969	0.982	0.985	0.979	0.969	0.851	0.913	0.965	0.972	0.973
	c	0.031	0.014	0.011	0.014	0.031	0.145	0.087	0.035	0.026	0.023
Pgi	*a*	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.002	0.000
	b	0.992	1.000	0.989	0.988	0.990	1.000	1.000	0.995	0.991	1.000
	c	0.008	0.000	0.011	0.010	0.010	0.000	0.000	0.005	0.007	0.000
*Adh1***	*a*	0.180	0.200	0.155	0.048	0.050	0.032	0.023	0.023	0.028	0.069
	b	0.820	0.800	0.845	0.952	0.950	0.968	0.977	0.977	0.969	0.931
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
Adh2	*a*	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.003	0.002	0.000
	b	1.000	1.000	1.000	1.000	0.993	1.000	1.000	0.997	0.998	1.000
*Acp2***	*a*	0.906	0.855	0.686	0.604	0.611	0.716	0.818	0.849	0.850	0.760
	b	0.094	0.145	0.302	0.382	0.368	0.223	0.166	0.142	0.145	0.137
	c	0.000	0.000	0.012	0.014	0.021	0.061	0.016	0.009	0.005	0.103
Got1	*a*	0.000	0.005	0.000	0.003	0.002	0.004	0.000	0.000	0.002	0.000
	b	1.000	0.995	1.000	0.997	0.998	0.996	1.000	1.000	0.998	1.000
Got3	*a*	0.000	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000
	b	1.000	1.000	1.000	0.999	0.998	1.000	1.000	1.000	1.000	1.000

Chi-squared test of independence: *significant ($P < 0.05$); **highly significant ($P < 0.01$)

Appendix III-3. Homozygote and heterozygote frequencies of spatial blocks for each locus.

Locus		b1	b2	b3	b4	b5	b6	b7	b8	b9	b10
Lap	Homozygote	0.313	0.291	0.318	0.444	0.394	0.397	0.472	0.380	0.412	0.328
	Heterozygote	0.688	0.709	0.682	0.556	0.606	0.603	0.579	0.620	0.588	0.672
6Pgdh2	Homozygote	0.375	0.473	0.598	0.540	0.571	0.560	0.559	0.633	0.640	0.641
	Heterozygote	0.625	0.527	0.402	0.460	0.429	0.440	0.441	0.367	0.360	0.359
6Pgdh3	Homozygote	0.359	0.491	0.523	0.506	0.478	0.454	0.462	0.503	0.521	0.466
	Heterozygote	0.641	0.509	0.477	0.494	0.522	0.546	0.538	0.497	0.479	0.534
Fum	Homozygote	0.813	0.817	0.598	0.403	0.488	0.461	0.467	0.446	0.427	0.562
	Heterozygote	0.188	0.183	0.402	0.597	0.512	0.539	0.533	0.554	0.573	0.438
Pgm2	Homozygote	0.938	0.964	0.970	0.958	0.945	0.702	0.836	0.930	0.943	0.947
	Heterozygote	0.063	0.036	0.030	0.042	0.055	0.298	0.164	0.070	0.057	0.053
Pgi	Homozygote	0.984	1.000	0.977	0.977	0.979	1.000	1.000	0.990	0.981	1.000
	Heterozygote	0.016	0.000	0.023	0.023	0.021	0.000	0.000	0.010	0.019	0.000
Adh1	Homozygote	0.641	0.600	0.689	0.903	0.900	0.936	0.954	0.955	0.938	0.863
	Heterozygote	0.359	0.400	0.311	0.097	0.100	0.064	0.046	0.045	0.062	0.137
Adh2	Homozygote	1.000	1.000	1.000	1.000	0.993	1.000	1.000	0.993	0.995	1.000
	Heterozygote	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.007	0.005	0.000
Acp2	Homozygote	0.813	0.800	0.698	0.571	0.641	0.642	0.755	0.755	0.738	0.702
	Heterozygote	0.188	0.200	0.302	0.429	0.359	0.387	0.245	0.245	0.262	0.298
Got1	Homozygote	1.000	0.991	1.000	0.995	0.997	0.993	1.000	1.000	0.995	1.000
	Heterozygote	0.000	0.009	0.000	0.005	0.003	0.007	0.000	0.000	0.005	0.000
Got3	Homozygote	1.000	1.000	1.000	0.997	0.997	1.000	1.000	1.000	1.000	1.000
	Heterozygote	0.000	0.000	0.000	0.003	0.003	0.000	0.000	0.000	0.000	0.000

Appendix IV. Kendall's coefficient for rank correlation (τ) for spatial blocks.

		Average Parameters				
Locus	Freq. homo[1]	*Hs*	*D*	*J′*	*Fis*	*NAC*
Lap	– 0.110	0.290	– 0.156	0.200	0.160	–
6Pgdh2	– 0.640**	0.380	0.510*	0.420	– 0.110	–
6Pgdh3	– 0.560*	– 0.240	– 0.200	– 0.070	– 0.560*	–
Fum	0.11	– 0.200	– 0.150	– 0.110	0.020	–
Pgm2	– 0.510*	0.330	– 0.150	– 0.380	– 0.510*	–
Pgi	– 0.240	– 0.290	– 0.070	– 0.020	–	–
Adh1	0.200	0.020	0.560*	0.420	– 0.200	–
Adh2	– 0.240	– 0.510*	– 0.640**	– 0.640**	–	–
Acp2	0.200	– 0.110	0.110	– 0.070	– 0.290	–
Got1	– 0.550	– 0.420	– 0.070	– 0.070	–	–
Got3	–	–	– 0.560*	– 0.550*	–	–
Total	–	0.07	0.02	0.020	– 0.110	– 0.160

[1] Frequency of homozygotes.
Null hypothesis (Ho): the parameter increases with the *x*-coordinate increase.*significant ($P < 0.05$); **highly significant ($P < 0.01$).
Data lacking one or more classes are not calculated.

Appendix V. Significant values for spatial autocorrelation, *I*(5).

	Distance class (m)													
Locus/Allele	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70
Adh1-a	++	++	++	++	++	–	–	–	–	–	–	–	–	–
Adh1-b	++	++	++	++	++	–	–	–	–	–	–	–	–	–
Adh1-c														
Lap-a														–
Lap-c	++	++		–	–	–		++	++	++	++	–	–	–
Lap-e	++	++		–	–	–		++	++	++	++	–	–	–
6Pgdh2-a	++	++	++		–	–		++	++	++	–	–	–	–
6Pgdh2-b	++	++	++		–	–		++	++	++	–	–	–	–
6Pgdh3-a				++	–									
6Pgdh3-b														
6Pgdh3-c														
Fum-a	++	++	++	++	++	–	–	–	–	–	–	–	–	–
Fum-b	++	++	++	–	–	–		++	++	++		–	–	–
Fum-c	++	++	++	++	–	–	–	–	–	–	–	–		++
Pgm-a														
Pgm-b	++	++	++	–	–	–	–	–	–	–		++	++	++
Pgm-c	++	++	++	–	–	–	–	–	–	–		++	++	++
Pgi-a														
Pgi-b			+											
Pgi-c			+											

Blank, not significant; +, positive significant value at 5% level; ++, positive significant value at 1% level; –, negative significant value at 5% level; —, negative significant value at 1% level.