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Genetic diversity in *Fagus crenata* (Japanese beech): influence of the distributional shift during the late-Quaternary

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Genetic diversity at 11 loci encoding nine enzymes was studied in 23 populations of Japanese beech *Fagus crenata* Blume distributed throughout the range of the species. Levels of genetic diversity were high for both within species (expected mean heterozygosity: 0.194) and within populations (expected mean heterozygosity: 0.187), whereas the level of genetic diversity among populations was low ($G_{ST} = 0.038$), as observed in various long-lived, woody plants. Despite the low differentiation among populations, geographical patterning of the variation was observed. Populations in south-western Japan tended to have greater within-population variation and to be more highly differentiated when compared with those in north-eastern Japan. In addition, allele frequencies observed at eight loci were significantly related to latitudinal and/or longitudinal gradients and showed clinal variation across the range of the species. Principal components analysis revealed that the populations tended to cluster according to their geographical locations. The nonrandom patterns of variation were probably shaped by relatively recent historical events such as late-Quaternary migration and founding events.

Keywords: allozymes, *Fagus crenata*, founding events, genetic variation, population differentiation, population expansion.

Introduction

Many allozyme studies examining genetic diversity of plant species have shown that various species maintain high levels of genetic diversity within natural populations. Among plant species, long-lived, woody species maintain higher levels of variation both within species and within populations and, further, show less genetic differentiation among populations compared with other plant species. In general, this tendency is related with longevity, large contiguous populations, allogamy and widely dispersed pollen and seed, as these life history and ecological traits tend to maintain high intrapopulation variation and prevent interpopulation differentiation (Brown, 1979; Hamrick *et al.*, 1979; Gottlieb, 1981; Hamrick & Godt, 1989; Hamrick *et al.*, 1992). Certainly, several life history and ecological traits of species are significantly related to the level and distribution of genetic diversity in plant species. However, these traits only account for less than one-half of the heterogeneity observed in genetic diversity among species (Hamrick & Godt, 1989; Hamrick *et al.*, 1992). Hamrick *et al.* (1992) noted that the remaining heterogeneity was probably explained by the evolutionary history of each species.

The Japanese Archipelago extends lengthwise from north-east to south-west with various mountain ranges running parallel to it. In general, past major climatic changes forced temperate plant species to migrate along either the Pacific Ocean side, the Japan Sea side or along the mountain slopes; i.e. during glacial periods they migrated either southwards or to lower altitudes into refugia, and during interglacial periods they expanded either northwards

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or to higher altitudes (Tsukada, 1980). Therefore, knowledge of the evolutionary history of temperate plant species, including shifts in distribution, fragmentation and population isolation, especially after the last glacial maximum, would help interpret the current genetic diversity.

Japanese beech forests, common in the cool temperate zone, are widely distributed from the Kuromatsunai lowland in Hokkaido to Mt. Takakuma in Kyushu (Horikawa, 1972; The Environment Agency, 1988; Fig. 1). The present centre of the distribution is in north-eastern Honshu on the Japan Sea side where beech forests remain in relatively large areas despite being fragmented in montane areas (mostly 200–1400 m elevation) because of post-World War II exploitation. On the other hand, the distribution from central Honshu on the Pacific Ocean side to south-western Japan is scattered and isolated in small montane areas mostly higher than 1000 m in elevation.

Relatively extensive data of radiocarbon-dated pollen analyses have been reported for *Fagus* spp. (mainly *F. crenata*, but including *F. japonica*) and Tsukada (1982a,b) reviewed them for an understanding of the late-Quaternary vegetation history. Takahashi *et al.* (1994) analysed allozyme variation in *F. crenata* populations in north-eastern Japan. However, there is no comprehensive allozyme study using natural populations sampled across the entire natural range of the species. In this report, we illustrate the genetic variation and population differentiation of *F. crenata* across its entire distribution, and elucidate the role of the late-Quaternary distributional shift in shaping the geographical variation revealed by allozyme markers.

Materials and methods

Plant materials

In this study we sampled 23 populations covering the naturally distributed areas of *F. crenata* (Fig. 1 and Table 3). To assess genetic diversity in the entire species distribution, the populations used in this study also included the 11 populations which were used by Takahashi *et al.* (1994) to estimate allozyme diversity of *F. crenata* forests in north-eastern Japan.

Winter buds were collected from 624 trees in the remaining 12 south-western populations during October and March; 20–30 winter buds were collected from each individual. To avoid sampling half-sib individuals sampled trees were separated by approximately 50 m and no attention was paid to the age or size of trees. Buds were stored at −80 °C prior to isozyme analysis.

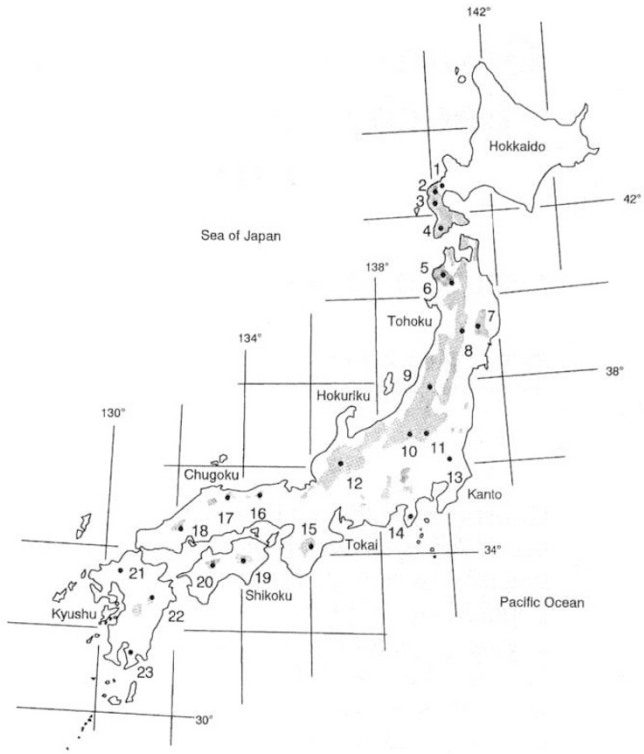

Fig. 1 Present distribution of *Fagus crenata* forests. Dots identify sample population locations; numbers correspond to population numbers in Table 3.

Enzyme electrophoresis

Enzyme extraction, electrophoresis and staining methods were similar to those described by Tsumura & Ohba (1993). Fifty milligrams of winter bud tissue was ground to a fine powder with a mortar and pestle using liquid nitrogen. Immediately after grinding, 50 mg of polyvinylpolypyrrolidone and 1 mL of extraction buffer (93 mM Tris-HCl (pH 7.5), 23.4 per cent glycerol, 0.6 per cent Tween 80, 12.0 mM DTT, 2.8 mM EDTA, 0.6 mM NAD, 0.5 mM NADP, 0.5 per cent 2-mercaptoethanol, 0.08 per cent BSA) were added. Individual homogenates were centrifuged at 30 000 *g* and 0°C for 40 min. Ten microlitres of supernatant per lane was used in polyacrylamide vertical slab gel electrophoresis (Davis, 1964; Ornstein, 1964) for 150 min at 4°C and 12.3 mA cm^{-2}. A running gel (7.5 per cent) and a spacer gel (3.75 per cent) were used. All samples were analysed for nine enzyme systems and scored for a total of 11 loci as follows: alcohol dehydrogenase (ADH, EC 1.1.1.1), *Adh-3*; malate dehydrogenase (MDH, EC 1.1.1.37), *Mdh-2* and *Mdh-3*; 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), *6Pg-2*; diaphorase (DIA, EC 1.8.1.4), *Dia*;

glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1), *Got*; amylase (AMY, EC 3.2.1), *Amy-3*; alanine aminopeptidase (AAP, EC 3.4.11.1), *Aap-1* and *Aap-2*; fumarase (FM, EC 4.2.1.2), *Fm*; and phosphoglucoisomerase (PGI, EC 5.3.1.9), *Pgi-1*. Genetic control of the expression of the various isozymes were inferred by Takahashi *et al.* (1994).

Statistical analysis

To estimate within-population variation, five parameters were estimated from allele frequencies: the proportion of polymorphic loci (P_l) at the 95 per cent level; the average number of alleles per locus (N_a); the effective number of alleles per locus (N_e; Kimura & Crow, 1964); the expected and observed average heterozygosities (H_e and H_o), where H_e was an unbiased estimate (Nei, 1978; Nei & Roychoudhury, 1974). The fixation index (F_{IS}) for polymorphic loci in each population and its averages across both populations and loci were determined (Wright, 1965; Nei, 1977). Deviations from Hardy–Weinberg equilibra were tested using χ^2 (Li & Horvitz, 1953).

Gene diversity statistics (Nei, 1973; Nei & Chesser, 1983) were estimated as follows: $H_T = H_S + D_{ST}$ and $G_{ST} = D_{ST}/H_T$, where H_T is the gene diversity in the total population, and H_S and D_{ST} are the average gene diversities within and between populations, respectively. G_{ST} is the relative extent of gene differentiation among populations. As suggested by Nei (1973), statistical averages were obtained over all loci to clarify a general mode of differentiation among populations. An average was also obtained for polymorphic loci only to compare with those of other species mentioned by Hamrick & Godt (1989) and Hamrick *et al.* (1992). The statistical significance of the deviation of G_{ST} from zero was tested using χ^2 (Workman & Niswander, 1970).

An additional measure of population differentiation was D_j (Gregorius & Roberds, 1986), defined as the genetic distance between the jth population and the remaining populations as follows:

$$D_j = \frac{1}{2}\sum_i |p_i(j) - \bar{p}_i(j)|,$$

where $p_i(j)$ and $\bar{p}_i(j)$ are the frequencies of the ith allele in the jth population and in the remaining populations, respectively. This measure indicates the proportion of alleles by which one population differs from all the other populations. If the jth population is identical to, or completely different from, the remaining populations, the values of D_j are 1 or 0, respectively.

Nei's unbiased genetic distances were calculated for all population pairs (Nei, 1972; Nei & Roychoudhury, 1974). To estimate the amount of gene flow among populations, the number of migrants exchanged per generation, Nm, was calculated indirectly from G_{ST} values at each locus and from the average values over all loci by applying Wright's (1931) infinite island formula: $Nm = (1-G_{ST})/4G_{ST}$, where N is the effective population size and m is the proportion of migrants exchanged per generation.

Significance tests in parametric linear regression (t-test) and in nonparametric regression (ordering test; Quenouille, 1952) were performed for the regression coefficients of the parameters (allele frequencies, P_l, N_e, H_e and D_j) against latitude and longitude of the 23 populations to identify relationships between genetic variation in populations and the latitudinal or longitudinal gradients. Furthermore, genetic relationships among populations were explored by principal components analysis based on a variance–covariance matrix of angular-transformed frequencies for all alleles in the 23 populations (SAS Institute, 1985).

Results

Number of alleles at each locus and patterns of allele distribution

A total of 44 alleles, at 11 loci, across 23 populations were detected. The number of alleles detected at each locus ranged from two at *Mdh-2* to seven at *Pgi-1*, the average being four. The allele frequencies for each population appeared relatively homogeneous; the most common alleles at each locus were identical over all populations. However, the differences among populations emerged as different frequencies of shared alleles and the presence and absence of rare alleles. Despite most of the frequencies fluctuating greatly along the geographical transect, 19 alleles (43 per cent) were found to be significantly related to latitudinal and/or longitudinal gradients throughout the species range (Table 1). Many rare alleles were detected in widely scattered localities. For the *Pgi-1* locus, however, allele *f* was detected only in south-western populations located in the Kanto, Tokai, Chugoku, Shikoku and Kyushu districts, and alleles *e* and *g* in more south-western populations located in the Chugoku, Shikoku and Kyushu districts.

Agreement with Hardy–Weinberg expectations

For estimating F_{IS}, 149 genotype distributions for each polymorphic locus in each population were

Table 1 Significance tests for regression coefficients of allele frequencies against latitude and longitude for 23 populations of *Fagus crenata*

			Latitude			Longitude		
			Parametric test†		Nonparametric test‡	Parametric test†		Nonparametric test‡
Locus	Allele	Range of the frequency	r^2	P	P	r^2	P	P
Adh-3	*a*	0.793–0.990	0.282	<0.01	<0.01	0.226	<0.05	<0.05
Mdh-3	*a*	0.000–0.214	0.633	<0.001	<0.001	0.689	<0.001	<0.001
	b	0.625–0.915	0.292	<0.01	<0.005	0.537	<0.001	<0.01
	c	0.027–0.235	0.202	<0.05	0.057	0.084	0.180	0.090
Dia	*a*	0.000–0.128	0.258	<0.05	<0.05	0.289	<0.01	<0.01
	b	0.856–1.000	0.333	<0.005	<0.05	0.364	<0.005	<0.005
Got	*a*	0.000–0.106	0.229	<0.05	<0.01	0.424	<0.001	<0.005
Amy-3	*b*	0.110–0.500	0.779	<0.001	<0.001	0.546	<0.001	<0.005
	e	0.440–0.860	0.706	<0.001	<0.001	0.557	<0.001	<0.001
Aap-1	*b*	0.830–1.000	0.161	0.058	<0.05	0.219	<0.05	<0.05
	c	0.000–0.152	0.104	0.133	0.131	0.201	<0.05	0.106
Fm	*a*	0.130–0.590	0.171	0.050	0.107	0.309	<0.01	<0.05
	b	0.410–0.870	0.171	0.050	0.107	0.309	<0.01	<0.05
Pgi-1	*b*	0.000–0.117	0.394	<0.005	<0.005	0.608	<0.001	<0.005
	c	0.680–0.968	0.498	<0.001	<0.005	0.460	<0.001	<0.05
	d	0.000–0.075	0.510	<0.001	<0.001	0.564	<0.001	<0.001
	e	0.000–0.080	0.367	<0.005	<0.001	0.508	<0.001	<0.001
	f	0.000–0.170	0.215	<0.05	<0.005	0.060	0.262	<0.01
	g	0.000–0.066	0.304	<0.01	<0.05	0.419	<0.001	<0.05

†*t*-test.
‡The ordering test (Quenouille, 1952).

used and compared with the expected genotype distributions under Hardy–Weinberg equilibrium (Table 2). For loci *Adh-3*, *Got* and *Aap-2*, F_{IS} was estimated in 18, 11 and four populations, respectively, of which 16 (89 per cent), five (45 per cent) and three (75 per cent) populations, respectively, had significantly positive values. At least one null allele was detected at the three loci each by no staining intensity on the same gel. In such cases, as heterozygotes with a null allele cannot be distinguished electrophoretically from homozygotes, F_{IS} values will be biased towards a positive deviation. Thus, deviation at these loci should be attributed to the null allele's occurrence, rather than to other evolutionary causes.

For loci *6Pg-2* and *Fm*, one of two populations and five of 23 populations, respectively, do not meet the expectation and they contributed significantly to mean positive F_{IS} values at both loci, indicating a significant excess of homozygotes compared with average expectations. Mean values at the remaining polymorphic loci *Mdh-3*, *Dia*, *Amy-3*, *Aap-1* and *Pgi-1*

Table 2 Fixation indices (F_{IS}) at polymorphic loci in 23 populations of *Fagus crenata* †

	No. of populations		
Locus	Estimated F_{IS}‡	Deviated from the expectations*	Mean F_{IS}
Adh-3	18	16	0.395***
Mdh-2	0	0	—
Mdh-3	23	3	0.003 NS
6Pg-2	2	1	0.179*
Dia	12	1	0.049 NS
Got	11	5	0.353***
Amy-3	23	1	0.001 NS
Aap-1	16	1	0.045 NS
Aap-2	4	3	0.411***
Fm	23	5	0.127***
Pgi-1	17	0	−0.064 NS

†Levels of significance: NS, not significant; *$P<0.05$; ***$P<0.001$.
‡F_{IS} for each locus of each population was estimated in the populations in which the loci were polymorphic.

Table 3 Proportions of polymorphic loci (P_l; 95 per cent criterion), average numbers of alleles per locus (N_a), effective numbers of alleles per locus (N_e), average observed heterozygosities (H_o) and fixation indices (F_{IS}) in 23 populations of *Fagus crenata*†

Population	Locality	Altitude (m)	N‡	P_l	N_a	N_e	H_o	F_{IS}§
1	Kuromatsunai, Hokkaido	40–90	80	54.5	2.18 (0.23)	1.28 (0.10)	0.157 (0.049)	0.166*
2	Mts. Kariba, Hokkaido	550–790	91	63.6	2.18 (0.30)	1.32 (0.10)	0.163 (0.052)	0.059
3	Mt. Urappudake, Hokkaido	190–350	62	54.5	2.55 (0.28)	1.30 (0.12)	0.191 (0.075)	−0.207
4	Mt. Sengendake, Hokkaido	50–650	111	54.5	2.36 (0.31)	1.25 (0.10)	0.141 (0.052)	0.049
5	Mts. Shirakami, Aomori	400–750	143	45.5	2.55 (0.31)	1.22 (0.09)	0.132 (0.041)	0.101
6	Mts. Shirakami, Akita	550–860	119	63.6	3.09 (0.44)	1.26 (0.10)	0.143 (0.048)	0.096
7	Mt. Hayachine, Iwate	660–1250	60	63.6	2.36 (0.34)	1.33 (0.13)	0.188 (0.067)	−0.100
8	Mt. Mahirudake, Iwate	460–910	105	54.5	2.73 (0.43)	1.27 (0.11)	0.161 (0.055)	−0.010
9	Mts. Iide, Yamagata	400–640	63	54.5	2.91 (0.37)	1.30 (0.11)	0.191 (0.061)	−0.094
10	Mt. Naeba, Niigata	780–1140	70	54.5	2.82 (0.30)	1.29 (0.10)	0.183 (0.058)	−0.039
11	Oze bog, Gumma	1200–1730	112	63.6	3.18 (0.40)	1.30 (0.09)	0.163 (0.048)	0.090
12	Mt. Hakusan, Gifu	800–1100	51	72.7	2.64 (0.34)	1.27 (0.11)	0.171 (0.053)	0.126*
13	Mt. Tsukuba, Ibaraki	800–876	48	54.5	2.36 (0.30)	1.24 (0.07)	0.135 (0.043)	0.034
14	Mt. Amagi, Shizuoka	1000–1100	53	45.5	2.82 (0.41)	1.23 (0.07)	0.150 (0.041)	−0.033
15	Mt. Ohdaigahara, Nara	1300–1600	57	45.5	2.55 (0.37)	1.20 (0.07)	0.130 (0.045)	0.085
16	Mt. Ohginosen, Tottori	1100–1300	50	72.7	2.55 (0.25)	1.35 (0.09)	0.194 (0.052)	0.011
17	Mt. Daisen, Tottori	900–1300	59	45.5	2.55 (0.25)	1.24 (0.08)	0.145 (0.043)	0.098
18	Mt. Jyakuchi, Yamaguchi	1100–1300	50	63.6	3.00 (0.36)	1.31 (0.08)	0.189 (0.043)	0.124
19	Mt. Tsurugi, Tokushima	600–700	50	72.7	2.73 (0.41)	1.30 (0.10)	0.183 (0.058)	−0.017
20	Mt. Ishizuchi, Ehime	1600–1900	52	63.6	2.73 (0.30)	1.32 (0.10)	0.176 (0.051)	0.193**
21	Mt. Seburi, Fukuoka and Saga	900–1055	49	72.7	3.00 (0.43)	1.41 (0.12)	0.225 (0.061)	0.028
22	Mt. Sobo, Miyazaki	1500–1757	55	54.5	2.64 (0.43)	1.33 (0.11)	0.199 (0.057)	0.003
23	Mt. Takakuma, Kagoshima	1000–1230	50	63.6	2.64 (0.39)	1.35 (0.12)	0.195 (0.063)	0.011
Mean			71.3	58.9	2.66 (0.35)	1.29 (0.10)	0.170 (0.053)	0.034

†Standard errors are in parentheses.
‡Sample size.
§F_{IS} values of each population were estimated from average F_{IS} values at loci apart from *Adh-3*, *Got* and *Aap-2*; levels of significance: $^*P<0.05$; $^{**}P<0.01$.

were not significant. The F_{IS} values were also averaged across loci in each population excluding those at *Adh-3*, *Got* and *Aap-2* because these loci had null alleles (Table 3). Population numbers 1, 12 and 20 had significantly positive values. However, neither the values in the remaining 20 populations nor the average value across all 23 populations (0.034) were significant. Therefore, as a whole and with only slight exceptions, these results suggest that *F. crenata* populations obey the Hardy–Weinberg Law.

Genetic variation within populations

Parameters of genetic variability within populations for the 23 populations are presented in Table 3 and Fig. 2. Averaged across all populations, the proportion of polymorphic loci (P_l) was 58.9. The average and effective numbers of alleles per locus (N_a and N_e) were 2.66 and 1.29, respectively. The average observed heterozygosity (H_o) was 0.170. For average expected heterozygosity (H_e), the average value and standard deviation across all populations was 0.187 ± 0.025. Figure 2 shows that the H_e (black dots) of populations tended to decrease with increasing latitude and longitude. In the regression tests, a significant relationship was found between H_e and longitude in the parametric test ($r^2 = 0.324$, $P<0.005$) and in the nonparametric test ($P<0.05$). No significant relationship between H_e and latitude was observed, although if the H_e value (0.140) of population number 15 (the lowest value among populations) was excluded from the test, a significant relationship emerged in the parametric test ($r^2 = 0.240$, $P<0.05$) and in the nonparametric test ($P<0.05$). N_e was also significantly related to longitudinal gradient in the parametric test ($r^2 = 0.272$, $P<0.05$) and in the nonparametric test ($P<0.05$). Therefore, populations in south-western Japan tend to show greater within-population variation than those in north-eastern Japan. No such relationships

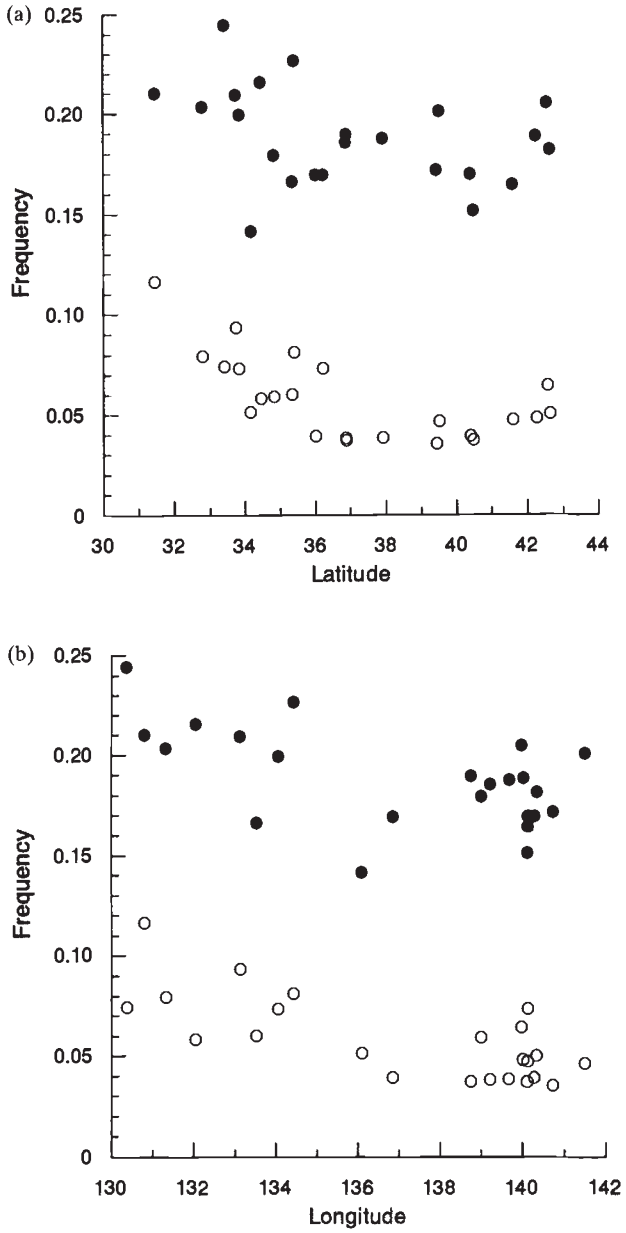

Fig. 2 Relationships of average expected heterozygosities (H_e; black dots) and the proportions of alleles by which one population differs from all the other populations (D_j; white dots) against latitude (a) and longitude (b) for 23 populations of *Fagus crenata*.

were found for combinations of P_1 and either latitude or longitude, or for N_e and latitude.

Population differentiation

Averaging values of D_j across all populations showed that the proportion of alleles by which a population differed from all the other populations was 0.057 ± 0.021 (SD). When D_j values in each population were plotted against latitude and longitude (Fig. 2, white dots) D_j tended to decrease with increasing latitude in the parametric test ($r^2 = 0.403$, $P < 0.005$) and in the nonparametric test ($P < 0.005$). Furthermore, D_j was also significantly related to latitudinal gradient in the parametric test ($r^2 = 0.529$, $P < 0.001$) and in the nonparametric test ($P < 0.005$).

All G_{ST} values for each locus were low (Table 4). However, apart from *6Pg-2*, the values of G_{ST} were statistically significant ($P < 0.01$ or < 0.001), indicating that significant heterogeneities of allele frequencies existed among populations at those loci. Both the mean G_{ST} values for all loci and only for polymorphic loci excluding *Mdh-2* were 0.038. Thus, only 4 per cent of the total variation resulted from differences between populations. The mean value of G_{ST} at polymorphic loci was also statistically significant ($P < 0.001$; Table 4). The number of migrants exchanged per generation among the 23 populations, *Nm*, ranged from 3.8 to 49.8 (mean 6.3; Table 4). As predicted by the small G_{ST} values, unbiased genetic distances between populations were small, ranging from 0.000 to 0.036 (mean 0.009).

Patterns of population relationship

Genetic relationships among populations were visualized by plotting the populations' first two principal components in two-dimensional space (Fig. 3). Strong regressions were revealed for population position on the first principal component against both latitude and longitude ($r^2 = 0.790$, $P < 0.001$; $r^2 = 0.823$, $P < 0.001$). In a further multiple regression analysis with the first principal component against latitude and longitude, 88.2 per cent of the variance of the first principal component was explained by latitude and longitude, i.e. by geographical locations of populations.

Discussion

Level of within-population and within-species genetic variation and extent of population differentiation

As summarized by Hamrick & Godt (1989) and Hamrick *et al.* (1992), *F. crenata* was found to maintain high genetic diversity comparable to other woody plants. When compared with other long-lived woody plants, the level of genetic variation within *F. crenata* populations was greater. The average values of parameters describing within-population variation

Table 4 Gene diversity statistics and the number of migrants exchanged per generation (*Nm*) for 11 loci in *Fagus crenata*†

Locus	H_T	H_S	D_{ST}	G_{ST}‡	*Nm*
Adh-3	0.195	0.191	0.004	0.018***	13.6
Mdh-2	0.002	0.002	0.000	0.019	12.9
Mdh-3	0.353	0.341	0.012	0.034***	7.1
6Pg-2	0.033	0.032	0.000	0.005 NS	49.8
Dia	0.114	0.113	0.001	0.012***	20.6
Got	0.118	0.115	0.004	0.030***	8.1
Amy-3	0.478	0.458	0.020	0.042***	5.7
Aap-1	0.135	0.133	0.002	0.013**	19.0
Aap-2	0.054	0.051	0.003	0.051***	4.7
Fm	0.435	0.408	0.027	0.061***	3.8
Pgi-1	0.217	0.208	0.009	0.043***	5.6
Mean					
Polymorphic loci	0.213	0.205	0.008	0.038***	
	(0.050)§	(0.047)	(0.003)	(0.006)	
All loci	0.194	0.187	0.007	0.038	6.3
	(0.049)	(0.046)	(0.003)	(0.006)	

†Gene diversity statistics: H_T is the gene diversity in the total populations; H_S and D_{ST} are the average gene diversities within and between populations, respectively; G_{ST} is the relative extent of gene differentiation among populations.
‡Levels of significance: NS, not significant; $^{**}P<0.01$; $^{***}P<0.001$. The test for *Mdh-2* could not be carried out because it was monomorphic.
§Standard errors are in parentheses.

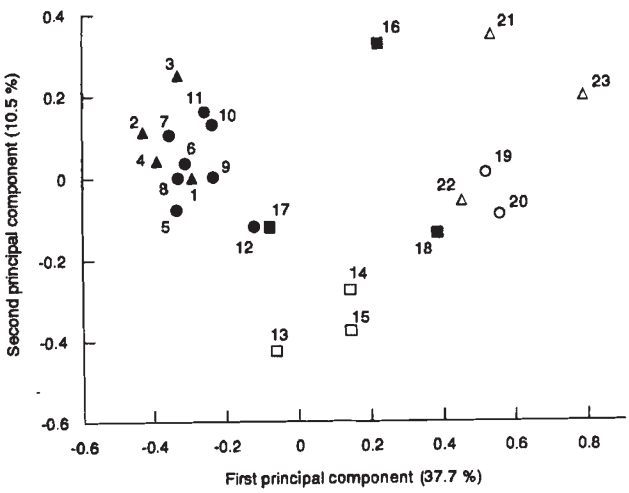

Fig. 3 Scatter diagram resulting from principal components analysis of angular-transformed allele frequencies from 23 populations of *Fagus crenata*. The two principal components explain 37.7 per cent and 10.5 per cent, respectively. Populations are given different symbols showing the locations in five geographical districts: ▲, Hokkaido; ●, Tohoku and Hokuriku; ■, Chugoku; □, Kanto and Tokai; ○, Shikoku; and △, Kyushu. Numbers refer to population numbers in Table 3.

for long-lived woody plants were: $P_l = 49.3$ per cent; $N_a = 1.76$; $N_e = 1.20$ and $H_e = 0.148$. These were lower than for *F. crenata* where $P_l = 58.9$ per cent; $N_a = 2.66$; $N_e = 1.29$ and $H_e = 0.187$. By contrast, genetic differentiation among populations of *F. crenata* was less than one-half the average of long-lived woody plants, as shown by G_{ST} values for polymorphic loci, 0.038 vs. 0.084, respectively. For total variation within species as indicated by H_{es} (Hamrick & Godt, 1989), equivalent to H_T over all loci in this study, *F. crenata* exhibited variation roughly similar to the average of long-lived woody plants (0.194 vs. 0.177).

In long-lived woody species, geographical range is the best indicator of genetic diversity (Hamrick *et al.*, 1992). In Japan, *F. crenata* forests are distributed within a regional range (Horikawa, 1972). Comparing the genetic diversity in *F. crenata* with that in long-lived woody plants also having regional ranges ($P_l = 69.2$ per cent; $N_a = 2.31$; $N_e = 1.26$; $H_e = 0.194$; $G_{ST} = 0.065$ and $H_{es} = 0.169$), genetic diversity in *F. crenata* was found to be generally similar for within-population and within-species variation but lower for among-population variation.

Additional characteristics influencing genetic diversity in plant species are the breeding systems and seed dispersal methods (Hamrick & Godt, 1989; Hamrick *et al.*, 1992). It is known that *F. crenata* outcrosses through wind-pollination. Beech nuts are dispersed by gravity and then scattered and hoarded by animals such as wood mice (*Apodemus speciosus* and *A. argenteus*; Miguchi, 1994) and Japanese nutcrackers (*Nucifraga caryocatactes japonicus*) and jays (*Garrulus gladnarius pallidifrons*; Watanabe, 1990). However, the extent of population differentiation in *F. crenata* is inconsistent with, and lower than, a typical wind-pollinated, outcrossing species (average $G_{ST} = 0.077$) or a typical species whose seed are dispersed by gravity (average $G_{ST} = 0.131$). Hamrick *et al.* (1992), however, did not summarize data for species with seed dispersal by caching. Although there are no data of *F. crenata* seed dispersal by birds, Johnson & Adkisson (1985) described seed dispersal of *F. grandifolia* by blue jays (*Cyanocitta cristata*). They found that about 75 blue jays transported and cached approximately 100 000 beech nuts over distances from a few tens of metres up to 4 km from a woodlot during September. In contrast, the dispersal distances of beech nuts by wood mice were relatively short, within 5 m for *F. crenata* (Miguchi, 1994) and 1–13 m for *F. sylvatica* (Jensen, 1985). As both pollen dispersal (Levin & Kerster, 1974) and seed dispersal by rodents is often effective only over short distances, long-range seed dispersal by birds may be an important source of gene flow between scattered populations (Vander Wall, 1990). Therefore, the high rate of gene flow generated especially by bird dispersal of beech nuts has possibly contributed to the low differentiation in *F. crenata*. It is more likely, however, that the recent evolutionary history of *F. crenata* has contributed to this low differentiation.

The average number of migrants exchanged per generation (Nm) was estimated to be 6.3. This parameter reflects historical gene flow rates that would vary from generation to generation. Although the estimates should be treated with caution (Slatkin & Barton, 1989), when considering many other species, this estimate of Nm is rather high (Govindaraju, 1988). Theoretical studies have shown that a relatively low gene flow, $Nm > 1$, is sufficient to hamper population differentiation caused by genetic drift at neutral loci (Wright, 1931; Maruyama, 1970; Levin & Kerster, 1974; Slatkin & Maruyama, 1975). Therefore, for *F. crenata* with a relatively large population size, the high rate of gene flow within and between populations may have prevented the loss of variation and differentiation between populations.

Geographical patterns of genetic diversity

Genetic diversity in *Fagus crenata* mostly corresponds to the generalization of genetic diversity in long-lived woody plants (Hamrick & Godt, 1989; Hamrick *et al.*, 1992), i.e. long-lived woody plants have relatively high genetic diversity within species, but most of the genetic diversity is within populations with little existing among populations. There are, however, remarkable characteristics in the genetic diversity of *F. crenata*, i.e. although there was low differentiation among populations, geographical patterning of the variation was clearly observed. First, the within-population variation tend to be lower in the more north-eastern populations, as indicated by H_e and N_e values, despite the south-western Japan populations being small and isolated, whereas those in north-eastern Japan are large and widespread. This fact is inconsistent with the general tendency for more widespread populations to retain greater genetic diversity than more geographically restricted populations.

Secondly, population differentiation appears to have proceeded less in north-eastern Japan, as indicated by D_j values. This fact was further confirmed by gene diversity analysis (Nei, 1973). Estimates of G_{ST} values were made for each of the eight south-western populations from the Chugoku, Shikoku and Kyushu districts and for the 12 north-eastern populations from the Hokkaido, Tohoku and Hokuriku districts. Population differentiation (mean $G_{ST} = 0.007 \pm 0.002$) in the north-eastern populations was less than one-quarter of that in the south-western populations (0.031 ± 0.013).

Thirdly, allele frequencies observed at eight loci showed significant clinal variation across the range of the species. Furthermore, this tendency was emphasized by the principal components analysis because the synthesized principal components resulted from the accumulated differences of frequencies for many alleles, most of which were the same alleles exhibiting the clinal variation. Indeed, the populations in the analysis tended to cluster according to their geographical locations. Noticeably, a high proportion (88.2 per cent) of the variance of the first principal component was explained by the geographical locations of the populations.

These characteristics are probably accounted for by the recent migration history of *F. crenata*. According to palynological studies (Tsukada, 1982a,b), multiple refugia were sparsely located only along coastal belts south of approximately 38°N latitude during the full-glacial period (25 000–15 000 years BP). Approximately 12 000 years ago, *Fagus* forests

began to expand rapidly northwards and to higher altitudes from the coastal refugia. It is particularly worthwhile to consider the following three distributional shifts of *Fagus* forests: (i) in south-western Japan, *Fagus* populations moved to higher altitudes and were isolated by about 7000 years ago; (ii) in central and north-eastern Japan, *Fagus* populations were fully established in their present distribution by about 7000 years ago; (iii) the new *Fagus* populations in north-eastern Japan must have been founded by migrants from the northern populations of its glacial refugia (approximately 38°N latitude). Because Japanese *Fagus* comprises two species, *F. crenata* and *F. japonica*, Tsukada's hypothesis included both species. Nevertheless, the inference about the distribution shift on the Japan Sea side would be for *F. crenata* as *F. japonica* is generally restricted to the Pacific side (Horikawa, 1972).

The north-eastern populations of *F. crenata* obviously originated from the peripheral refugia north of the last glacial distribution. These peripheral refugia possibly maintained a lower diversity than the multiple refugia located on the more south-western coasts. In present populations of various tree species, the tendency has been found for marginal populations to maintain lower diversity than in central populations (Guries & Ledig, 1982; Michaud *et al.*, 1995). The distributional expansion allowed by climatic amelioration after the glacial maximum was probably accomplished by repeated founding events, presumably through dispersal by birds (Vander Wall, 1990). Consequently the variation in new populations founded with small population size may have further decreased because of genetic drift during the succeeding postglacial migration. When population size is reduced, the genetic variation is expected to decline because of genetic drift. The rate of decline depends on the effective population size (Wright, 1931; Nei *et al.*, 1975). Such a distribution in the last glaciation and the following migration events could explain the result that variation within *F. crenata* populations declined in more north-eastern populations. In a study of *Pinus contorta*, the results of allozyme analysis clearly suggest that stochastic genetic drift in long-distance founding events during its postglacial spread resulted in the reduced allelic diversity in the populations founded toward the northern distribution limit (Wheeler & Guries, 1982a,b; Cwynar & MacDonald, 1987). Similar results have been reported for *Picea abies* where central European populations have consistently low genetic variability, most probably as an effect of severe population size reductions during the last glaciation (Lagercrantz & Ryman, 1990).

Fagus crenata populations in south-western Japan, when compared with geographically restricted populations of long-lived woody plants, revealed relatively high within-population variation despite the small population size in the mountains. Therefore, even now, variation in the south-western populations may reflect the high variation presumably maintained in the multiple refugia or the descendent populations (Hiebert & Hamrick, 1983; Niebling & Conkle, 1990), i.e. because of genetic drift, the present populations may not have lost completely the variation within the ancestral populations.

If the process of population expansion in north-eastern Japan had really happened, population differentiation may have proceeded between the newly established populations. Nevertheless, very limited differentiation was found among north-eastern Japan's present populations, indicating that gene flow may have been so high that the population differentiation was prevented because of the relatively continuous range of *F. crenata* in north-eastern Japan. This has probably contributed to the low between-population differentiation of *F. crenata* at the species level. Alternatively, for populations in south-western Japan, isolated at least since early in the postglacial period, the interpopulational differentiation has been somewhat greater. Comps *et al.* (1990) also reported that genetic differentiation in *Fagus sylvatica* was greater in the Mediterranean region compared with that in the Continental region because the populations in the Mediterranean region had been spread out from several sources isolated from each other during glaciations.

Clinal variation of allele frequencies along latitude and/or longitude has been reported for several tree species (e.g. *F. sylvatica* (Leonardi & Menozzi, 1995); *Picea abies* (Lagercrantz & Ryman, 1990); *Quercus petraea* (Zanetto & Kremer, 1995)). These authors have generally proposed that geographical variation patterns in allele frequencies resulted from postglacial migration and founding events. The geographical clines of *F. crenata* allele frequencies probably also reflect the distribution of refugia in the last glacial maximum, subsequent migration and founding events. Clines in allele frequencies can be produced both by unidirectional and successive founding events through migration and by fusion through migration from two refugia that have become differentiated during separation. The adaptive significance of allozymes along environmental gradients cannot be completely rejected, because even if the studied allozymes are neutral or nearly neutral, natural selection can indirectly affect allozymes if they are linked to genes on which selection

is acting. Nevertheless, the effect of late-Quaternary evolutionary events on *F. crenata* may provide the most reasonable explanation for the geographical pattern of allozymes found in this study.

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