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Population Biology of *Fagus crenata* Blume I. Demographic Genetic Differentiations of Lowland and Montane Populations in Toyama, Central Honshu, Japan

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Abstract **Based on demographic genetic differentiations, six isolated small lowland populations and one continuous large montane population of the Siebold's beech forests (*Fagus crenata*) in Toyama Prefecture, Honshu, Japan, were examined using isozymes as genetic markers. The results of within- and among-population genetic variabilities revealed that all six lowland populations, which are fragmented and isolated from each other at present, showed significantly lower percentages of polymorphic loci (*P*) and lower mean numbers of alleles per locus (*A*) than the montane population. Fluctuations in allele frequencies were observed in lowland populations. Alleles that were rare in the montane population showed unexpectedly higher frequencies in some lowland populations. The mean F_{ST} value of the lowland populations (0.116) was significantly higher than that of the montane population in Tateyama (0.005) and several other populations analyzed. All these facts suggest that small isolated Siebold's beech populations remaining on the foothills in the lowlands of Toyama Prefecture have been exposed to climate shifts and strong human disturbances, which result in fragmentation and isolation of local populations. On the other hand, the large continuous montane population on Tateyama maintains a higher genetic variability, with genetic reshuffling within and among subpopulations. Spatial genetic localizations and the resulting changes in the size-class structure in lowland populations were also discussed in relation to the demographic changes.**

Key words: **demographic genetics, *Fagus crenata*, population biology, population fragmentation, random genetic drift.**

Local populations of long-lived woody species have been exposed to severe environmental constraints and have also experienced occasional fluctuations in population size due to competitive interactions among the woodland elements which have adapted to warmer or colder climatic conditions during past climatic shifts. Particularly in small populations at the margins of the large geographical range of a species, the effects of environmental changes have operated more intensively. Such changes in environmental and ecological conditions might have also influenced the genetic variability of the population (Soule, 1973; Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Hoffmann and Blows, 1994). One of the major consequences of environmental change is reduction in population size. Small populations have a stochastic risk of loss of allelic diversity, induced or even accelerated high genetic differentiation among populations, reduced gene flow among neighboring local populations (Hamrick and Nason, 1995), and also in some instances elevated inbreeding. Under such circumstances genetic bottlenecks and/or random genetic drift would have an effect on genetic structures (Wright, 1922; Lande, 1988; Young et al., 1996).

It has been well documented that, during Pleistocene geological history in the northern hemisphere, considerable geographical shifts in their range distributions have occurred repeatedly for major woody species such as beeches, oaks, maples, and birches (Tsukada, 1982a, b; Delcourt and Delcourt, 1987). On the other hand, more recent habitat fragmentations and isolations in many natural populations, in both animal and plants, due to extensive human disturbance and exploitation of their habitats have caused much more drastic changes in their demographic genetic structures (Soule et al., 1988; Young et al., 1993). Therefore, critical research on genetic variation in fragmented populations, investigating how and to what extent it reflects environmental changes in past geological periods as well as recent human exploitation, is important for understanding the ecological and genetic consequences of habitat and forest fragmentations, and also the

effects on local substructurings of populations at various levels (Templeton et al., 1990).

The results of previous genetic analyses based upon allozyme variability of the Siebold's beech, *Fagus crenata* (Tomaru et al., 1996), showed considerably high levels of genetic diversity within local populations, but the extent of genetic differentiation among overall populations was relatively low (G_{ST}=0.005 to 0.061). On the other hand, unique localized genetic variabilties and substructurings were recently discovered in several studies (Kitamura et al., 1997a, b; Kawano and Kitamura, 1997) of local populations in Ogawa located on the border of Ibaraki and Fukushima on the Pacific coast. In this series of studies, they have adopted a systematic sampling strategy, mapping all individuals and recording the size distributions (DBH or DGH) of all individuals within sampling plots. This critical population analysis revealed exceedingly unique demographic genetic substructurings, and indicated quite small effective population size and the presence of remarkably small-scale genetic substructurings among neighboring patch populations (Kawano and Kitamura, 1997). Somewhat similar situations were also shown by Young et al. (1993) from fragmented sugar maple populations (also cf. Young and Merriam, 1994). These results show that the sampling strategy is very critical to demonstrate within- or among-population genetic structures (Epperson, 1989) for sedentary organisms, such as woody plants which have life spans often exceeding several hundred years. Traditional random sampling disregarding either spatial positions and/or growth stages of individuals within a patch or a local population is not sufficient to reveal local population structures from the demographic genetic point of view.

It is important to understand whether or not spatio-temporal genetic structures of local or patch populations of the study area are reflecting past regional geohistorical events or are reflecting recent consequences of forest fragmentation due to intensive human disturbances (Boyle et al., 1990; Knowles et al., 1992; Young and Merriam, 1994; Young et al., 1993; Templeton et al., 1990; Kawano and Kitamura, 1997). Therefore, in sampling the populations we have paid special attention not only to spatial but also to size- (or age-) distributions of individuals within each selected population.

In the present study, we have first examined demographic structures of several lowland populations and then analyzed genetic structures using isozymes as genetic markers. Based upon all the evidence obtained, we attempted to examine the effects of population fragmentation and isolation on genetic variabilities and differentiations among local beech populations isolated in the lowlands of Toyama Prefecture on the Japan Sea side of Honshu. Second, we have selected a montane population on Tateyama, which still maintains a more or less large continuous breeding population, and analyzed its demographic genetic structures.

The results obtained from lowland and montane populations were then compared, and the consequences of forest fragmentation and isolation on genetic variability and substructurings of beech populations in Toyama were critically analyzed in comparison with those of the Tohoku and Kanto districts as well (Ohkawa et al., unpubl. data and in preparation).

Materials and Methods

1. *Study Species*

The Siebold's beech (*Fagus crenata* Blume) is one of the climax canopy species in cool-temperate deciduous forests in Japan. Its geographical range extends widely in the lowlands to the montane zone from Kuromatsunai (the northernmost limit) in Oshima Peninsula, Hokkaido (42° lat.), to Honshu, Shikoku, and Kyushu, covering broad low montane zones, although populations in Shikoku and Kyushu are somewhat sporadically isolated on the mountains. The southernmost limit of its range is Mt. Takakuma (31.5° lat.) in Kyushu (Horikawa, 1972). However, several extremely isolated small beech populations are also found on the foothills at low elevations (10–300 m above sea level) in several scattered foothills along the Ishikawa-Toyama border in the Hokuriku district. These small patches or local populations were once much larger continuous populations, and have been fragmented due to recent human activities and disturbances (Tsukada, 1982a, b).

2. *Study Populations and Field Samplings*

Six lowland populations on the foothills in Toyama Prefecture, Japan, were selected in order to investigate demographic genetic structures (Table 1; Fig. 1). In the lowland populations, field surveys and samplings were conducted during May and June 1996, which was the year just after the mass flowering and fruiting of the Siebold's beech on the Japan Sea side. Masting with several years' interval is well known in the Siebold's beech (Kikuchi, 1968). The exact locations of all individual trees, including seedlings, juveniles, and mature trees within the plot, and diameters at breast height (DBH) or at the ground level (DGL, but both denoted as DBH here for convenience) were recorded, and then several fresh leaves were collected from every tree for isozyme analyses. Genotypes were determined for all sampled individuals, so that parametric values rather than sample estimates for measures of genetic variability were obtained in these lowland populations.

All lowland populations were smaller than 1.0 ha, with a small number of mature individuals (Table 1); associated with the Siebold's beech were evergreen oaks such as *Quercus acuta* or *Q. salicina* as co-dominant

Table 1. Study plots of *Fagus crenata* in six lowland and five montane populations in Toyama Prefecture.

Study stand	Altitude (m)	Total surveyed area (ha)	Number of trees investigated				Density of matures (ha^{-1})	Locality
			Total	Seedling	Established tree			
				[1] 1st yr., [2] 2nd yr.	Juvenile (DBH < 30)	Mature (DBH > 30)		
Lowland populations								
Unazuki (UNA)	220	0.2	33	5[1]	23	5	25.0	Hachiman shrine, Unazuki-cho
Mt. Futagami (FUT)	270	0.7	97	76[1]	12	9	12.9	Futagami, Takaoka-shi
Mt. Ojiro (OJI)	120	0.2	139	85[1]	47	7	35.0	Ojiro, Oyabe-shi
Kurikara-toge (KUR)	250	0.3	108	62[1]	28	18	60.0	Kurikara toge, Oyabe-shi
Ichitani (ICH)	200	0.2	293	267[1]	15	11	55.0	Ushidake shrine, Tonami-shi
Soyama (SOY)	560	0.2	69	21[1]	32	16	80.0	Soyama, Taira-mura
Montane populations in Tateyama								
T1	1150–1200	0.5	68	–†	–†	68	113.3	Buna-daira, Tateyama-cho
T2	1200–1250	1.5	55	–†	–†	55	36.7	Buna-daira, Tateyama-cho
T3	1200–1250	0.8	36	–†	–†	36	45.0	Buna-daira, Tateyama-cho
GAP(gap in plot T3)	1200–1250	0.2	257	112[2]	145	–†		Buna-daira, Tateyama-cho
T4	1100–1150	2.5	67	–†	–†	67	26.8	Buna-zaka, Tateyama-cho
T5	1100–1150	0.5	53	–†	–†	53	101.9	Buna-zaka, Tateyama-cho

–† not investigated.

species in the canopy layer. These stands have remained by chance at the ridges of foothills in urban areas or have been preserved as sacred forests of shrines or temples. These populations are, as a matter of course, isolated at great distances from each other; hence gene flow among these populations can hardly be expected.

On Mt. Tateyama, field surveys and samplings were conducted in five subpopulations in 1992 (plot T1), 1997 (plot T2), and 1998 (plots T3-T5). In these subpopulations, trees with DBH > 30 cm in each study plot were investigated. A gap larger than 800 m^2, which is regarded as the largest size category found in the beech forests (Yamamoto, 1989), was found within plot T3. A study plot of 20 m × 35 m was established in the center of this gap (plot GAP); all seedlings and juveniles (at most 23.5 cm) in the GAP plot were also investigated and leaves were sampled.

All sampled leaves were placed immediately in an icebox in the field, taken to the laboratory, and then stored at −80°C until enzyme extraction.

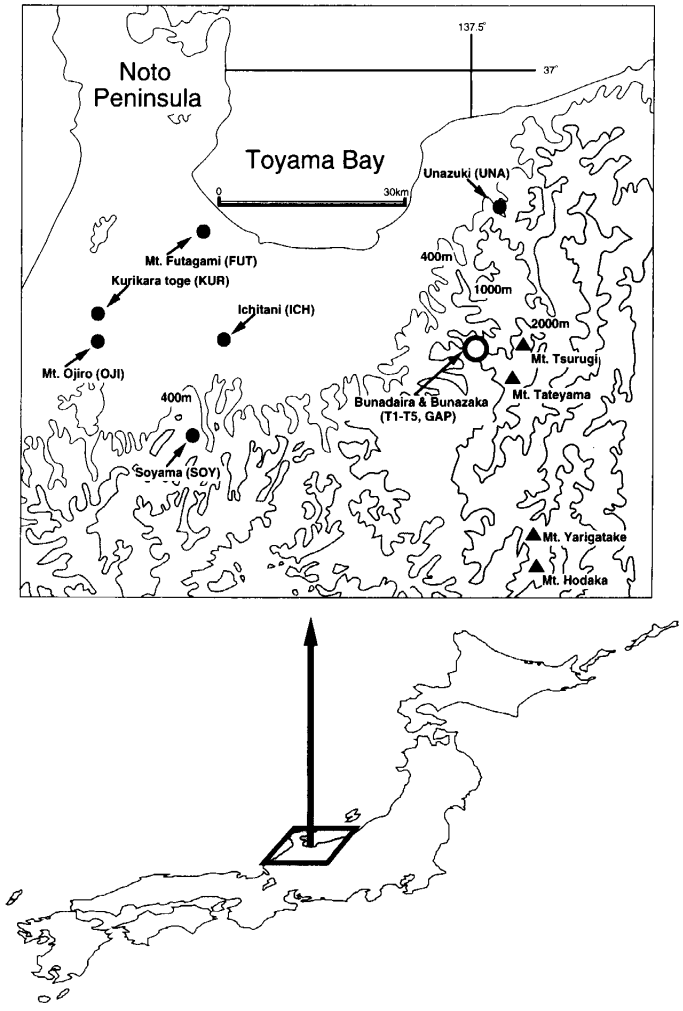

Fig. 1. Locations of six lowland populations (filled circles) and five montane subpopulations (included ina large open circles) of *Fagus crenata* in Toyama Prefecture, Honshu, Japan.

3. *Allozyme Analysis*

About 100 mg of fresh leaf tissue was frozen in liquid nitrogen and crushed with mortar and pestle. Immediately after crushing, 1 ml of extraction buffer (93 mM Tris-HCl (pH 7.5), 23.4% glycerol, 0.6% Tween 80, 12.0 mM DTT, 0.5% 2-mercaptoethanole) and 75 mg of polyvynilpolypyroridon were added. After centrifugation of homogenates at 15,000 rpm at 0°C for 10 minute, 12 μl of supernatant per enzyme was used for electrophoresis.

Vertical discontinuous polyacrylamide slab gel electrophoresis (Shiraishi, 1988) was conducted for genotyping of all samples. The following 11 enzyme systems and 13 allozyme loci were analyzed in this study: alanine aminopeptidase (*Aap*), asparate aminotransferase (*Aat-1* and *Aat-2*), amylase (*Amy-1*), diaphorase (*Dia*), glutamate dehydrogenase (*Gdh*), fumarase (*Fum*), isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh*), phosphoglucoisomerase (*Pgi*), phosphoglucomutase (*Pgm-1* and *Pgm-2*), and 6-phosphogluconate dehydrogenase (*6Pgdh*).

Staining procedures followed or were minor modifications of Shiraishi (1988) and Richardson et al. (1986).

Alleles were assigned alphabetically by electrode speed and were also denoted so as to maintain consistency with my genetic studies of other Siebold's beech populations.

4. *Data Analysis*

(1) *Genetic variability within populations*

Before conducting genetic analyses on the lowland populations, we distinguished two main growth stages: seedlings and established trees. Established trees include all individuals except first-year seedlings, and most of them were thought to be older than several years (period since the last masting). The reason we have distinguished different growth stages is that changes in genetic variability might take place accompanied by drastic thinning of individuals, especially between the seedling and succeeding juvenile stages. If any significant genetic changes occur during growth processes, we will be able to detect the changes in genetic diversities of overall growth stages even in small populations; notably, it is important to analyze the occurrence of rare alleles in the juvenile stages. Also, in order to solve the statistical problems due to small sample size in fragmented and isolated populations, it is useful to discriminate growth stages, especially between the seedlings and subsequent juvenile stages. Genetic variabilities for this established stage in each lowland population were regarded as population mean values and used for analyses at the population level.

The following measures of genetic variability were calculated for each population: percentages of polymorphic loci (*P*, 100% and 95% criteria), mean number of alleles per locus (*A*), effective number of alleles per locus ($Ae = 1/(1-He)$), observed (*Ho*) and expected (*He*)

heterozygosity (unbiased; Nei, 1978), and Wright's inbreeding coefficient (F_{IS})(Wright, 1922). All statistics, except for F_{IS}, were calculated for the established tree stage in lowland populations. Average values of *A*, *Ae*, *Ho*, and *He* were calculated for all loci and for polymorphic loci (100% criterion) separately. Differences of means of these statistics at all loci between lowland and montane populations were tested using the Mann-Whitney *U*-test. Inbreeding coefficients were tested for significance with chi-square goodness-of-fit, $\chi^2 = N F^2 (a\text{-}1)$, with $df = a(a\text{-}1)/2$, where *N* is the total sample size and *a* is the number of alleles at a locus (Li and Horvitz, 1953).

Since all lowland populations are composed of small numbers of individuals, the low levels of genetic diversity found in each population might be explained only by the present small population size, not by genetic drift or severer bottleneck effect which once may have occurred. Therefore we conducted simple simulations to examine the relationships between sample size and genetic diversity. Simulations were conducted based on the study site on Mt. Tanzawa in Kanagawa Prefecture, where we have already surveyed spatio-temporal genetic structures of the Siebold's beech population in a large (2.25 hectare) plot based on the same allozyme loci used in this study (Ohkawa et al., unpublished data). Simulation procedures are as follows: Five arbitrary populations of randomly sampled 5, 10, 20, 40, and 80 trees with DBH>10 cm were produced, and then genetic diversity measures such as *P*, *A*, and *He* were calculated in each simulated population. This procedure was repeated 1000 times; then grand mean, minimum, and maximum values of each statistic were calculated. The correlations between grand means of these simulated diversity measures and logarithm transformed population sizes were significantly positive ($P = 0.379 + 0.152 \ln(N)$, $r = 0.968$, $p < 0.01$; $A = 1.022 + 0.412 \ln(N)$, $r = 0.995$, $p < 0.001$; $He = 0.169 + 0.008 \ln(N)$, $r = 0.935$, $p < 0.05$;), and these were then compared to the actual values obtained from the lowland populations. Comparing the results of these simple simulations, we are able to estimate the relative importance of genetic drift in the present genetic diversity of lowland populations.

(2) *Genetic differentiation among populations*

Genetic differentiation among populations was analyzed using *F*-statistics (Wright, 1965), following the methods of Weir and Cockerham (1984), using the computer software FSTAT (Goudet, 1995). The G_{ST} was also calculated as the ratio of among-population genetic diversity (D_{ST}) to total genetic diversity (H_T) (Nei, 1973). Confidence intervals at the 95% level of *F*-statistics and G_{ST} values were obtained by bootstrapping over loci for the multilocus estimates. Mean G_{ST} and 95% confidence interval over loci were also calculated for the previous report (Tomaru et al., 1996) and for my unpublished data among natural large beech populations.

Gene flow among populations was estimated using Wright's indirect method (1951): the number of migrants per generation was calculated as $Nm = (1\text{-}G_{ST}) / 4 G_{ST}$.

(3) *Spatial autocorrelation analysis*

Spatial genetic structures within populations were analyzed by Moran's *I* (Sokal and Oden, 1978a, b) using the Visual Basic program developed by T. Ohkawa. In the lowland populations, this analysis was applied for seedlings and for both juveniles and mature trees with DBH>10 cm, separately. In the study plot GAP in the montane zone, Moran's *I* were calculated for four size classes, seedlings (SD) and juveniles (JV) with DBH<5 cm, 5 cm<DBH<10 cm, and 10 cm<DBH<25 cm, respectively, in order to estimate the changes of genetic structures in different growth stages. In other montane populations, Moran's *I* were calculated for mature trees with DBH>30 cm. We used the distance class with regular intervals of 5 m for seedlings and juveniles, and 10 m for mature trees.

(4) *Genetic heterogeneity among size classes*

Observed and expected heterozygosities and inbreeding coefficients in each lowland population were compared between seedling and established tree stages and tested by Wilcoxon rank correlation over all polymorphic loci. Allele frequency heterogeneities were tested among three size classes in the lowland populations. The three size classes were as follows: 1, 1st yr. seedling; 2, juveniles (or mature trees) with DBH<30 cm; 3, mature trees with DBH>30 cm. In FUT populations, DBH>20 cm was adopted to size class 3 instead, because almost all individuals had coppice sprouted trunks in their stamps.

In GAP plot, genetic heterogeneity was also tested among the four size classes which were used in spatial autocorrelation analysis.

Results

1. *Genetic Variabilities of Lowland and Montane Populations*

A total of 1156 individuals, 739 collected from the lowland, and 417 from the montane populations, were investigated. The histograms of size-class distribution for lowland populations are shown in Fig. 2. As was expected for the succeeding year of mast-fruiting, numbers of seedlings were extremely high in FUT, KUR, ICH, and OJI. In general, typical L-shaped distribution was observed; however, it is notable that the numbers of seedlings in UNA and SOY were small. It is also notable that small peaks in numbers of intermediate s-

ize-classes were observed in five lowland populations: 20–30 cm in FUT, under 10 cm in UNA, 30–40 cm in KUR, 30–40 cm in ICH, and 20–30 cm in SOY.

Allele frequencies at 13 polymorphic loci are listed in Appendices 2, 3, and 4. Of these thirteen allozyme loci, *Aat-1* and *Pgm*-2 loci were fixed in all the lowland populations, and *Gdh* locus showed polymorphisms only in the Soyama (SOY) population. Specific alleles showed extremely high frequencies in particular lowland populations (Especially, less frequent alleles at several loci in montane populations represented unexpected higher frequencies in some lowland populations). For example, frequencies of allele *b* at *Aat-2, b* and *e* at *Dia*, and *b* at *Pgi* were considerably high only in FUT, FUT, UNA, and ICH populations, respectively (Fig. 3a, b, e). For *Fum, Idh*, and *Pgm-1*, allele frequencies fluctuated among lowland populations while montane subpopulations showed consistent variation (Fig. 3c, d, f).

Genetic diversity measures for the lowland and the montane populations are listed in Table 5 and summarized for population groups in Table 6. The proportion of polymorphic loci at 100% criterion (P_{100}) ranged from 38.5 to 76.9 for the lowland populations and from 76.9 to 92.3 for the montane populations, with mean values of 59.0 and 83.1, respectively. Total numbers of alleles per population and mean numbers of alleles per locus (A) ranged from 18 to 27, 1.38 to 2.08, for the lowland populations, and from 28 to 32, 2.15 to 2.46, for the montane populations, respectively. Means of these values were significantly different between lowland and montane populations ($p<0.05$ in Mann-Whitney U-test). The effective number of alleles per locus (Ae) and expected (He) and observed heterozygosities (Ho) over all loci showed the same levels of diversity in both population groups (Table 2).

Average inbreeding coefficients (F_{IS}) for each population did not differ among populations within a population group (Tables 2, 4 and 5). Average F_{IS} for both lowland and montane populations showed no deviation from 0 (Table 3). However, significant deviations of F_{IS} were found in specific loci and/or size-classes. For established trees in lowland populations, excesses (SOY) or deficits (ICH) of homozygotes were found at *Idh* loci (Table 5).

For established trees, the excesses (*Idh* in SOY, *Pgm*-2 in T1, *Aat-2* in T4, and *Aap* in GAP) and deficits (*Idh* in ICH and in T1) of homozygotes were also found in lowland and montane populations (Table 5). For seedlings, three cases in lowland populations (*Fum* in FUT and ICH, and *Pgi* in FUT) and two cases in montane populations (*Dia* and *Idh* in GAP) showed excess homozygotes compared to the Hardy-Weinberg equilibrium (Table 6).

Based on the simulation, relationships between genetic diversity measures and population sizes revealed

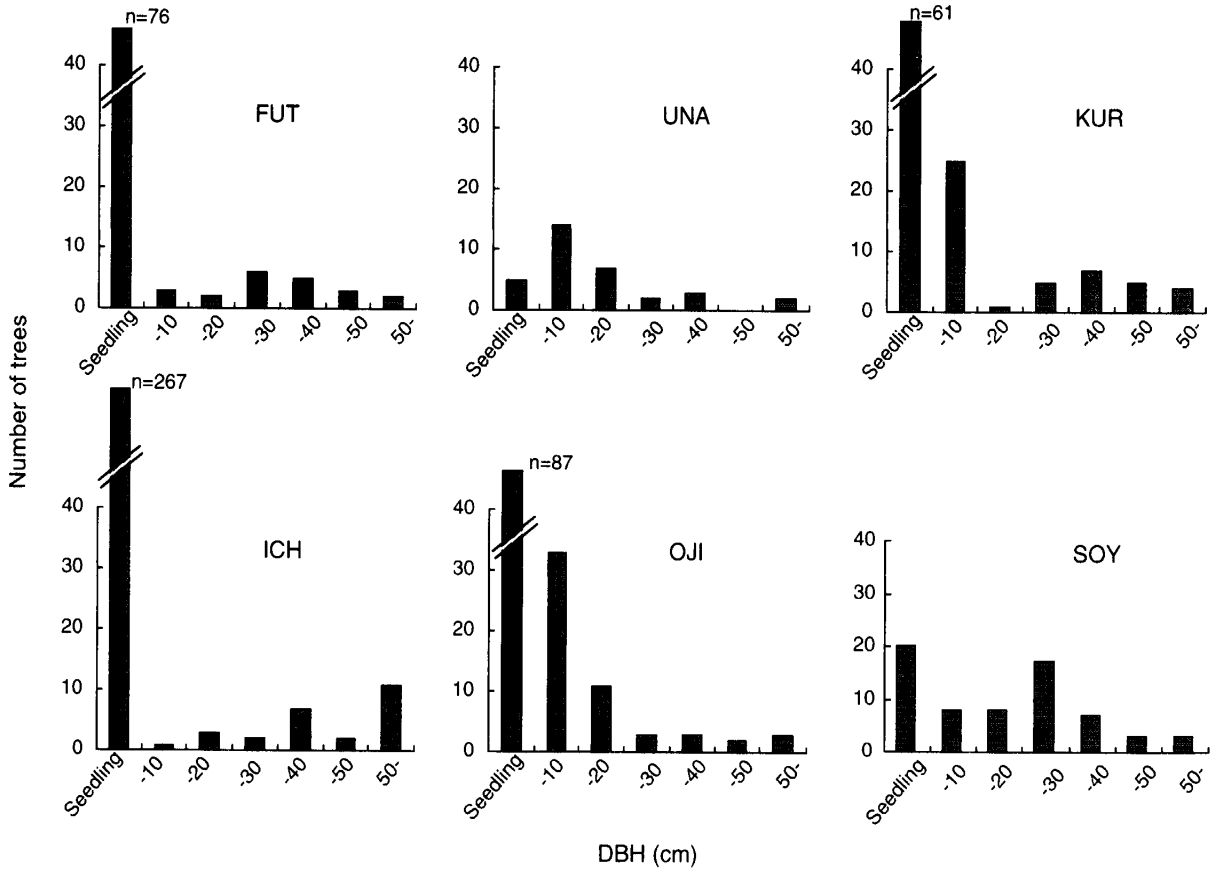

Fig. 2. Size-class distributions of the Japanese beech in six lowland populations in Toyama Prefecture, Honshu, Japan.

significant deficits of genetic diversity in the lowland populations (Fig. 4). In genetic diversity measures of *P* and *A* (Figs. 4a, b), lowland populations obviously showed lower diversities than those simulated for a large population with the same sample size levels. Five populations with the notable exception of FUT showed much lower values than the minimum estimation in 1000 times replicates in the simulations. In the diversity measures (*He*), less genetic variability was also found in the lowland populations (Fig. 4c). Only the FUT population had the same level of diversity as that simulated for a large population in the same sample size

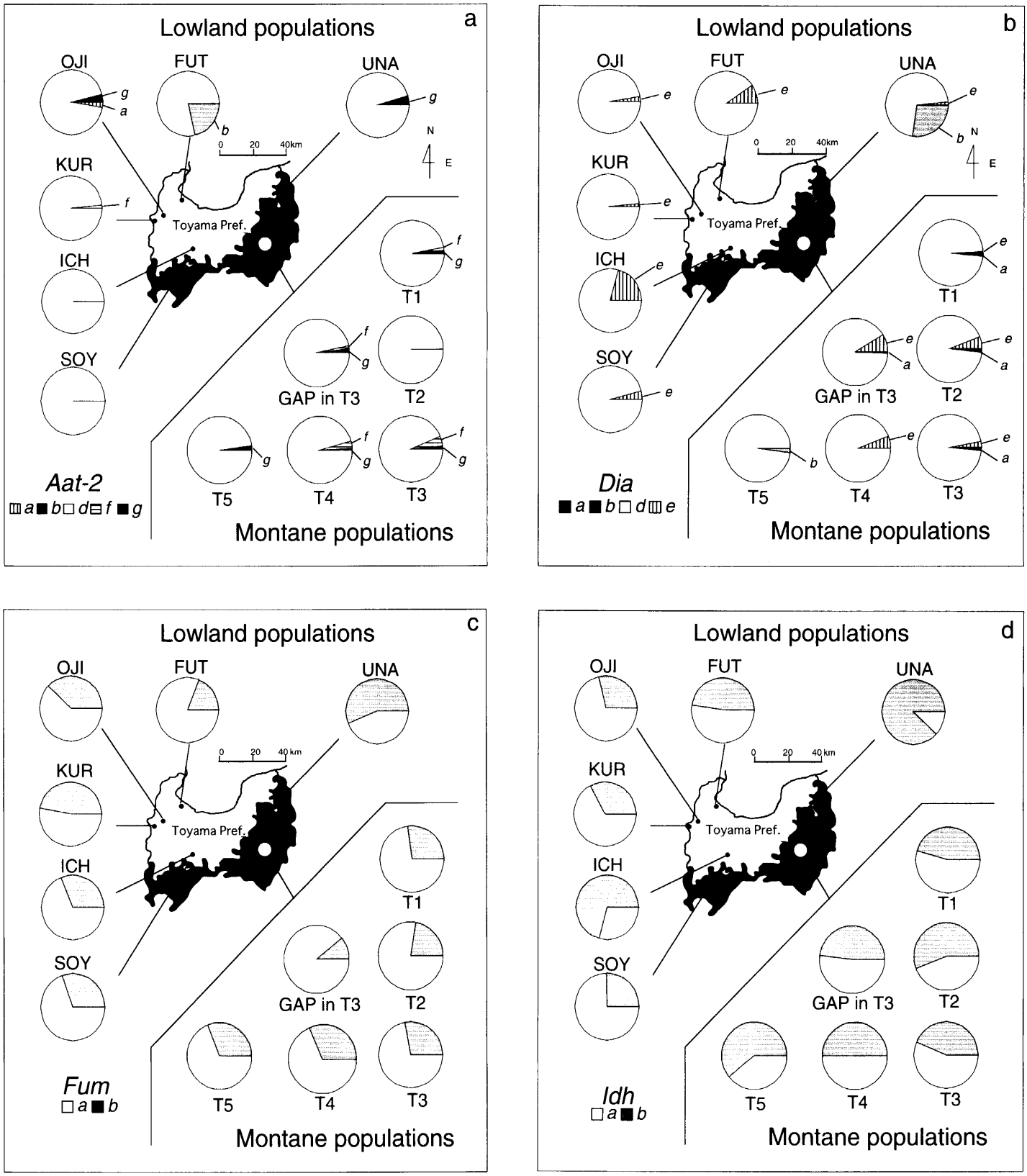

Fig. 3a-d

range.

2. *Genetic Differentiations among Lowland and Montane Populations*

The mean fixation index (F_{ST}) value over all loci was 0.116 with 95% CI ranging from 0.060 to 0.179 among the lowland populations, and 0.005 with 95% CI from 0.001 to 0.014 among the montane populations. Significant F_{ST} were found at all loci except *6 Pgdh* in lowland populations. However, significant F_{ST} was observed only in *Pgm-2* for montane populations. Non-overlapping of 95% CI of mean F_{ST} and G_{ST} values was found between the lowland population groups and the montane population groups.

3. *Spatial Genetic Structures within Populations*

The results of spatial autocorrelation analyses using Moran'*I* statistics from first to fifth distance classes are summarized in Table 10 for different size classes. Correlograms of Moran'*I* values for 5 shorter distance classes of two representative alleles in some populations are also shown in Fig. 6.

In montane populations T1 to T5, positive autocorrelations for mature trees with DBH>30 cm were observed in less than 6% of alleles analyzed for first distance class. In GAP, seedling size class showed no spatial genetic structure in the first distance class. For the juvenile size class of DBH<5 cm, 17.4% of analyzed alleles showed a positive autocorrelation in the first distance class of 5 m. However, this spatial structure completely disappeared in subsequent size class, 5 cm<DBH<10 cm (Table7; Fig. 6). On the other hand, in all the lowland populations except OJI, positive autocorrelations, which were found also in the seedling stage, were maintained in the juvenile stage with DBH>10 cm. In the ICH population, which had a very low level of genetic diversity, 4 of 5 alleles in seedlings (80%) and 2 of 5 alleles in mature trees (40%) showed significant positive autocorrelations in the first distance class of 5 and 10 m, respectively (Table 7). In other lowland populations, more than 10% of alleles showed significant positive autocorrelations at the first distance class in the mature stage (UNA, 10%; FUT, 20.0%; KUR, 14.3%; SOY, 36.4%).

No significant autocorrelation was obtained in the mature stage of the OJI population, but spatial genetic structures were observed within this study plot. The OJI plot was divided in half into two sub-plots, and allele frequencies were compared between the two. Heterogeneities in allele frequencies were found for *Aat-2* and *Fum* (Fig. 7). This simple genetic structure with no positive autocorrelation in the spatial analysis may represent the restricted mother trees with uncommon alleles.

4. *Temporal Genetic Heterogeneity*

Between the first-year seedling and mature stages in the lowland populations, we found no differences in

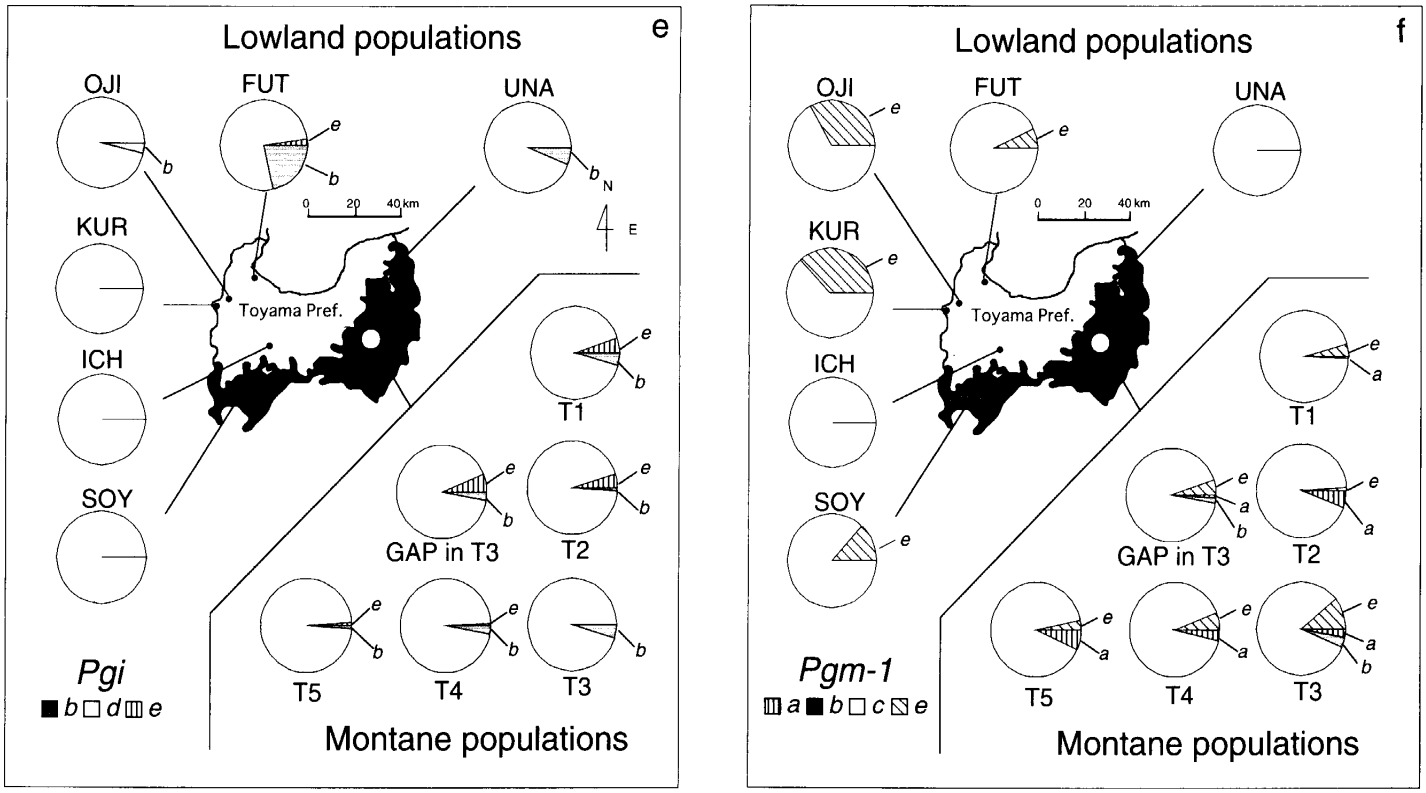

Fig. 3a-f. Allele frequencies at highly variable six loci, (a) *Aat-2*, (b) *Dia*, (c) *Fum*, (d) *Idh*, (e) *Pgi*, and (f) *Pgm-1*. Shaded areas on the map show the current distribution of the montane beech forests in Toyama Prefecture, Honshu, Japan.

Ho, He, or F_{IS} values for all loci (Wilcoxon rank correlation tests, $p>0.05$).

Among size classes in lowland populations, significant heterogeneities of allele frequencies were found

Table 2. Genetic variability for *Fagus crenata* based on 13 loci in six lowland and five montane populations.

Population	P_{100}	*A*	*Ae*	*Ho*	*He*	F_{IS}
Lowland						
UNA	53.8	1.69	1.24	0.154	0.146	−0.048
		(0.75)	(0.35)	(0.204)	(0.184)	
FUT	76.9	2.08	1.37	0.175	0.209	0.049
		(1.04)	(0.42)	(0.172)	(0.197)	
OJI	61.5	1.85	1.33	0.196	0.182	−0.080
		(0.90)	(0.44)	(0.260)	(0.212)	
KUR	53.8	1.62	1.33	0.182	0.176	−0.025
		(0.65)	(0.46)	(0.210)	(0.230)	
ICH	38.5	1.38	1.23	0.160	0.141	−0.128
		(0.51)	(0.31)	(0.217)	(0.184)	
SOY	69.2	1.92	1.33	0.184	0.191	0.367
		(1.04)	(0.45)	(0.202)	(0.200)	
Montane						
T1	76.9	2.31	1.32	0.194	0.190	0.025
		(0.95)	(0.40)	(0.213)	(0.190)	
T2	84.6	2.15	1.28	0.173	0.174	−0.029
		(0.69)	(0.37)	(0.178)	(0.178)	
T3	92.3	2.46	1.31	0.208	0.197	−0.012
		(0.88)	(0.34)	(0.200)	(0.169)	
T4	84.6	2.31	1.29	0.159	0.171	0.061
		(1.03)	(0.40)	(0.174)	(0.192)	
T5	76.9	2.15	1.24	0.152	0.149	0.001
		(0.90)	(0.34)	(0.177)	(0.181)	

*Standard deviations are in parentheses

Table 3. Genetic variability in six lowland and five montane beech populations.

Genetic diversity statistics		Lowland population		Montane population		*U*-test
		Mean	SD	Mean	SD	
Mean number of alleles per population		22.7		29.6		$p<0.01$
Percentage of polymorphic loci, *P*	100%	59.0		83.1		$p<0.01$
	95%	46.2		52.3		
Number of alleles per locus, *A*	All loci	1.74	(0.24)	2.28	(0.13)	$p<0.05$
	Polymorphic loci	2.26	(0.16)	2.54	(0.12)	
Effective number of alleles per locus, *Ae*	All loci	1.30	(0.06)	1.28	(0.03)	ns
	Polymorphic loci	1.52	(0.07)	1.33	(0.04)	
Observed heterozygosity, *Ho*	All loci	0.174	(0.021)	0.177	(0.024)	ns
	Polymorphic loci	0.309	(0.068)	0.209	(0.026)	
Expected heterozygosity, *He*	All loci	0.174	(0.021)	0.181	(0.019)	ns
	Polymorphic loci	0.302	(0.044)	0.212	(0.021)	
Inbreeding coefficient, F_{IS}	Polymorphic loci	0.023	(0.178)	0.009	(0.035)	

ns: not significant at $p<0.05$.

Table 4. Wright's *F*-statistics calculated among six lowland and among five montane beech populations.

Locus	Lowland population							Montane population						
	F_{IS}	F_{IT}	F_{ST}	H_S	H_T	G_{ST}	*Nm*	F_{IS}	F_{IT}	F_{ST}	H_S	H_T	G_{ST}	*Nm*
Aap	−0.137	−0.038	0.087	0.053	0.060	0.118	1.9	0.044	0.048	0.005	0.132	0.134	0.013	19.1
Aat-1	−†	—	—	—	—	—	—	−0.011	−0.007	0.003	0.027	0.028	0.010	24.1
Aat-2	−0.012	0.091	0.102	0.098	0.111	0.113	2.0	0.102	0.107	0.005	0.061	0.062	0.014	17.6
Amy-1	0.030	0.068	0.040	0.484	0.505	0.043	5.6	0.039	0.039	0.001>	0.497	0.501	0.008	29.5
Dia	−0.208	−0.013	0.161	0.181	0.210	0.138	1.6	−0.043	−0.033	0.009	0.088	0.089	0.014	17.8
Fum	−0.076	−0.028	0.045	0.435	0.464	0.063	3.7	0.048	0.045	0.001>	0.402	0.404	0.005	49.2
Gdh	0.242	0.284	0.054	0.023	0.024	0.062	3.8	—	—	—	—	—	—	—
Idh	−0.014	0.222	0.233	0.386	0.500	0.228	0.8	−0.010	−0.001	0.009	0.491	0.500	0.017	14.7
Mdh	0.000	0.086	0.085	0.241	0.258	0.066	3.5	0.067	0.067	0.001>	0.209	0.211	0.007	34.5
Pgi	−0.088	0.055	0.132	0.086	0.099	0.132	1.6	−0.049	−0.040	0.008	0.100	0.101	0.014	17.7
Pgm-1	−0.001	0.173	0.173	0.208	0.259	0.197	1.0	−0.055	−0.044	0.011	0.179	0.182	0.018	13.3
Pgm-2	—	—	—	—	—	—	—	0.274	0.323	0.068	0.057	0.060	0.059	4.0
6Pgdh	−0.009	−0.004	0.005	0.015	0.015	0.015	16.2	−0.017	−0.010	0.006	0.029	0.030	0.012	19.9
Mean	−0.028	0.091	0.116	0.170	0.193	0.107	3.8	0.025	0.030	0.005	0.189	0.192	0.016	21.8
±SE	0.034	0.032	0.020	0.051	0.057	0.020	1.3	0.016	0.015	0.003	0.051	0.051	0.004	3.3
95% CI	−0.079	0.017	0.060	0.087	0.117	0.068		−0.004	0.004	0.001	0.118	0.103	0.011	
	0.008	0.159	0.179	0.308	0.293	0.147		0.057	0.066	0.014	0.287	0.306	0.025	

−† Locus is monomorphic; ** *F*-statistics significantly different from zero ($p<0.01$).

Table 5. Fixation indices (F_{IS}) at polymorphic loci for established trees (a), for mature trees (b), and for juveniles (c).

Locus	Lowland population (a)						Montane population (b)					GAP(c)
	UNA	FUT	OJI	KUR	ICH	SOY	T1	T2	T3	T4	T5	
Aap	-†	−0.024	—	−0.186	—	−0.089	0.134	−0.109	0.198	−0.047	−0.034	0.182**
Aat-1	—	—	—	—	—	—	—	−0.020	−0.032	−0.008	−0.011	−0.018
Aat-2	−0.024	0.010	−0.051	−0.014	—	—	−0.023	0.000	−0.053	0.307**	−0.023	−0.024
Amy-1	0.408	−0.081	0.097	−0.019	−0.038	0.027	0.114	−0.023	−0.201	0.124	−0.051	−0.049
Dia	−0.348	−0.084	−0.029	−0.014	−0.238	−0.043	−0.018	−0.068	−0.032	−0.063	−0.023	0.067
Fum	0.028	0.382	0.017	−0.204	−0.031	−0.249	−0.069	−0.067	0.072	0.168	0.067	−0.120
Gdh	—	—	—	—	—	0.231	—	—	—	0.000	—	—
Idh	−0.105	−0.145	−0.222	−0.101	−0.405**	0.409**	−0.283*	0.165	−0.143	0.075	0.112	−0.132
Mdh	−0.200	0.319	−0.137	—	−0.038	0.069	0.127	−0.133	0.210	0.085	−0.001	0.162
Pgi	−0.024	−0.149	−0.038	—	—	—	−0.080	−0.053	−0.049	−0.032	−0.017	−0.035
Pgm-1	—	−0.077	0.083	0.021	—	−0.153	−0.049	−0.056	−0.143	0.006	−0.084	0.123
Pgm-2	—	—	—	—	—	—	0.328**	−0.010	−0.032	0.000	—	−0.032
6Pgdh	—	−0.024	—	—	—	−0.032	—	−0.030	−0.032	−0.015	—	−0.014

-† Locus is monomorphic.

* $p<0.05$; ** $p<0.01$ indicate significant deviation from the expected heterozygosity under Hardy-Weinberg equilibrium.

Table 6. Fixation indices (F_{IS}) at polymorphic loci for seedlings.

Locus	Lowland population						Montane population
	UNA	FUT	OJI	KUR	ICH	SOY	GAP
Aap	•	−0.056	—	0.222	—	−0.111	−0.032
Aat-1	•	−†	—	—	—	—	−0.057
Aat-2	•	−0.089	−0.096	—	—	—	0.103
Amy-1	•	0.061	0.043	−0.137	−0.039	−0.099	0.030
Dia	•	0.079	—	−0.014	0.019	−0.053	0.196*
Fum	•	0.282*	−0.185	0.183	0.157*	−0.254	0.029
Gdh	•	—	—	—	—	−0.081	—
Idh	•	−0.218	−0.139	0.085	0.067	−0.004	0.178*
Mdh	•	−0.078	−0.133	—	0.079	0.286	0.020
Pgi	•	0.263*	−0.012	—	—	—	−0.052
Pgm-1	•	−0.048	0.105	0.110	—	−0.143	0.060
Pgm-2	•	—	—	—	—	—	−0.009
6Pgdh	•	−0.027	—	—	—	—	−0.014

• not calculated because of small seeding number.
−† monomorphic locus
* $p<0.05$ indicate significant deviation from the expected heterozygosity under Hardy-Weinberg equilibrium.

for *Pgm*-1 locus in the OJI and *Amy*-1 locus in the ICH population (*G*-test, $p<0.05$, Table 8), in a total of 78 heterogeneity tests (monomorphic locus is automatically recognized as homogeneous in allele frequency). In GAP of the montane population, three of the 13 loci showed significant heterogeneities in allele frequencies among size classes (Table 8).

Discussion

1. *Low Levels of Genetic Diversities within Lowland Populations*

Population genetic theory predicts that small, isolated populations will experience higher levels of genetic drift and lower levels of gene flow than large, continuous populations (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). As a consequence of such effects, small populations inevitably show less genetic diversity. Using allozyme data, it has been demonstrated that small

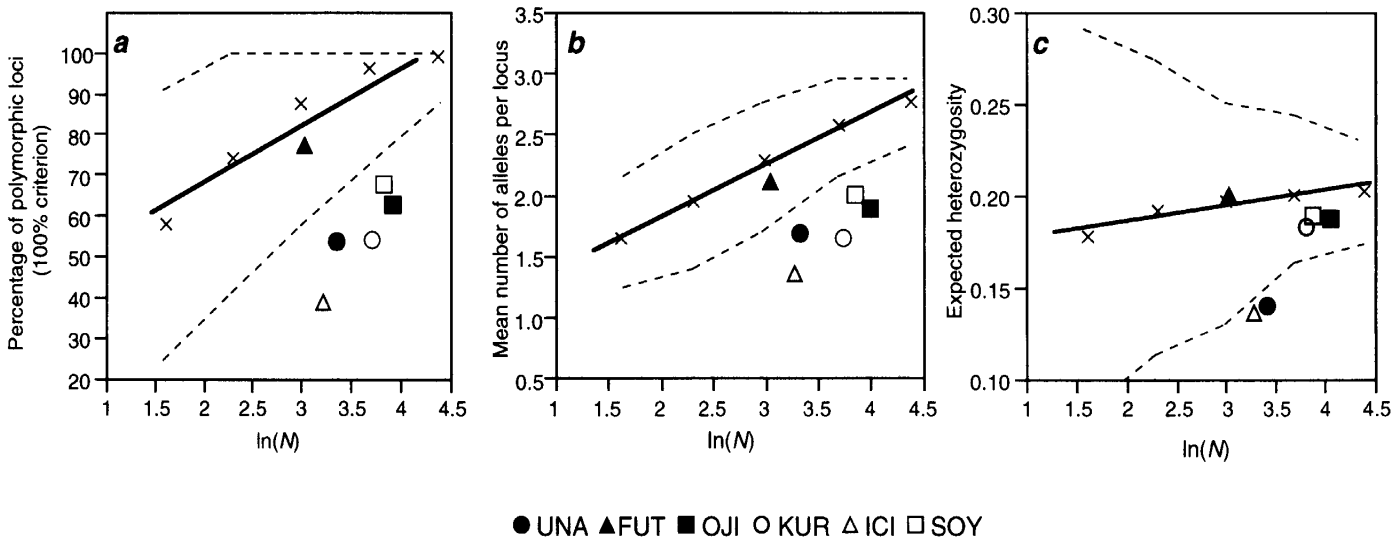

Fig. 4. Relationships between genetic diversities in lowland populations against population size (log transformed). a) *P*, b) *A*, c) *He*. The Xs represent the grand mean of 1000 times replicates in the simulations based on Tanzawa population. Solid lines represent the regression lines for their grand means. Broken lines connect minimum or maximum values in 1000 times replicates in the simulation in each arbitrary population.

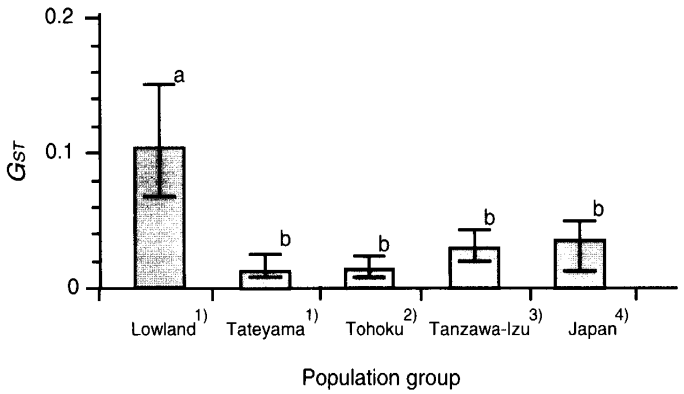

Fig. 5. G_{ST} values and 95% C for different population groups of *Fagus crenata*. 1) present study, 2) among five local populations in Yamagata Pref. (Ohkawa et al., unpublished data), 3) among four local populations in Kanagawa and Shizuoka Pref. (Ohkawa et al., unpublished data), and 4) among 23 populations widely collected in Japan (Tomaru et al., 1996). The same isozyme loci were analyzed in 2) and 3) population groups. Different letters on the column represent non overlapping of 95% CI.

populations exhibit less genetic variation, both within and among populations, than large populations (Moran and Hopper, 1983; McClenagham and Beauchamp, 1986; Sampson et al., 1988; Bilington, 1991). Low levels of genetic diversity can also be expected in marginal populations of species with a wide geographic range (Soule, 1973; Hoffman and Blows, 1994; Rossum et al., 1997). Lowland beech populations examined in this study are scattered and isolated on the foothills, contain small numbers of mature trees, and are located altitudinally at the marginal zone. These populations are obviously remnant populations that were much larger at least 2000 years ago, and were fragmented due to strong human disturbances, and have been isolated for a long period of time (Tsukada, 1982a, b). Therefore, extremely low but localized genetic diversities are to be expected to occur in these lowland beech populations. Indeed, the genetic diversity measures *P* and *A* over all loci averaged for lowland populations were considerably lower than those of the montane populations (Table 3). The result of simulations also suggests that severe genetic erosion has occurred in these lowland populations (Fig. 4). The population genetic parameters *He* and *Ae*, were however, not much different between lowland and montane population groups. The most significant evidence of genetic reductions observed in the lowland populations was the lack of less frequent alleles. The loss of rare alleles, however, will not greatly influence the changes in genetic diversity measures such as *He* and *Ae* (Young et al., 1996).

The relationships between population size and genetic diversity have been reported for *Salvia pratensis* (van Treuren et al., 1991), *Scabiosa columbaria* (van Treuren et al., 1991), and *Eucalyptus albens* (Prober and Brown, 1994). Lowland beech populations in Toyama are already considerably small in size, and thus the low genetic diversities may possibly be due to the small numbers of sampled trees. However, simulations conducted to reveal the relationship between the sample size and genetic diversity in beech populations have indicated that genetic diversities of lowland populations were lower than expected values of simulated alternatives. Proportions of polymorphic loci and mean numbers of alleles per locus in lowland populations other than FUT were considerably low.

The current states of considerably localized genetic diversity maintained in these six lowland populations are well exhibited in Figs. 3a-f. The results obtained here in small isolated lowland populations suggest that random genetic drift (Wright, 1948; Crow and Kimura, 1972; Kimura and Crow, 1964) or severer bottleneck effects (Nei et al., 1975) may have operated on reducing the genetic diversities and fluctuating allele frequencies, as is clearly shown in the values of *P* and *A* (Table 2).

2. *The Effects of Population Fragmentation to* F_{IS}

The Siebold's beech is assumed to be an obligate outbreeder. Rossi et al. (1996) and Kitamura et al. (1999) reported high outcrossing rates in the European and American beeches, respectively. But, the exact outcrossing rates for Siebold's beech populations have not yet been precisely estimated.

A study on the reproductive biology of *Fagus sylvatica*, the European beech, reported that isolated individuals produced a high proportion of empty seeds, and a negative correlation was found between the proportion of empty seed production and forest stand areas (Nilsson and Wastljunk, 1987). A similar phenomenon is also known in the Siebold's beech (Kamata, 1996). Thus, in light of all this evidence, the levels of self-pollination even in the mast flowering year would be very limited in small isolated local populations. Although selfing is not likely to occur, mating among restricted numbers of mother trees and the effect of overlapping generation may distort F_{IS} to positive (Table 6).

3. *High Levels of Genetic Differentiation among Lowland Populations*

A number of earlier studies on allozyme variability for outcrossing wind-pollinated tree species have demonstrated that most of the genetic variabilities existed within populations (Hamrick and Godt, 1989; Hamrick et al., 1992). Average values of G_{ST} for the above-mentioned species is very low (0.084; Hamrick et al., 1992). For the genus *Fagus*, the same patterns of genetic variation have also been recognized (Comps et al., 1991; Leonardi and Menozzi, 1995; Tomaru et al., 1996).

In the montane populations on Tateyama, more than

98% of the allozyme variation was restored within populations. The G_{ST} values of this population group (0.016) were very small, since sampled populations were distributed in adjacent sites, forming a more or less continuous large population. The G_{ST} value reported previously for populations in the whole range of the Siebold's beech is 0.038 (Tomaru et al., 1996), and for other local population groups examined in the present series of studies the values are 0.023 in the Tohoku district (Ohkawa et al., unpubl. data), and 0.047 in Kanagawa and Shizuoka Prefectures (Ohkawa et al., unpubl. data). These values are slightly lower than average values for forest tree species (Hamrick et al., 1992).

Several alleles showed extremely high frequencies in some lowland populations. This type of fluctuation of allele frequency was found at four loci, *Aat-2, Dia, Pgi,* and *Pgm-1* in the present study. Allele frequencies of

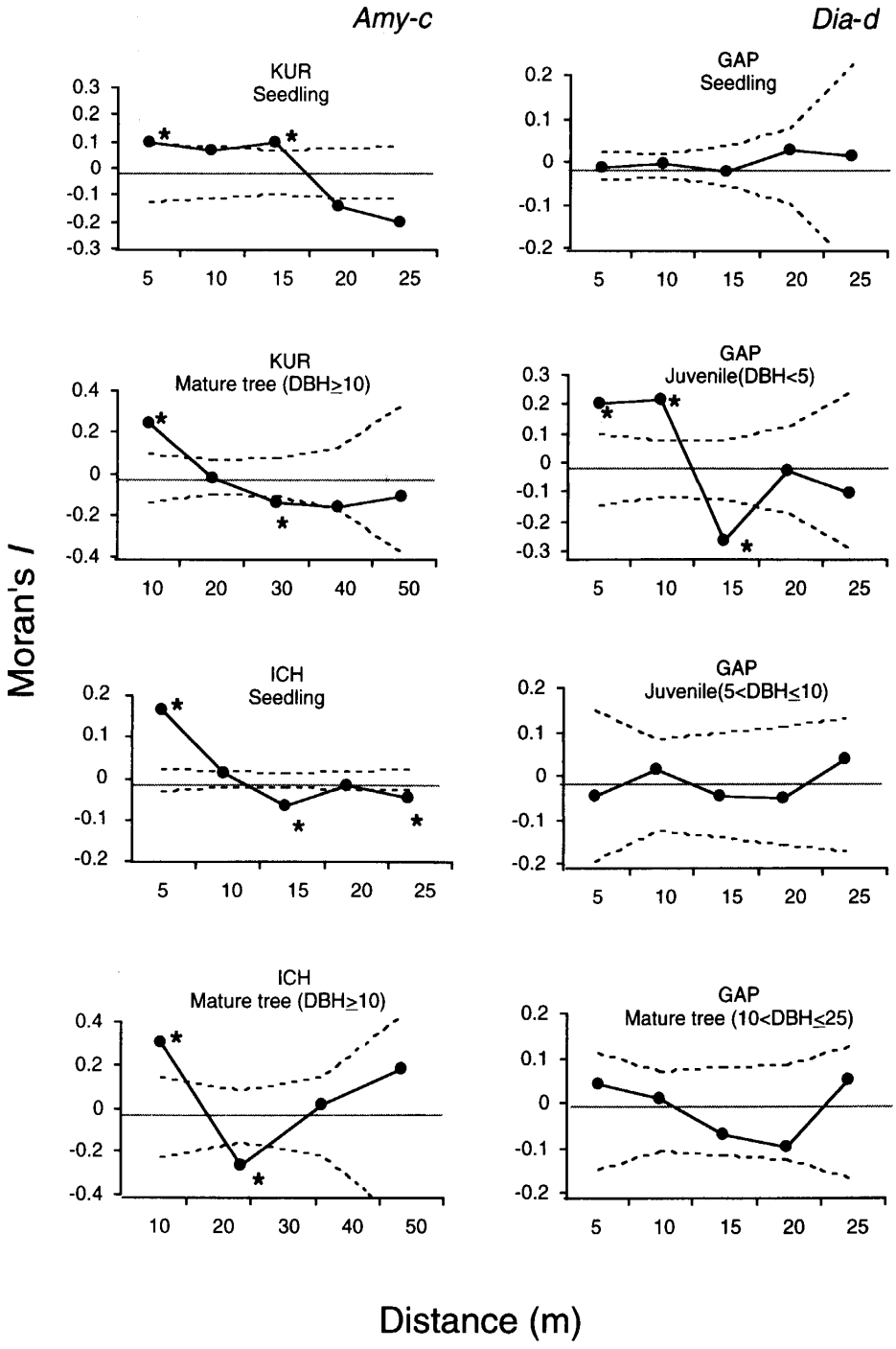

Fig. 6. Correlograms for representative alleles; *Amy-c* in the lowland populations (four correlograms on the left) and *Dia-d* in GAP plot in montane region (four correlograms on the right). A pair of correlograms for seedlings and mature tree are presented for the lowland populations, and change of genetic structure along four size classes are shown for GAP population. Dashed lines represent 95% CI of Moran's / values; Asterisks represent significant positive or negative Moran's / values in each distance class.

Table 7. Results of spatial autocorrelation analysis by Moran's *I*. Mean Moran's *I* values and percentages of positively autocorrelated alleles (in parentheses) from 1st to 5th distance classes are listed.

Distance class[a]	Lowland population										
	UNA	FUT		OJI		KUR		ICH		SOY	
	AD[c]	SD	AD	SD	AD	SD	AD	SD	AD	SD	AD
(Total No.)[b]	10	16	15	12	10	9	7	5	5	10	11
1st	−0.095	0.015	0.080	0.080	−0.018	0.043	0.014	0.082	0.162	−0.001	0.276
	(10.0)	(6.3)	(20.0)	(41.7)	(0.0)	(37.5)	(14.3)	(80.0)	(40.0)	(10.0)	(36.4)
2nd	−0.037	−0.005	−0.042	0.020	−0.043	0.009	0.003	0.036	−0.022	−0.001	0.009
	(0.0)	(0.0)	(0.0)	(25.0)	(0.0)	(12.5)	(14.3)	(40.0)	(20.0)	(0.0)	(0.0)
3rd	0.026	−0.021	−0.091	0.004	−0.044	−0.002	−0.124	−0.033	−0.064	−0.110	0.033
	(0.0)	(0.0)	(0.0)	(25.0)	(0.0)	(0.0)	(14.3)	(20.0)	(0.0)	(0.0)	(9.1)
4th	−0.205	−0.037	−0.063	−0.042	−0.136	−0.061	−0.361	−0.026	−0.069	−0.122	−0.086
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(9.1)
5th	−0.054	−0.006	−0.131	−0.048	−0.216	−0.064	−0.758	−0.041	−0.188	0.122	−0.067
	(0.0)	(6.3)	(0.0)	(0.0)	(10.0)	(0.0)	(0.0)	(20.0)	(0.0)	(0.0)	(0.0)

Distance class[a]	Montane population								
	GAP				T1	T2	T3	T4	T5
	SD	JV(D<5)	JV(D<10)	AD(D<25)	AD	AD	AD	AD	AD
(Total No.)	24	23	21	23	18	16	21	17	12
1st	−0.008	0.055	−0.038	−0.071	0.092	0.026	−0.025	0.010	−0.033
	(0.0)	(17.4)	(0.0)	(26.1)	(5.6)	(0.0)	(4.8)	(5.9)	(0.0)
2nd	−0.017	−0.022	−0.002	−0.020	−0.018	−0.066	−0.010	0.021	−0.021
	(0.0)	(4.3)	(4.8)	(4.3)	(0.0)	(0.0)	(9.5)	(5.9)	(8.3)
3rd	−0.016	0.004	−0.035	−0.041	0.004	0.008	−0.033	−0.015	−0.029
	(0.0)	(4.3)	(0.0)	(4.3)	(5.6)	(6.3)	(0.0)	(5.9)	(0.0)
4th	−0.004	−0.001	−0.025	−0.040	−0.018	−0.020	0.001	−0.032	0.002
	(4.2)	(8.7)	(0.0)	(0.0)	(5.6)	(0.0)	(9.5)	(0.0)	(0.0)
5th	−0.001	−0.046	−0.043	−0.018	−0.020	0.005	−0.005	−0.016	0.024
	(0.0)	(0.0)	(0.0)	(4.3)	(0.0)	(12.5)	(0.0)	(0.0)	(16.7)

[a] Distance class: 5 m for SD and JV, 10 m for AD; [b] Total number of alleles tested.
[c] Size class categories are as follows; SD: Seedlings, JV: Juveniles, AD: adult trees with DBH > 10 cm for lowland populations and DBH > 30 for mountain populations.

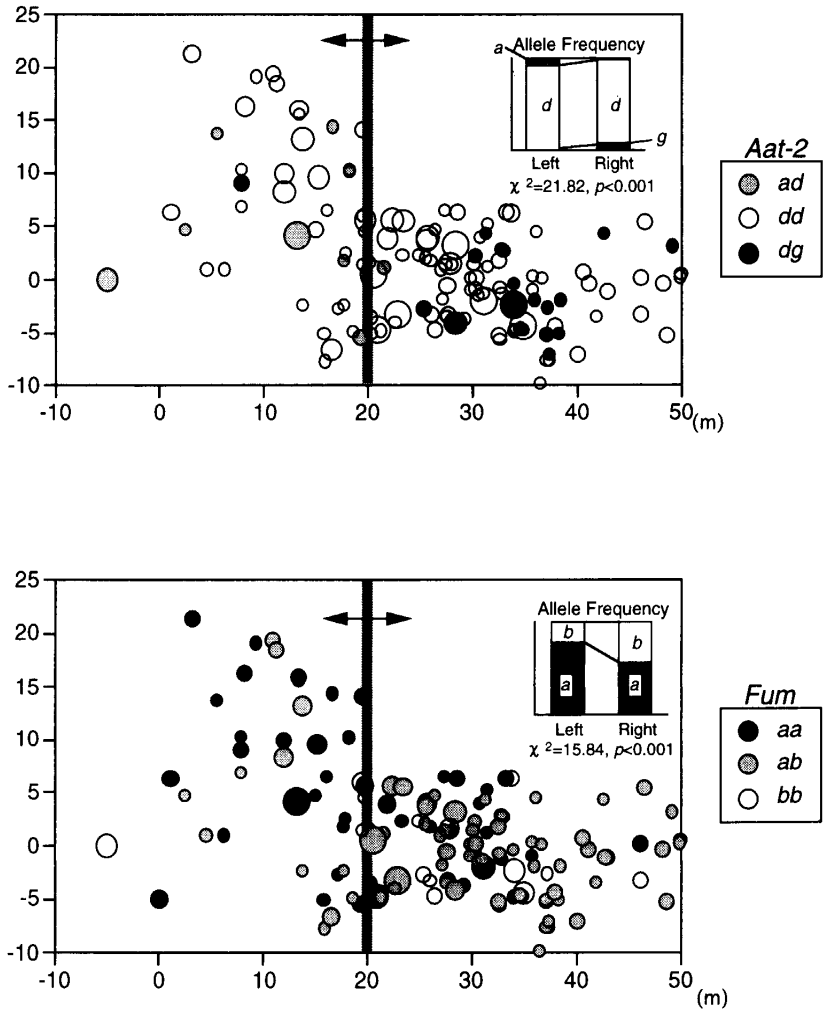

Fig. 7. Spatial distributions of genotypes at *Aat-2* and *Fum* in OJI population. These maps also show the simple genetic structures in small populations. Allele frequencies at these loci are sigtnificantly differed between two subplots.

Table 8. Results of *G*-test for allele frequency heterogeneity among size classes within populations.

Locus	Lowland population						Montane population
	UNA	FUT	OJI	KUR	ICH	SOY	GAP
Aap	–†	ns	–	ns	–	ns	$p<0.05$
Aat-1	–	–	–	–	–	–	ns
Aat-2	ns	ns	ns	ns	–	–	ns
Amy-1	ns	ns	ns	ns	$p<0.05$	ns	$p<0.01$
Dia	ns	ns	ns	ns	ns	ns	ns
Fum	ns	ns	ns	ns	ns	ns	$p<0.05$
Gdh	–	–	–	–	–	ns	ns
Idh	ns	ns	ns	ns	ns	ns	ns
Mdh	ns	ns	ns	–	ns	ns	ns
Pgi	ns	ns	ns	–	–	–	ns
Pgm-1	–	ns	$p<0.05$	ns	–	ns	ns
Pgm-2	–	–	–	–	–	–	ns
6Pgdh	–	ns	–	–	–	ns	ns

–† Locus is monomorphic; ns: not significantly different among size classes ($p>0.05$).

other allozyme loci also highly fluctuated, which obviously reflects the effect of genetic drift on the loss of alleles or changes of their frequencies in lowland populations. In contrast, the G_{ST} value for the lowland populations was evidently much higher (0.107) (Table 4). Non-overlapping of the 95% confidence interval of mean G_{ST} values obtained bootstrapping over loci between the lowland populations in Toyama and the other population groups, e.g., the montane populations in Tateyama as well as those in the Tohoku and Kanto districts (Ohkawa et al., unpubl. data, and see also Tomaru et al., 1996), indicate significant genetic differentiations among lowland populations (Fig. 5; Table 4). Estimates of gene flow among the lowland populations ranged from 0.8 to 5.6 (only *6Pgdh* locus showed extremely high values, 16.2), with a mean value of 3.8. These values were also much lower than those estimated for the populations collected over a wide geographical range ($Nm=6.3$; Tomaru et al., 1996).

4. *Extreme Cases of Isolated Populations*

Extreme cases of isolation were well demonstrated in lowland populations. The size-class distribution of UNA and SOY populations(Fig. 2) suggested a specific size-class structure mostly consisting of individuals of intermediate size-classes. Other lowland populations, with a good number of seedlings, also showed evidence of pooling in intermediate size-classes. These results of skewness in size-class structure were characteristic of small isolated populations.

The numbers of multilocus genotypes are fewer in UNA and SOY populations than in the other lowland populations (Table 9). The main reason for reduction in multilocus genotype is the small number of seedlings, which have the potential to introduce new genetic diversity into the local population.

The FUT population, however, maintains high genetic diversity relative to other lowland populations, despite the fact that small samples were examined (Fig. 3; Table 2). Almost all mature beech trees in this population were multi-stemmed, but they were scattered sparsely here and there, which suggests rather recent human disturbances of this mixed forest stand (Kamitani, 1986; Koop, 1987). A somewhat similar situation was observed in the SOY population, but this population maintains the natural conditions (Fig. 1; Table 2).

The spatial distributions of trees in UNA and FUT show characteristic patchiness (Fig. 8). The development of patchiness within a small isolated population would give rise to limited gene dispersal and high degrees of genetic substructurings. If genetic variation within population is highly structured, more genetic diversity could be lost during the habitat fragmentation (Hamrick and Nason, 1995). The genetic structures found even in mature stages in the lowland populations, therefore, would have directly reduced genetic diversity when fluctuation occurs in population size.

5. *Changes in Spatial Genetic Structures within Lowland Populations*

With respect to the spatial genetic structures in natural populations of the genus *Fagus*, weakly positive autocorrelation at informative shorter distance classes has been reported (Merzeau et al., 1994; Leonardi and Menozzi, 1996). Similar patterns were also reported for other long-lived tree species (Epperson and Allard, 1989; Knowles, 1991; Perry and Kowles, 1991; Young and Merriam, 1994; Bacilieri et al., 1994). The results of spatial autocorrelation analyses on the montane

Table 9. Numbers of trees for each multilocus genotype (Percentages of trees for each category are in parentheses).

Population	N	Numbers of trees for each multilocus genotype													
		1	2	3	4	5	6	7	8	9	10	11	12	18	24
FUT	97	70 (72.2)	3 (6.2)	3 (9.3)	–	1 (5.2)	–	1 (7.2)	–	–	–	–	–	–	–
UNA	33	19 (57.6)	7 (42.4)	–	–	–	–	–	–	–	–	–	–	–	–
ICH	294	32 (10.9)	15 (10.2)	9 (9.2)	8 (10.9)	3 (5.1)	3 (6.1)	5 (11.9)	3 (8.2)	1 (3.1)	2 (6.8)	2 (7.5)	1 (4.1)	1 (6.1)	–
OJI	139	80 (57.6)	12 (17.3)	4 (8.6)	3 (8.6)	1 (3.6)	1 (4.3)	–	–	–	–	–	–	–	–
KUR	107	66 (61.7)	11 (20.6)	5 (14.0)	1 (3.7)	–	–	–	–	–	–	–	–	–	–
SOY	69	49 (71.0)	7 (20.3)	2 (8.7)	–	–	–	–	–	–	–	–	–	–	–
GAP+T3	289	148 (51.2)	20 (13.8)	8 (8.3)	4 (5.5)	2 (3.5)	1 (2.1)	–	–	–	1 (3.5)	1 (3.8)	–	–	1 (8.3)

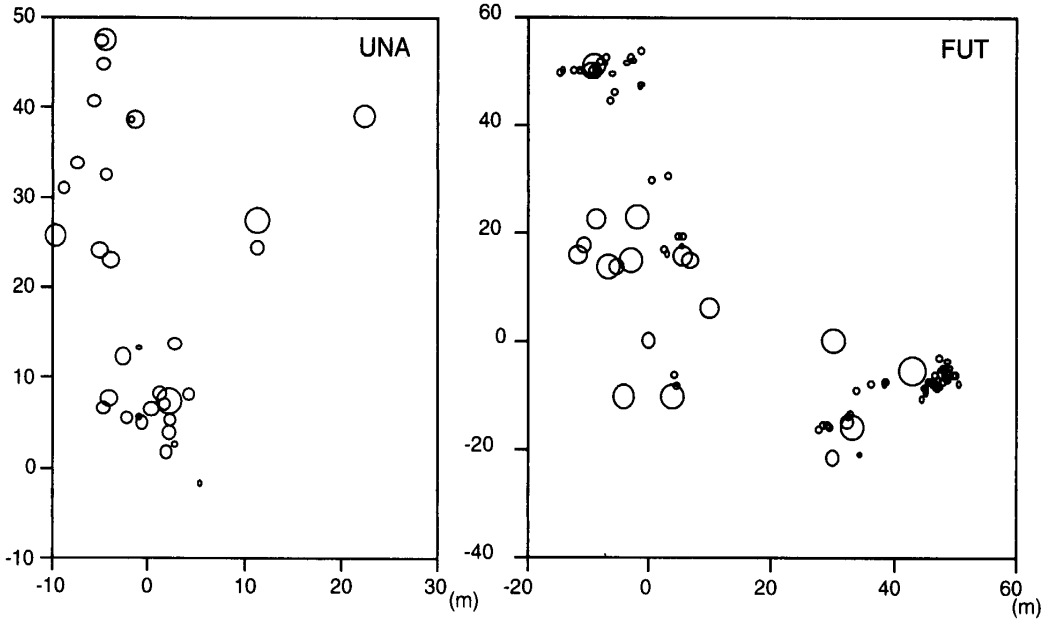

Fig. 8. Spatial distributions of individuals for two lowland populations. a) UNA and b) FUT.

beech populations in this study were also in agreement with the results of previous studies, less than 6% of alleles showing positive autocorrelations at the shortest distance class (Table 7). This result suggests virtually random distributions of alleles in the mature stages in large continuous populations, indicating that the effect of isolation by distance (Wright, 1943) is not significant on a large spatial scale in terms of mature tree distributions.

Additional evidence indicates that the temporal changes in genetic components of populations are related spatial elements. Understanding the mutual effect of spatial and temporal scales would be very instructive, especially in long-lived perennial plants (Young and Merriam, 1994). The changes in spatial genetic structures within a gap population (GAP) might depict the development of genetic substructures in the early growth stage and their disappearance in later growth stages. Genetic substructures developed in the juvenile stage with DBH$<$5 cm completely disappear in the next growth stage, probably reflecting stochastic survivorships of genotypes during the 'gap' regeneration processes (cf. Kitamura et al., 1997a). However, a few alleles that represent positive autocorrelation in mature stages in the montane population may exhibit the possibility of maintenance of genetic substructures through the regeneration processes or local adaptation.

More notable spatial genetic substructures were found in both seedling and mature stages in the lowland populations. In the seedling stage, most of the populations had a high proportion of positive autocorrelated alleles at the shortest distance class. If within-population seed dispersal is quite limited and density of mother trees is low, it should lead to clumping of related individuals (Young and Merriam, 1994). The clumping of related genotypes found in the seedling stage in lowland populations would have been forecast by the limited seed dispersal range (Maeda, 1988).

The genetic localization patterns found in lowland populations were maintained in the mature stages (Table 7). One possible reason for the differences in maintenance of genetic structure between the lowland and montane populations is that the size class discriminations were different in the montane and lowland populations. In the lowland populations, due to the small numbers of individuals within plots, trees with DBH$>$10 cm were simultaneously analyzed. This category, therefore, may include pairs of half-sib progenies and their mother trees, and these will produce clearer spatial genetic structures. The proportions of the positive autocorrelated alleles in mature stages were found to be higher in the lowland populations than in montane populations. This may be due to the limited number of reproductive individuals, which are genetically related to each other. This extreme case is the genetic structure in OJI population. Although no significant results were obtained in spatial autocorrelation analysis, more simple genetic structure was found developing in this population (Fig. 7).

6. *Temporal Genetic Heterogeneity*

Overlapping generations of long-lived tree species are predicted to show genetic heterogeneity among growth stages, and will slow down the rates of loss of genetic variation in fragmented populations by genetic drift (Hamrick and Nason, 1995). In *F. crenata*, significant differences of allele frequencies among size classes are reported (Kitamura et al., 1997b). Kitamura et al. (1997b) also indicated that heterogeneity of allele frequencies in the Ogawa population of this species was primarily owing to the role of juvenile stages as a genetic sink population. In the present study, hetero-

geneity in allele frequencies was also found in a regenerated gap population in the montane zone (Table 8). Therefore, genetic variabilities are maintained among growth stages, as indicated by a previous study (Kitamura et al., 1997b). In the lowland populations, on the other hand, genetic heterogeneity among growth stages was somewhat weak. Only two *G*-tests on 78 cases, including monomorphic loci, showed heterogeneity of allele frequencies among size classes. These allele frequency differences are mainly observed in the seedling stage; thus it is not the same situation as the juvenile sink populations found in the montane populations.

7. *Geo-historical Background of Population Differentiations of* Fagus crenata *Populations*

Based on the analyses of radiocarbon-dated fossil pollens collected from Miike at sea level in Ishikawa Prefecture, Tsukada (1982a, b) presumed that the last glacial refugia for *Fagus crenata* forests in the lowlands of this region disappeared until about 4000 yr. ago and that subsequently, evergreen broad-leaved tree species have gradually increased and taken the place of beech forests. Beech populations analyzed in the present study in Toyama Prefecture are all distributed on the foothills at low elevations, about 100–200 m (−500 m) above sea level, and include some evergreen oaks, such as *Quercus acuta* or *Q. salicina*, as co-dominant canopy tree species. The current beech forests in this region are higher than about 600 m above sea level; therefore, it is certain that these lowland populations were fragmented into small populations and have been isolated from large and continuous mainland populations, although present small population sizes may be mostly due to recent large-scale human exploitations. In these populations, significant genetic erosion was found because of severe bottleneck effects and/or genetic drift. Genetic analysis of these long-term isolated populations should be valuable and may be a first report on long lived-tree species.

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Appendix 1. Allele frequencies for established trees at 13 loci in six lowland beech populations.

Locus	Allele	Lowland population					
		UNA	FUT	OJI	KUR	ICH	SOY
Aap	*a*	–	0.024	–	0.163	–	0.082
	b	1.000	0.976	1.000	0.837	1.000	0.918
Aat-1	*d*	1.000	1.000	1.000	1.000	1.000	1.000
	f	–	–	–	–	–	–
Aat-2	*a*	–	–	0.028	–	–	–
	b	–	0.214	–	–	–	–
	d	0.946	0.786	0.935	0.986	1.000	1.000
	f	–	–	–	0.014	–	–
	g	0.054	–	0.037	–	–	–
Amy-1	*b*	–	0.095	0.074	–	–	0.010
	c	0.785	0.619	0.630	0.663	0.788	0.520
	e	0.054	0.071	–	0.130	–	0.020
	f	0.161	0.167	0.139	0.207	0.212	0.367
	g	–	0.048	0.157	–	–	0.082
Dia	*a*	–	–	–	–	–	–
	b	0.304	–	–	–	–	–
	d	0.678	0.905	0.972	0.989	0.808	0.959
	e	0.018	0.095	0.028	0.011	0.192	0.041
Fum	*a*	0.446	0.810	0.620	0.522	0.712	0.694
	b	0.554	0.190	0.380	0.478	0.288	0.306
Gdh	*a*	–	–	–	–	–	0.071
	b	1.000	1.000	1.000	1.000	1.000	0.929
Idh	*a*	0.071	0.524	0.713	0.598	0.288	0.745
	b	0.929	0.476	0.287	0.402	0.712	0.255
Mdh	*a*	0.161	0.119	0.120	–	0.212	0.296
	b	0.839	0.881	0.880	1.000	0.788	0.704
	c	–	–	–	–	–	–
Pgi	*b*	0.036	0.214	0.037	–	–	–
	d	0.964	0.762	0.963	1.000	1.000	1.000
	e	–	0.024	–	–	–	–
Pgm-1	*a*	–	–	–	–	–	–
	b	–	–	–	–	–	–
	c	1.000	0.953	0.667	0.598	1.000	0.867
	e	–	0.047	0.333	0.402	–	0.133
Pgm-2	*a*	–	–	–	–	–	–
	b	1.000	1.000	1.000	1.000	1.000	1.000
	c	–	–	–	–	–	–
6pgdh	*b*	1.000	0.976	1.000	1.000	1.000	0.969
	c	–	0.024	–	–	–	0.031

Appendix 2. Allele frequencies for mature trees at 13 loci in five montane beech populations.

Locus	Allele	Montane population				
		T1	T2	T3	T4	T5
Aap	*a*	0.076	0.098	0.109	0.045	0.033
	b	0.924	0.902	0.891	0.955	0.967
Aat-1	*b*	–	–	–	–	0.011
	d	1.000	0.980	0.969	0.993	0.989
	f	–	0.020	0.031	0.007	–
Aat-2	*a*	–	–	–	–	–
	b	–	–	–	–	–
	d	0.970	1.000	0.938	0.955	0.978
	f	0.015	–	0.047	0.037	–
	g	0.015	–	0.016	0.007	0.022
Amy-1	*b*	–	–	–	0.015	0.011
	c	0.591	0.637	0.688	0.612	0.722
	e	0.015	–	0.016	0.007	–
	f	0.311	0.255	0.234	0.306	0.233
	g	0.083	0.108	0.063	0.060	0.033
Dia	*a*	0.015	0.020	0.016	–	–
	b	–	–	–	–	0.022
	d	0.977	0.922	0.953	0.940	0.978
	e	0.008	0.059	0.031	0.060	–
Fum	*a*	0.727	0.775	0.719	0.687	0.689
	b	0.273	0.226	0.281	0.313	0.311
Gdh	*a*	–	–	–	–	–
	b	1.000	1.000	1.000	1.000	1.000
Idh	*a*	0.545	0.441	0.563	0.500	0.389
	b	0.455	0.559	0.438	0.500	0.611
Mdh	*a*	0.167	0.118	0.094	0.090	0.100
	b	0.833	0.882	0.891	0.910	0.889
	c	–	–	0.016	–	0.011
Pgi	*b*	0.045	0.010	0.047	0.030	0.011
	d	0.902	0.941	0.953	0.963	0.978
	e	0.053	0.049	–	0.007	0.011
Pgm-1	*a*	0.008	0.059	0.031	0.037	0.067
	b	–	–	0.031	–	–
	c	0.947	0.931	0.828	0.903	0.900
	e	0.045	0.010	0.109	0.060	0.033
Pgm-2	*a*	0.106	–	0.031	–	–
	b	0.886	0.990	0.969	1.000	1.000
	c	0.008	0.010	–	–	–
6Pgdh	*b*	1.000	0.971	0.969	0.985	1.000
	c	–	0.029	0.031	0.015	–

Appendix 3. Allele frequencies for seedling at 13 loci in six lowland and one montane beech populations.

Locus	Allele	Lowland population						Montane population
		UNA	FUT	OJI	KUR	ICH	SOY	GAP
Aap	*a*	–	0.053	–	0.221	–	0.100	0.031
	b	1.000	0.947	1.000	0.779	1.000	0.900	0.969
Aat-1	*d*	1.000	1.000	1.000	1.000	1.000	1.000	0.946
	f	–	–	–	–	–	–	0.054
Aat-2	*a*	–	–	0.035	–	–	–	–
	b	–	0.158	–	–	–	–	–
	d	1.000	0.842	0.888	1.000	1.000	1.000	0.965
	f	–	–	–	–	–	–	0.022
	g	–	–	0.076	–	–	–	0.013
Amy-1	*b*	–	0.086	0.059	–	–	–	0.004
	c	0.800	0.592	0.612	0.566	0.592	0.550	0.652
	e	–	0.072	–	0.238	–	0.025	0.036
	f	0.200	0.217	0.129	0.197	0.408	0.300	0.134
	g	–	0.033	0.200	–	–	0.125	0.174
Dia	*a*	–	–	–	–	–	–	0.006
	b	0.100	–	–	–	–	–	–
	d	0.900	0.908	1.000	0.984	0.762	0.950	0.908
	e	–	0.092	–	0.016	0.238	0.050	0.086
Fum	*a*	0.400	0.862	0.647	0.393	0.781	0.675	0.848
	b	0.600	0.138	0.353	0.607	0.219	0.325	0.152
Gdh	*a*	–	–	–	–	–	0.075	–
	b	1.000	1.000	1.000	1.000	1.000	0.925	1.000
Idh	*a*	0.400	0.684	0.612	0.639	0.361	0.775	0.509
	b	0.600	0.316	0.388	0.361	0.639	0.225	0.491
Mdh	*a*	0.200	0.072	0.118	1.000	0.191	0.300	0.080
	b	0.800	0.928	0.882	–	0.809	0.700	0.911
	c	–	–	–	–	–	–	0.009
Pgi	*b*	0.200	0.151	0.012	–	–	–	0.049
	d	0.800	0.842	0.988	1.000	1.000	1.000	0.947
	e	–	0.007	–	–	–	–	0.004
Pgm-1	*a*	–	–	–	–	–	–	0.013
	b	–	–	–	–	–	–	0.022
	c	1.000	0.954	0.518	0.574	1.000	0.875	0.925
	e	–	0.046	0.482	0.426	–	0.125	0.040
Pgm-2	*a*	–	–	–	–	–	–	0.009
	b	1.000	1.000	1.000	1.000	1.000	1.000	0.991
	c	–	–	–	–	–	–	–
6Pgdh	*b*	1.000	0.974	1.000	1.000	1.000	1.000	0.986
	c	–	0.026	–	–	–	–	0.014
LAP	*a*	–	–	–	–			
	b	–	0.048	0.343	0.900			
	c	1.000	0.238	0.019	0.100			
	d	–	0.619	0.259	–			
	e	–	0.095	0.324	–			
	f	–	–	0.056	–			