MICROSATELLITE GENETIC VARIATION IN RARE ISOLATED POPULATION OF *MAGNOLIA SIEBOLDII*[1]

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ABSTRACT

M. *sieboldii* spp. *japonica,* living on restricted habitat, often forms small isolated populations. In order to evaluate the effect of genetic drift and other factors in determining genetic variation in these small populations, microsatellite genetic variation was compared among two remote mountain regions, the Ohmine and the Ishizuchi. Significant positive correlation between population size and genetic diversity and significant or nearly significant isolation-by-distance in each of the regions suggested that genetic drift was predominant in determining genetic variation within each of the regions in *M. sieboldii,* and that populations were at or near drift-gene flow equilibrium. However, these relationships differed among the regions, with low genetic diversity in the Ohmine populations. Although we could not determine the real factor changing the level of genetic variation among the regions, weaker and not strictly significant IBD in the Ishizuchi populations might suggest more frequent gene flow or recolonization processes in the Ishizuchi region, and narrower allele size ranges in the Ohmine might reflect historical bottlenecks. Moreover, an interruption of microsatellite repeat motifs at one of the locus found in the Ohmine populations suggested different mutation rate at microsatellites among the regions.

Key words: *Magnolia sieboldii*, microsatellite, genetic drift, population genetic diversity, gene flow, mutation rate

INTRODUCTION

Population genetic structures result from selection, mutation, migration and genetic drift, interacting with the species' ecological and historical traits. Random genetic drift will be important when populations are small (BARRET & KOHN 1991). Smaller populations within a species are likely to contain less variation than larger populations, showing also higher levels of among-population differentiation, since the effects of genetic drift may vary with population size (ELLSTRAND & ELAM 1993). Some empirical studies on plant population genetics, mainly using allozymes, detected positive association between population size and genetic variation in rare or endemic plant species, although others have failed to detect such relationships (BARRET & KOHN 1991, ELLSTRAND & ELAM 1993, OOSTER-MEIJER 1996).

Genetic drift is likely to impact not only smaller populations but also geographically isolated populations within a species (DOLAN 1994, ROWE *et al.* 1999), where the loss of genetic variation due to drift may not be counterbalanced by gene flow from external pollen/seed sources, although the effects of population isolation on gene flow are ambiguous (WEIDEMA *et al.* 2000). Moreover, association between population size and genetic diversity can be altered when historical factors are more important than current population size in determining patterns of diversity. For example, populations may have not reached their evolutionary equilibrium since past demographic changes such as bottlenecks and founding events.

Assessment of the effects of isolation an drift from genetic data is an important tool for conservation of populations/species, as severely bottlenecked or isolated populations prone to genetic depauperation/deletion, inbreeding depression and/or demographic fluctuation may be identified. Recently hypervariable microsatellites are getting more used as a tool of population genetics, and they may be also useful in this context. Indeed, their high mutation rates ensures that variation is abundant and

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quickly restore after bottlenecked, so the effects of more recent demographic events can be detected more likely than by other genetic markers such as allozymes (WAYNE *et al.* 1991).

In this study, microsatellite loci were surveyed in rare isolated populations of *Magnolia sieboldii* spp. *japonica* across its distributed range, to assess the effect of genetic drift and other factors in determining the level and the distribution of population genetic variation. *M. sieboldii* spp. *japonica* is a deciduous shrub which reaches to the height of 3 m, and is distributed mainly in western Japan, extending from Yakushima Island to North Kanto area. Populations of *M. sieboldii* are usually small and isolated, growing at high elevations in mountainous regions, 1,000–2,000 m a. s. l. and living in restricted areas such as narrow stony ridges, among rocks, or on the edges or in openings of upper cool-temperate-deciduous to subalpine-coniferous forests, and also in limestone or serpentine areas (UEDA 1980).

MATERIALS AND METHODS

Seven populations were sampled in each of two remote mountain ranges; *i.e.*, the Ishizuchi region and the Ohmine region (Fig. 1). Populations within regions were 0.3–14 km apart. Population size was roughly estimated by counting all individuals higher than 20 cm in a clump of *M. sieboldii* shrubs.

For each population leaves were collected from 11 to 56 shrubs, and DNA was extracted using a CTAB method (MILLIGAN 1992). Four microsatellite loci (M6D3, M6D4, M10D8 and M17D5), selected from 11 loci identified by ISAGI *et al.* (1999) in *M. obovata*, were assayed. PCR reactions were carried out as described in ISAGI *et al.* (1999). The PCR products were electrophoresed on the ABI 377 instrument, and product size was determined using Genescan^{TM} analysis software.

Genetic polymorphism was measured as the mean number of alleles per locus (A), observed heterozygosity (H_O) and heterozygosity expected from Hardy-Weinberg assumptions (H_E). These indices of population genetic diversity were compared with observed population size. Weir & Cockerham's F_{ST} values were calculated with the TFPGA program (MILLER 1997) to estimate interpopulational genetic differentiation. In addition, population pairwise F_{ST} values were estimated using the programs FSTAT (GOUDET 1995) to test an isolation-by-distance (IBD) model.

RESULTS

In total, 12, 8, 17 and 12 alleles were detected at loci M6D3, M6D4, M10D8 and M17D5, respectively. In the Ishizuchi region, numbers of alleles per locus ranged from 2.75 to 6, with an average of 4.64, while in the Ohmine it ranged from 2 to 3.5 with an average of 2.82. Regional F_{ST} was 0.286 (95% C.I. 0.227–0.343) among the Ishizuchi populations and 0.314 (95% C.I. 0.243–0.394) among the Ohmine populations, indicating a limited gene flow even among nearby populations. None of the population genetic parameters was significantly correlated with population size when all populations were analyzed together. Positive correlation between genetic diversity and population size was detected when populations coming from each region were separately considered (Fig. 2). Fig. 2 also illustrates the difference in the regression slopes between regions. The three population genetic parameters increased more rapidly with increasing population size in the Ishizuchi region than in the Ohmine. The slope of regression was significantly different between the regions for A (p = 0.0112) and H_O (p = 0.0021), but not for H_E(p = 0.1062). by ANCOVA.

Isolation-by-distance was also detected within regions. Pairwise F_{ST} was correlated with geographic distance for populations from the same regions (r = 0.475, p = 0.0013) and for the Ohmine region r = 0.832, p = 0.0009), but not for the Ishizuchi region (r = 0.402, p = 0.0705) (Fig. 3). The regression slopes were not significantly different between the regions (p = 0.109) by ANCOVA, although the Ohmine populations appeared to depict more rapid pattern of isolation-by-distance.

DISCUSSION

Microsatellite genetic variation in *M. sieboldii* was characterized by low population genetic diversity (A = 3.74 and H_O =0.366 on average) and quite high genetic differentiation even among nearby populations within regions (F_{ST} = 0.286 in the Ishizuchi and 0.314 in the Ohmine). Although genetic investigation on a comparable spatial scale are limited in literature for plants, some studies using microsatellites or allozymes reported a lower genetic differentiation at this spatial scale, (*e.g.*, WHITE *et al.* 1999, SOEJIMA *et al.* 1998). Such low genetic diversity and high differentiation were considered mainly due to genetic drift. In this study, a significant correlation between genetic diversity estimates

Table 1 Observed population size, number of alleles per locus (A), observed (H_O) and expected heterozygosity (H_E) in 14 populations of *Magnolia sieboldii*.

Population	Observed population size	A	H_o	H_E
Mts. Ishizuchi				
ISZ1	30	5.25	0.492	0.645
ISZ2	29	3.75	0.409	0.499
ISZ3	53	4.50	0.463	0.555
ISZ4	121	6.00	0.642	0.652
ISZ5	35	4.25	0.331	0.378
ISZ6	92	6.00	0.597	0.634
ISZ7	28	2.75	0.292	0.308
Mean		4.643	0.461	0.524
Mts. Ohmine				
OMN1	487	3.50	0.490	0.524
OMN2	18	2.50	0.308	0.385
OMN3	169	3.50	0.368	0.420
OMN4	84	2.75	0.313	0.328
OMN5	90	2.50	0.280	0.303
OMN6	31	2.00	0.268	0.254
OMN7	20	3.00	0.232	0.406
Mean		2.821	0.323	0.374
Total mean		3.732	0.392	0.449

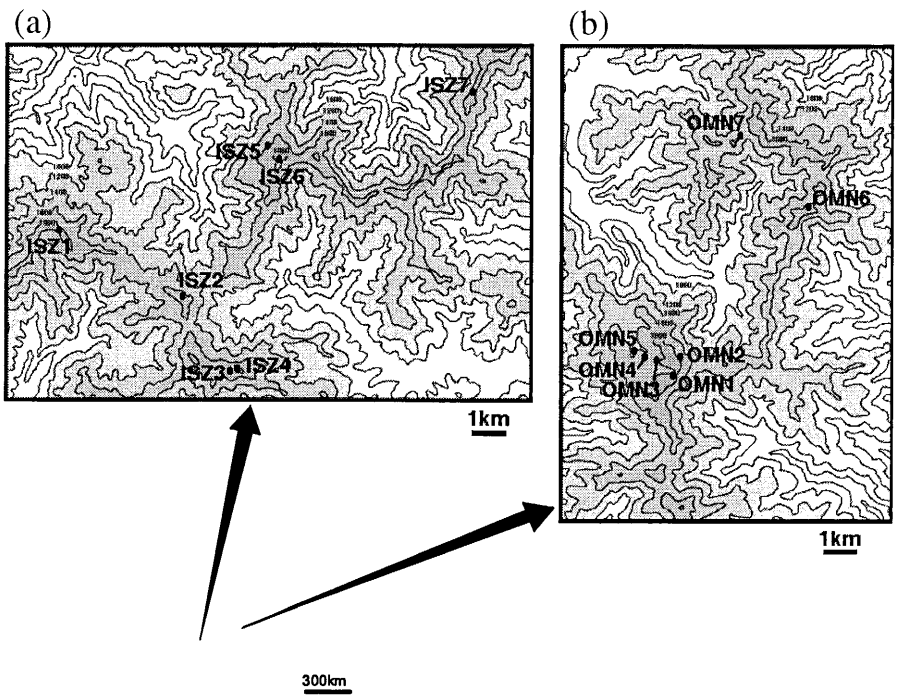

Figure 1. Geographical maps of two sampling regions, the Ishizuchi mountain range (a), and the Ohmine mountain range (b).

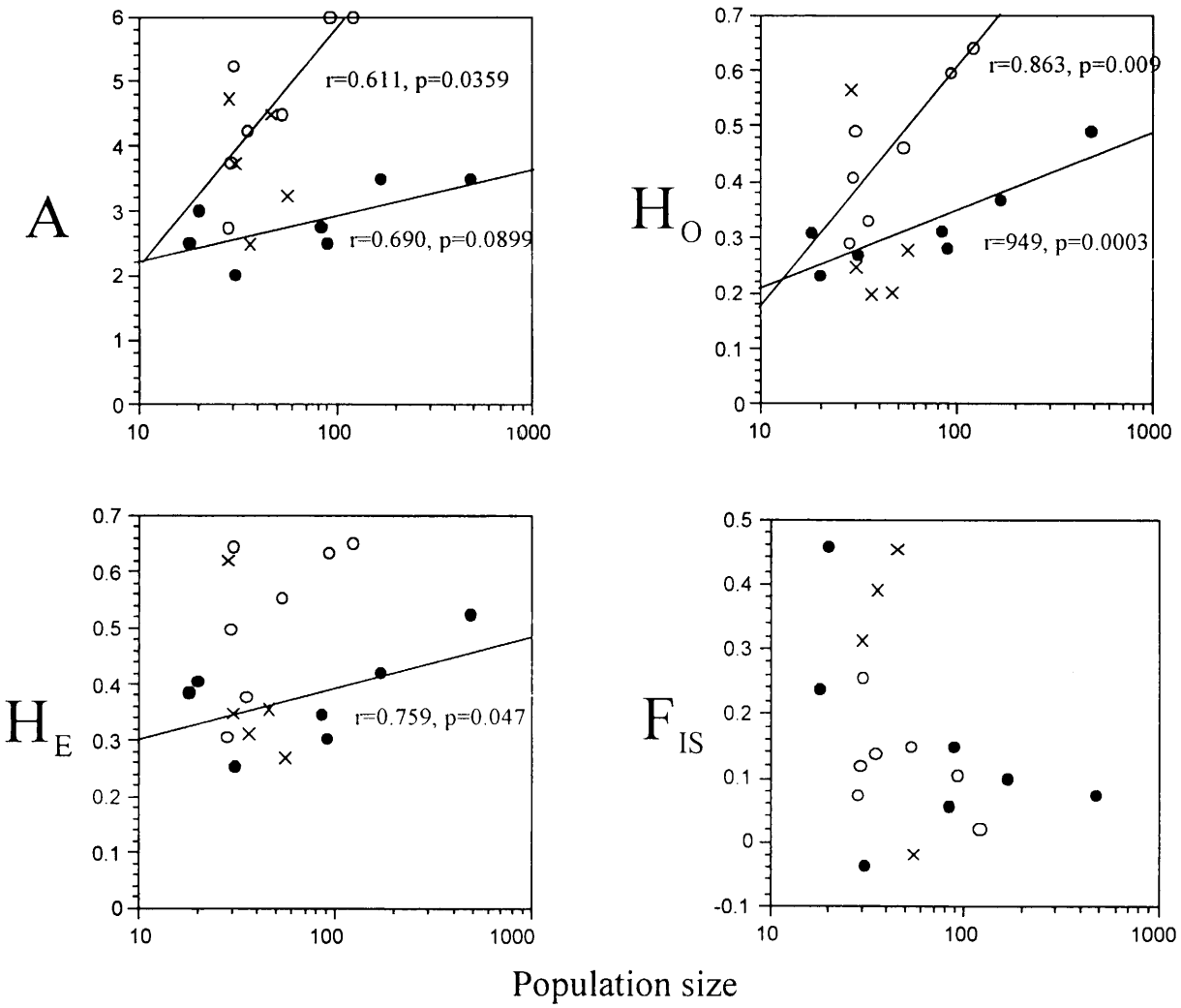

Figure 2. Relationships between population size and measures of population genetic diversity and inbreeding coefficient F_{IS}. Open circles and solid circles indicate population from the Ishizuchi and the Ohmine, respectively.

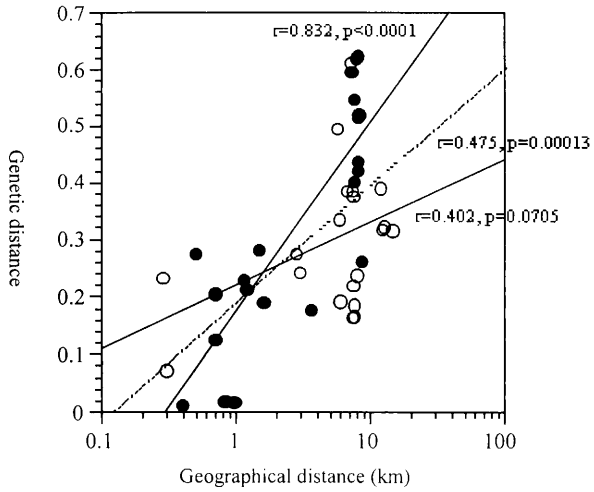

Figure 3. Relationships between the geographic and genetic distance within the Ishizuki (open circles) and the Ohmine (solid circles).

and population size was detected when populations from the Ishizuchi and Ohmine regions were considered independently, though the same relationship was not significant when populations from the two regions were pooled together (Fig. 2). Such correlation between current population size and measures of genetic diversity suggests that genetic drift played a major role in determining population genetic variation for *M. sieboldii* on a regional scale.

However, a possibility that current genetic variation is still influenced by founder effects should not be omitted. M. sieboldii is an early-succesional species living in relatively open spaces-like forest edges and gaps-, and sometimes along paths through forests. The observed absence of aged adults and young seedlings in several sites (data not shown) suggests that some if the populations considered in this study might be of recent origin from a small

number of founders.

When compared mong the regions, relationships between population genetic diversity and population size seemed different, with lower levels of genetic variation in the Ohmine populations (Fig. 2). Some hypothesis may be formulated here to explain the observed differences between regions in the level of population genetic variation. One possibility is that the levels of current gene flow were different among regions. Smaller gene flow among the Ohmine populations might have accelerated the loss of genetic diversity by random genetic drift. The IBD patterns, which slightly differed between regions, supported this hypothesis. The IBD slope of the Ohmine populations was slightly, though not significantly (p = 0.110), steeper than that of the Ishizuchi populations (Fig. 3), suggesting that genetic differentiation due to genetic drift were eased by the higher levels of gene flow. Second, the correlation in Ishizuchi populations was relatively weak and not strictly significant (r = 0.402, p = 0.0705). It might suggest that the Ishizuchi populations had not reached the equilibrium since recent demographic changes. The study populations in Ishizuchi may have been recently bottlenecked or recolonized.

Another possibility is that historical factors altered the level of genetic variation across the region. Total range in allele size as well as allelic richness was much smaller in Ohmine than in Ishizuchi. If microsatellites mainly follow stepwise mutation model, it may suggests that mutation of alleles have not recovered from past bottlenecks.

Finally, molecular factors should not be dismissed. When microsatellite regions were sequenced for some individuals from the Ishizuchi and the Ohmine at these four loci, an interruption of repeat motifs was detected at the locus M17D5 for all of the individuals from the Ohmine, with a C substituting a G in the (GA) repeats. It is likely that the lowest allelic richness at the locus M17D5 in the Ohmine (data not shown) was partly due to lower mutation rate, caused by interruption in the repeat motifs. However, trends still hold when the locus M17D5 was removed from analysis (A, p = 0.0094; Ho, p = 0.0261; He, p = 0.1687). Thus it is regarded that the interruption of the repeat motifs could hardly explain the difference in the population genetic diversity among the regions, although it might partly contribute to the small allelic richness at the locus M17D5 in the Ohmine.

Many studies have the relationships between population size and genetic variation. However, they did not always detect significant correlations between them (*e.g.*., COATES 1988, DOLAN 1994, HURTREZ-BOUSSÉS 1996, MAKI *et al.* 1996, BERGE *et al.* 1998). In some cases, it was argued that recent demographic changes, gene flow and historical factors could hidden the correlation between population size and genetic variation. However, analytical studies on the relative importance of these micro-evolutionary process in shaping the genetic variation in wild plant populations are rare.

In this study, the comparison among different regions revealed that the effect of genetic drift was predominant within the regions, and that other factors such as gene flow, historical bottlenecks and mutation rate at microsatellite loci might alter the level of genetic diversity across the entire region. Further studies are needed in order to determine the relative importance of these factors.

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