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# Genetic Structure in the Northernmost Marginal Population of Japanese Beech (*Fagus crenata* BLUME): Influence of the Founding Event on Genetic Structure

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**Abstract**

We examined the genetic variability and structure within the northernmost marginal population of Japanese beech (*Fagus crenata* BLUME), located in the Kuromatsunai lowland (Utasai), Hokkaido Island, Japan, with special regard to the influence of the founding process on its genetic structure. From palynological records, it appears that the population was established between 350 and 680 years ago. We investigated 119 trees, using 11 isozyme loci, which encode eight enzyme systems. The proportion of polymorphic loci, average number of alleles per locus, effective number of alleles per locus, expected heterozygosity and observed heterozygosity in the population were 0.64, 2.5, 1.30, 0.182 and 0.178, respectively. The average $F_{IS}$ value was 0.046. Significant linkage disequilibrium was found for two pairs of loci ($P < 0.05$), probably related to founder effects during and after establishment of the population. Genetic structure in the population was examined by Moran's *I*, standard normal deviate (SND), and the number of alleles in common (NAC). The genetic clustering in the population was weaker and less clear than in previously studied populations. The genetic structure commonly seen in populations of this species has probably not yet emerged here because of the small number of generations since it was founded.

*Key words: Fagus crenata,* founder effect, Japanese beech, genetic variation, linkage disequilibrium, marginal population, spatial autocorrelation, within-population genetic structure

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## Introduction

Abundance and distribution patterns of genetic variation within and among plant populations are functions of genetic processes such as mating system, gene flow, random genetic drift, mutation and selection (LOVELESS and HAMRICK, 1984; HAMRICK *et al.,* 1992). Similarly, spatial genetic structure within plant populations is a function of the same genetic processes (SOKAL *et al.,* 1997; SOKAL and JACQUEZ, 1991). Thus, spatial autocorrelation analysis (SOKAL and ODEN, 1978a,b) can be valuable for both examining the genetic structure of a population and identifying the main genetic processes responsible for the structure. The genetic structure of a wide range of forest tree populations has been studied (BERG and HAMRICK, 1995; EPPERSON and ALLARD, 1989; EPPERSON and ALVAREZ-BUYLLA, 1997; KNOWLES *et al.,* 1992; YOUNG and MERRIAM, 1994). Early studies on conifers found very little clustering of genetic variation, and even when detected, the degree of clustering found was slight (EPPERSON and ALLARD, 1989; KNOWLES, 1990; XIE and KNOWLES, 1991). In contrast, genetic clustering was frequently found in species in which seed dispersal was limited, such as *Camellia japonica* (UENO *et al.,* 2000), *Quercus* spp. (BACILIERI *et al.,* 1994; BERG and HAMRICK, 1995; GEBUREK and KNOWLES, 1994; UBUKATA *et al.,* 1999) and *Fagus crenata* (KAWANO and KITAMURA, 1997; OHKAWA *et al.,* 1998; TAKAHASHI *et al.,* 2000). Thus, within-population genetic structure is obviously influenced by the mechanism of seed dispersal (HAMRICK *et al.,* 1993).

Founding events of populations have major effects on within-population genetic variation and structure. The effect generally reduces genetic variation, with respect to the source population, although clinal distributions of genetic variation along geographic ranges can arise from a series of successive founding events (LEDIG, 2000; MIYAMOTO *et al.,* 2001; SUYAMA *et al.,* 1997; TOMARU *et al.,* 1997). Several studies have indicated that within-population genetic structure can also be influenced by re-founding processes after forest cutting (DAYANANDAN *et al.,* 1999; KNOWLES *et al.,* 1992; TAKAHASHI *et al.,* 2000).

Beech has been well studied with respect to genetic structure within populations (KAWANO and KITAMURA, 1997; KITAMURA *et al.,* 1997a, b, 1998; LEONARDI and MENOZZI, 1996; OHKAWA *et al.,* 1998; TAKAHASHI *et al.,* 2000) as well as phylogeography (DEMESURE *et al.,* 1996; MERZEAU *et al.,* 1994; OHKAWA *et al.,* 1998; TOMARU *et al.,* 1997, 1998). Phylogeographic studies of *F. crenata* populations covering its whole geographic range have found little differentiation ($G_{ST}$ = 0.038) in the isozymes, but clear differentiation ($G_{ST}$ = 0.963) has been detected in maternally inherited mitochondrial DNA. These results suggest that a much higher rate of gene flow is associated with pollen flow than with seed dispersal (TOMARU *et al.,* 1998). Similar results have also been obtained in *F. sylvatica* (COMPS *et al.,* 1990; DEMESURE *et al.,* 1996). Thus, most of the nuclear genetic variation in *Fagus* has been retained within populations rather than among populations. Studies of the intra-population genetic structure have revealed that genetic clustering in *F. crenata* populations is probably due to limited seed dispersal (TAKAHASHI *et al.,* 2000).

The distribution shift of *F. crenata* after the last glacial period is relatively well known through studies of palynological records (IGARASHI, 1990, 1994; IGARASHI and YASUMURA, 1989; SAKAGUCHI, 1989; TAKIYA and HAGIWARA, 1997; TSUKADA, 1982,1983). The *Fagus* forests were restricted to areas more southerly than 37–38° N, and were abundant in the coastal regions of southwestern Japan during the peak of the last glacial period (ca. 20,000 yr. B.P.; TSUKADA, 1982, 1983). At about 12,000 yr. B.P., *Fagus* began to extend its distribution

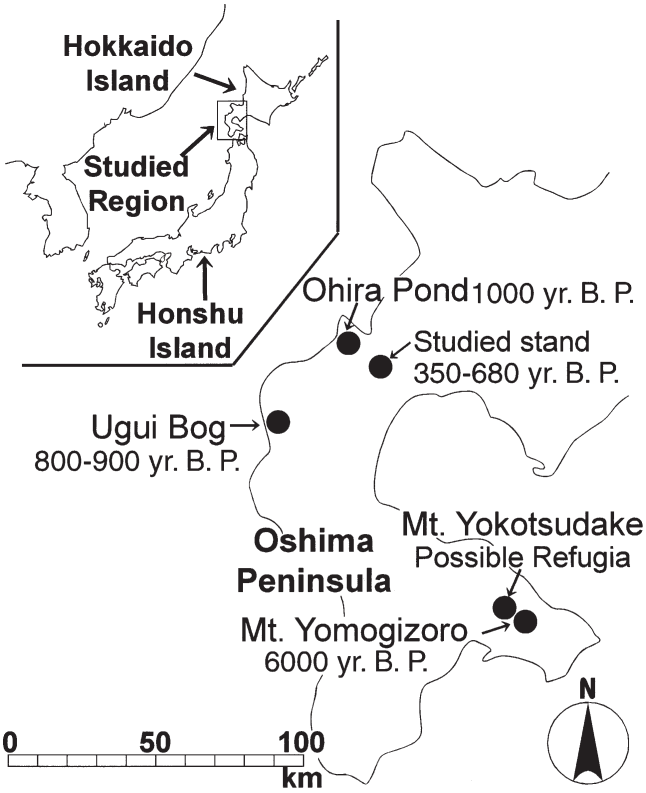


*Figure 1.* – Location of the studied *Fagus crenata* stand, at Utasai, and the historical periods when *F. crenata* forest was most likely to have been established at five locations on Oshima Peninsula, Hokkaido Island, according to palynological analysis by IGARASHI (1994) and TAKIYA and HAGIWARA (1997).

both northwards and inland to higher elevations, arriving at the northern end of Honshu Island in ca. 9,000 yr. B.P. (TSUKADA, 1982). According to these studies, the forest subsequently reached the southern end of Oshima Peninsula, Hokkaido Island in ca. 6,000 yr. B.P., and its current northern limits between 350 and 680 yr. B.P. (*Fig. 1*; IGARASHI and YASUMURA, 1989; SAKAGUCHI, 1989). However, another possibility, proposed by TAKIYA and HAGIWARA (1997), that a *F. crenata* refugium was present during the last glacial period at Mt. Yokotsudake in the south of Oshima Peninsula, southwestern Hokkaido, and this was the origin of the current northernmost population. However, both hypotheses suggest that the current marginal population, i.e. the Utasai population, was established by immigrant nuts from populations located further south somewhere between 350 and 680 yr. B.P. This implies that the Utasai population has undergone only a few generations since it was founded. Simulation studies by SOKAL and WARTENBERG (1983) and EPPERSON (1990) indicate that a considerable number of generation times (30–50 generations) are needed to reach quasi-stationary status. Therefore, the Utasai population may exhibit little or no within-population genetic structure because of the very few generation cycles that have passed since it was founded.

Here we present a study of genetic variation and structure in the most northerly, marginal population of *F. crenata* and discuss the relationship between its within-population genetic structure and founding process. The population is especially suitable for such analysis because of its well-recorded forest history. Acquiring greater knowledge of how genetic structure changes with time is important, since it will increase our overall understanding of the genetic structure of forest tree species.

## Materials and Methods

*The studied population, sampling and isozyme analysis*

We studied a beech (*Fagus crenata* BLUME) population located in the Kuromatsunai lowland on Oshima Peninsula, Hokkaido Island, Japan (80 m; 42°38'56"N, 140°20'0"E; designated Utasai or UT). This represents the northernmost habitat of the species. A 100 m x 130 m survey plot was delineated, and all 119 trees in the plot taller than 3 m were mapped. The height and diameter at breast height (DBH) of the 119 trees were measured. Height ranged from 3–30 m (mean and standard deviation; 20.9 ± 7.6 m), while the DBH ranged from 4–98 cm (mean and standard deviation; 44.8 ± 23.4 cm). A histogram of DBH is presented in *Fig. 2*. The tree density and basal area covered by *F. crenata* in this population were 91.5 trees/ha and 18.4 $m^2$/ha, respectively. Winter buds were collected from all the mapped trees, and used for the analysis. The following 11 loci, encoding eight enzyme systems, were analyzed: *Mdh-2* and *Mdh-3* (E.C.1.1.1.37), *6Pg-2* (E.C.1.1.1.44), *Dia-1* and *Dia-2* (E.C.1.8.1.4), *Got* (E.C.2.6.1.1), *Amy-3* (E.C.3.2.1.1), *Aap-1* and *Aap-2* (E.C.3.4.11.2), *Fm* (E.C.4.2.1.2) and *Pgi-1* (E.C.5.3.1.9). Details of the procedures for isozyme analysis have been described previously (TOMARU *et al.,* 1997; TSUMURA *et al.,* 1990)

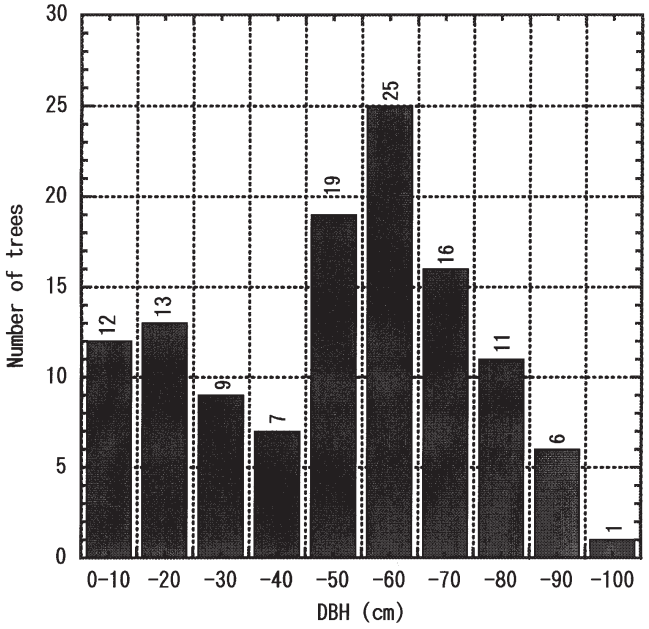


*Figure 2.* – Distribution of diameter at breast height (DBH) in the Utasai population of *Fagus crenata*.

*Data analysis*

Polymorphic loci were defined as loci where the frequency of the most prevalent allele was less than 0.95. The proportion of polymorphic loci (*Pl*), the average number of alleles per locus (*Na*), the effective number of alleles per locus (*Ne*; KIMURA and CROW, 1964), the average observed heterozygosity ($H_o$) and the expected heterozygosity ($H_e$; NEI, 1987) were calculated and used to determine the degree of genetic variability.

The inbreeding coefficient ($F_{IS}$) was calculated as the average of $f_{is}$ weighted by the sample sizes for each locus, where $f_{is} = 1\text{-}h_o/h_e$; $h_e$ being the expected heterozygosity; and $h_o$ the observed heterozygosity of the locus. Only variable loci with two or more alleles were included in the calculation of $F_{IS}$. The significance of the $f_{is}$ values was tested according LI and HORVITZ (1953). Deviation in the genotype frequencies from Hardy-Weinberg expectations was examined at each of the variable loci, and the possibility of linkage disequilibrium between all pairs of the variable loci was examined. Both tests were performed using the chi-square test of the GDA version 1.0 program (LEWIS and ZAYKIN, 1999). Alleles with frequencies less than 0.05 were pooled together as one allele before the two tests.

We examined the genetic structure of the population at three levels at the allelic level using Moran's *I* correlograms, at the genotypic level using standard normal deviate (SND; SOKAL and ODEN, 1978a) analysis and at the multi-locus level by calculating the number of alleles in common (NAC; BERG and HAMRICK, 1995; SURLE *et al.,* 1990). The values, their expectations and variances were calculated over 12 distance classes of 5 m intervals (0–5 m, 5–10 m and so on). For Moran's *I* analysis, we used only the most frequent allele at each polymorphic loci. An average correlogram of *I*, which was simply the arithmetic mean of the individual correlograms, was produced. The variances of average Moran's *I* values were calculated using a bootstrap procedure (sampling alleles with replacement one thousand times). For SND determinations, genotypes whose frequencies lay outside the range 0.05 to 0.95, and whose expected numbers of joins were less than unity, were excluded from the calculation. Joins between individuals having identical genotypes were referred to as like joins, and joins between individuals having different genotypes were considered unlike joins. Eleven like joins and 15 unlike joins satisfied our criteria, and were used for the calculation of SND. The overall significance of the individual correlogram of *I* and SND correlograms was assessed using the Bonferroni procedure. A grand mean of NAC was also calculated by averaging all possible pairwise NAC values without respect to distance. The grand mean represents the null hypothesis of spatial randomness of alleles. To assess the significance of NAC excesses and deficits, we calculated the variances of NAC values in each distance class using a bootstrap procedure (joins belonging to each class were sampled up to the original join number in the respective class, with replacement one thousand times).

To evaluate the characteristics of genetic variability and within-population genetic structure at the Utasai population we compared the results with two previously studied beech populations, that is, an old-growth beech population at Mt. Kurikoma (KU) and a secondary beech population at Mt. Akita-komagatake (AK), which was subjected to forest cutting during the 1920s (TAKAHASHI *et al.,* 2000). Statistical differences of polymorphic indices among the three populations were tested using pairwise *t*-tests.

## Results

*Genetic variability and linkage disequilibrium*

In total, 27 alleles were detected among 11 loci in 119 mapped *Fagus crenata* trees of the Utasai population (*Table 1*). The average values and standard errors of the *Pl*, *Na*, *Ne*, $H_e$, and $H_o$ parameters describing the genetic variability at the 11 loci in the population were 0.64, 2.5 ± 0.3, 1.30 ± 0.11, 0.182 ± 0.056 and 0.178 ± 0.057, respectively (*Table 2*). The $f_{is}$ values ranged from –0.072 to 0.248. Most of the $f_{is}$ values were not statistically significant although the $f_{is}$ at the *Dia-1* locus was significant ($P < 0.01$), indicating an excess of homozygotes at the locus. The $F_{IS}$ value for the Utasai population was 0.046 ± 0.031 (mean and standard error; not statistically significant). No heterogeneity in genotype frequencies was observed at the nine variable loci. Linkage disequilibrium was examined at all possible locus pairs among nine variable loci (36 pairs), and

*Table 1.* – Detected alleles and their frequencies at the 11 loci examined in the Utasai *Fagus crenata* stand.

| | Locus | Allele | | | | |
|---|---|---|---|---|---|---|
| | | *a* | *b* | *c* | *d* | *e* |
| 1 | *Mdh-2* | 1.000 | | | | |
| 2 | *Mdh-3* | | 0.725 | 0.275 | | |
| 3 | *6Pg-2* | | 0.004 | 0.996 | | |
| 4 | *Dia-1* | 0.055 | 0.941 | 0.004 | | |
| 5 | *Dia-2* | 0.046 | 0.924 | 0.030 | | |
| 6 | *Got* | 0.008 | 0.013 | 0.958 | 0.021 | |
| 7 | *Amy-3* | | 0.378 | 0.013 | | 0.609 |
| 8 | *Aap-1* | 0.029 | 0.920 | 0.051 | | |
| 9 | *Aap-2* | | 1.000 | | | |
| 10 | *Fm* | 0.391 | 0.609 | | | |
| 11 | *Pgi-1* | | 0.013 | 0.924 | 0.063 | |

*Table 2.* – Parameters of genetic variability and inbreeding coefficients at the 11 loci examined in the Utasai *Fagus crenata* stand.

| No. | Locus | *Pl* | *Na* | *Ne* | $H_e$ | $H_o$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| 1 | *Mdh-2* | - | 1 | 1.00 | 0.000 | 0.000 | - |
| 2 | *Mdh-3* | poly † | 2 | 1.66 | 0.401 | 0.347 | 0.134 |
| 3 | *6Pg-2* | - | 2 | 1.01 | 0.008 | 0.008 | 0.000 |
| 4 | *Dia-1* | poly | 3 | 1.13 | 0.112 | 0.084 | 0.248** |
| 5 | *Dia-2* | poly | 3 | 1.17 | 0.143 | 0.126 | 0.120 |
| 6 | *Got* | - | 4 | 1.09 | 0.082 | 0.084 | -0.026 |
| 7 | *Amy-3* | poly | 3 | 1.94 | 0.488 | 0.504 | -0.034 |
| 8 | *Aap-1* | poly | 3 | 1.18 | 0.151 | 0.151 | -0.005 |
| 9 | *Aap-2* | - | 1 | 1.00 | 0.000 | 0.000 | - |
| 10 | *Fm* | poly | 2 | 1.91 | 0.478 | 0.513 | -0.072 |
| 11 | *Pgi-1* | poly | 3 | 1.16 | 0.142 | 0.134 | 0.053 |
| | Average | 0.64 | 2.5 | 1.30 | 0.182 | 0.178 | 0.046 |
| | | | (0.3) | (0.11) | (0.056) | (0.057) | (0.031) |

Abbreviations: *Pl*, the proportion of polymorphic loci (0.95 criterion); *Na*, the average number of alleles per locus; *Ne*, the effective number of alleles per locus; $H_e$, the expected heterozygosity; $H_o$, the observed heterozygosity and $F_{IS}$, the inbreeding coefficient. Figures in parentheses show standard errors.
** $P<0.01$
†The 'poly' means that the locus was polymorphic.

was found to be significant ($P < 0.05$) for two locus pairs (*Mhd-3* & *Amy-3* and *Mdh*-3 & *Fm*).

*Genetic structure*

As mentioned in *Materials and Methods*, we used only the most frequent allele at each polymorphic locus for calculating Moran's *I* values. The following seven alleles were used for the calculation: *Mdh-3*$^b$, *Dia-1*$^b$, *Dia-2*$^b$, *Amy-3*$^e$, *Aap-1*$^b$, *Fm*$^b$, and *Pgi-1*$^c$. The average Moran's *I* value for the first distance class was 0.143 (not significant) and the value decreased as distance increased, indicating that genetic similarity decreases as distance increases (*Fig. 3*). The correlograms of *Mdh-3*$^b$ and *Dia-2*$^b$ showed positively significant *I*s in the shortest distance class, although the two correlograms were not significant with respect to the Bonferroni criteria. The proportion of positively significant *I*s in the shortest distance class was 0.29. The number of significant alleles in the 12 distance classes is presented in *Fig. 4*. No negatively significant *I*s were detected at distance classes less than 25 m. None of the seven individual correlograms showed an *I* value that differed significantly from that of the average correlogram, suggesting no selection was operating on the seven studied loci. Of the 11 like joins considered, only the *b*/*c* genotype at the *Mdh-3* (SND = 2.735) was positively significant ($P < 0.01$) in the first distance class. Therefore, the proportion of positively significant like joins was 0.09 in the first distance class. Among the 15 unlike joins, two pairs were significantly negative in the first distance class (SND (*Mdh-3; b*/*b* – *c*/*c*) = 2.434; SND (*Fm; a*/*b* – *b*/*b*) = –2.212). Thus, the proportion of negatively significant unlike joins was 0.13 in the first distance class. Negatively significant unlike joins are

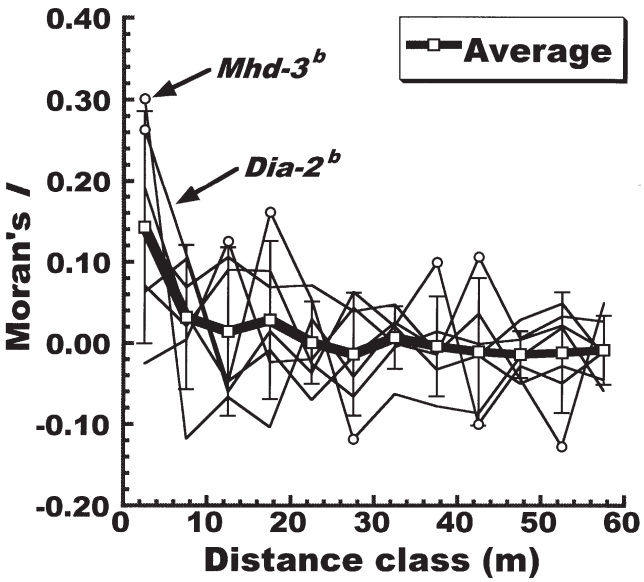


*Figure 3.* – Individual and average Moran's *I* correlograms of the Utasai *Fagus crenata* stand, Oshima Peninsula, Hokkaido Island.
Average values are shown by the bold line Error bars denote standard deviations. Open circles denote significant values at the 5 % probability level. Alleles with positively significant values in the first distance class are shown by name. Variances of average correlogram were estimated using the bootstrap procedure.

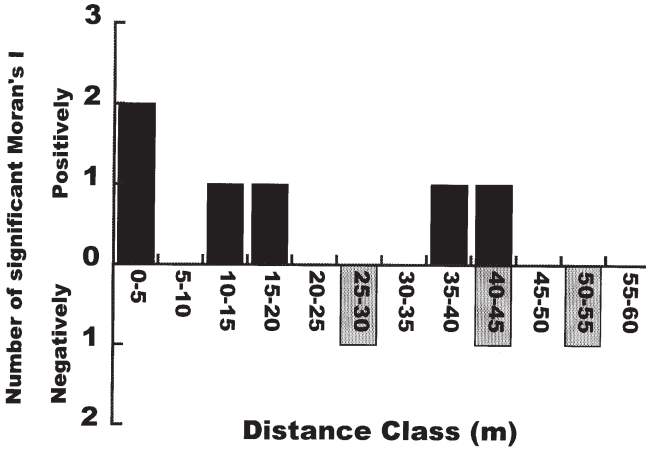


*Figure 4.* – Numbers of positively and negatively significant Moran's *I* values in the Utasai *Fagus crenata* stand, Oshima Peninsula, Hokkaido Island.

thought to reflect the cumulative effects of the positive association of like joins. None of the positively and negatively significant correlograms were significant according to the Bonferroni criteria. The grand mean and standard deviation of the NAC was 1.629 ± 0.002. The correlogram of the NAC values also showed a decrease in genetic similarity with increasing distance, although no NAC significantly differed from the grand mean NAC value (*Fig. 5*). Thus, this multilocus analysis indicated that there was no significant genetic clustering in the plot.

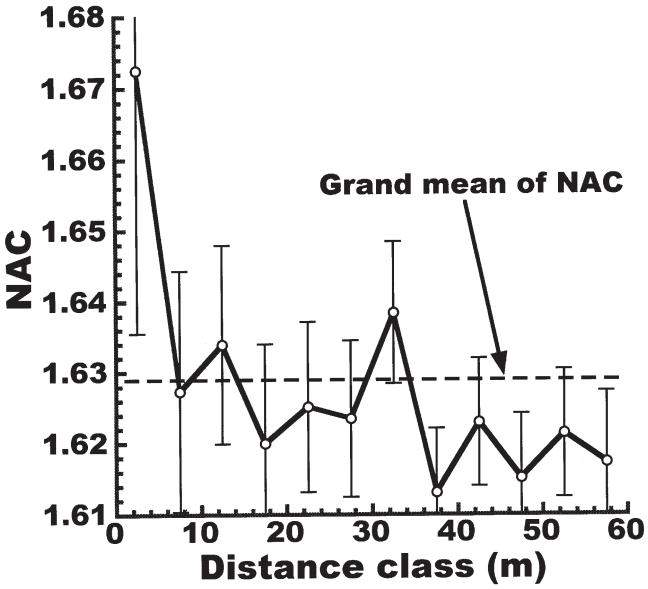


*Figure 5.* – NAC values over 12 distance classes in the Utasai *Fagus crenata* stand, Oshima Peninsula, Hokkaido Island.
The grand NAC mean is shown by the dashed-line. No NAC values significantly differed from the grand mean at the 5% probability level. Variances of the NAC values were estimated using the bootstrap procedure. Error bars show standard deviations.

## Discussion

### *Genetic variability and linkage disequilibrium*

We compared the genetic variability of UT with that of the KU population, an old-growth population, using information on isozyme frequencies at nine common loci (excluding the *Mdh*-2 and *Aap-2* data; *Table 3*). Most parameters were similar between the two populations, suggesting that UT had similar genetic variability to the KU population. However, the number of alleles at the *Amy-3* was only three in the UT, while the corresponding value was seven in KU. The *Na* for UT was slightly (but not significant) lower than that for KU, perhaps due to the putative founder effects that influenced the Utasai population. This tendency is consistent with results from a previous study by TAKAHASHI *et al.* (1994), who found that rare alleles present at low frequencies in populations on Honshu Island were absent in populations on Hokkaido Island. The cited authors suggested that this tendency could be due to founder effects that occurred during the colonization process as *F. crenata* extended its range northwards after the last glacial period. Similar reductions in the number of alleles in shifts from putative refugia to current marginal regions, presumed to be due to a series of founding events, have been found in *Pinus coulteri* (LEDIG, 2000) and *Alnus trabeculosa* (MIYAMOTO *et al.,* 2001).

Linkage disequilibrium was found at two pairs of loci in UT, but none were observed in the old-growth population, KU. The proportion of significant locus pairs in UT (0.056) was quite close to what may be expected by chance alone. Thus, the linkage disequilibrium in UT would be regarded as being very weak. Nine old-growth populations sampled throughout the northern Honshu Island did not show any significant linkage disequilibrium in the two locus pairs (*Mhd-3 & Amy-3* and *Mdh-3 & Fm*), suggesting those three loci would not actually be linked (unpublished data). Founder effects can cause not only reductions in the number of alleles but also linkage disequilibrium (HARTL and CLARK, 1997), so the disequilibrium observed in the Utasai population could be related to events in the founding process of the population. Once linkage disequilibrium has been established, several generations are needed to dissipate it, even if the loci are not actually linked on a chromosome (HARTL and CLARK, 1997). Since the Utasai population appears to have been founded some time around 350–680 yr. B. P., the population may not yet have had enough time to dissipate linkage disequilibrium generated during its foundation, but it would be expected to diminish in future generations.

*Table 3.* – Comparison of genetic variability and inbreeding coefficients at nine loci common to two *Fagus crenata* stands.

| Stand | *Pl* | *Na* | *Ne* | $H_e$ | $H_o$ | $F_{IS}$ |
|---|---|---|---|---|---|---|
| Utasai | 0.78 | 2.8 | 1.36 | 0.223 | 0.217 | 0.063 |
| (UT) | | (0.2) | (0.12) | (0.060) | (0.063) | (0.039) |
| Mt. Kurikoma | 0.78 | 3.3 | 1.33 | 0.203 | 0.193 | 0.042 |
| (KU) | | (0.5) | (0.13) | (0.057) | (0.052) | (0.031) |

Averages are shown with standard errors in parentheses. Abbreviations for three stands are also shown in parentheses. For abbreviations for genetic indices, see *Table 2*.

### *Genetic structure*

The Moran's *I* and NAC correlograms showed that values of these coefficients decreased as the distance increased. Two allelic and three genotypic (one like join and two unlike joins) correlograms were significant in the first distance class. These results suggest that genetic clustering was present in the Utasai population. However, none of the correlograms for UT was significant according to Bonferroni criteria, although the proportion of significant correlograms based on these criteria was 0.43 in KU (TAKAHASHI *et al.,* 2000). In UT, the proportions of positively significant *I,* positively significant like joins, and negatively significant unlike joins in the first distance class were 0.29, 0.09, and 0.13, respectively. The corresponding proportions in KU were 0.43 (when the first two distance classes were pooled), 0.29 and 0.09 (TAKAHASHI *et al.,* 2000). Thus, the genetic structure in UT is weaker and less clear than in KU. These findings can be explained by conclusions derived from simulation studies (SOKAL and WARTENBERG, 1983; EPPERSON, 1990). SOKAL and WARTENBERG (1983) examined temporal aspect of genetic structure in a series of Monte-Carlo simulations, and found that correlograms became significant within the first five generations of foundation, even when starting from a random distribution of genetic variation (SOKAL and WARTENBERG, 1983). However, the *I* values in the first distance class and patch sizes, as defined by the X-intercepts of the correlograms, continued to increase thereafter. The simulations showed 30–50 generations were needed before the correlograms reach a quasi-stationary status (EPPERSON, 1990). The UT population can be considered a very young population in terms of generations since its foundation. The number of generations that UT has undergone may not be sufficient for it to reach the quasi-stationary status in genetic structure that the species

would be expected to develop eventually, i.e. it could still be progressing towards its inherent genetic structure endpoint.

The UT population and the AK population (which was 're-founded' from a few remnant trees after forest cutting during the 1920s) showed interesting, divergent, differences from the KU population. The AK population retained stronger linkage disequilibrium (12 out of 36 locus pairs) than UT (two out of 36 locus pairs). Furthermore, the proportions of positively significant like joins (0.09) and allelic correlograms (0.00; according to the Bonferroni criterion) in the first distance class in the UT population were smaller than those of KU (0.29 and 0.43), while the corresponding for AK (0.38 and 0.86) were larger than those of KU. No NAC values were significant in UT, while NAC values of the other two populations were positively significant at shorter distance classes. If we assume the genetic structure for KU, an old-growth forest, to be typical of primary populations of the species, the genetic structure in UT was weaker than primary forest, whereas that in AK was stronger.

The within-population pattern of genetic variation is not independent of previous generations, especially in species that have limited seed dispersal mechanisms (HAMRICK *et al.,* 1993). Instead, the genetic structure of current generations may be influenced by the cumulative effect of the genetic clustering of many preceding generations (KNOWLES *et al.,* 1992). KNOWLES *et al.* (1992) studied two tamarack (*Larix laricina*) populations that have markedly different anthropological disturbance histories. One population had regenerated from a site with scattered remnant trees but with no other seed source nearby. The other had regenerated from a site that had no remnant trees, but it was surrounded by abundant sources of seed. The former showed spatial autocorrelation, whereas the latter did not. The cited authors also drew attention to the role that preceding generations could play in the genotypic arrays of the remnant trees. The Utasai population was likely to be founded from seeds randomly cached by birds from more southern populations that may be genetically unrelated to each other. In contrast, in AK, the genotypic array of the population should reflect the genetic clustering of the trees present prior to the forest cutting. Thus, the UT and AK populations resulted from very different founding processes (or, more precisely, 're-founding' in the case of AK) in terms of the influence of genetic structure before their foundation. One plausible explanation for the differences in genetic structure between the UT and AK populations is that UT may have been established at a place where *F. crenata* had not existed previously, so it was not influenced by a pre-existing genetic structure, while AK would be expected to reflect, at least in part, the genetic structure of the preceding generations.


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# Variation in Nutrient Utilization and Juvenile Growth in Open-pollinated Families of *Picea sitchensis* (Bong.) Carr. Grown in a Phytotron and Correlations with Field Performance

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**Abstract**

The purpose of this study was to estimate genetic variation in nitrogen (N) and phosphorus (P) utilization (= amount of biomass produced per unit nutrient in the needles), and growth traits in seedlings from 27 open-pollinated families of *P. sitchensis*. Further, the purpose was to estimate juvenile – mature correlations between these traits and breast height diameter in field trials. The seedlings were grown for two growth periods in climate chambers. There were two treatments: free access and restricted access to nutrients. The nutrient treatment in restricted access was chosen to result in a growth of approximately one third of the growth in the free access treatment. Height, shoot, root and needle dry weights, as well as amount of N and P in the needles and N and P utilization were assessed. There was a strong treatment effect of nutrients on all height and above-ground biomass traits. They were statistically different at the 1% level. There was a significant family effect for N and P utilization and for all other traits studied under restricted access to nutrients. On the contrary, no significant family effects were noted for nitrogen and phosphorus utilization under free access to nutrients, this may be attributed to luxury consumption of nutrients. The precision of the family variance estimates and heritabilities were slightly higher in restricted access than in the free access treatment. The family x nutrient interaction was significant for most of the traits studied, which resulted in non-significance for most of the family effects in the joint analyses of data from the two treatments. Selection of families that responded strongly to a high availability of nutrients could be useful at regeneration of sites with high soil fertilities. The family mean correlations between juvenile traits and breast height diameter in field were all weak ($R^2 \leq 0.2$).

*Key words:* Nutrient utilization, *Picea sitchensis*, genetic correlations, growth chamber.

## Introduction

Sitka spruce (*Picea sitchensis*) is economically the most important conifer tree species in Ireland where it accounts for about 60% of the current forest estate and about 65% of current afforestation (ANONYMOUS, 1996). It has been included in Irish tree breeding since 1960 (O'DRISCOLL, 1977). Future breeding will benefit from a deeper genetic-physiological understanding of variation in growth.

Nutrients, and particularly phosphorus and nitrogen, are often lacking under Irish forest conditions. In Ireland it is almost standard practice to apply phosphate to sites prior to planting and during the growth of the crop, but current concerns about nutrient run-off have lead to more regulated applications. There are several reports on conifers indicating genetic variation in response to different nutrient levels (e.g. ROBERDS *et al.*, 1976; NAMBIAR 1984, LI *et al.*, 1991; JONSSON *et al.*, 1997). Therefore, it is of interest to study genetic variation in the Irish breeding population of *P. sitchensis* with respect to growth at different availability of nitrogen and phosphorus.

There is some confusion as regards the terminology of nutrient efficiency. We use this term to cover uptake of nutrients,

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