Fine-Scale Spatial Genetic Structure within Continuous and Fragmented Populations of *Trillium camschatcense*

Hiroki Yamagishi, Hiroshi Tomimatsu, and Masashi Ohara

From the Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan (Yamagishi and Ohara); and the Department of Biological Sciences, Tokyo Metropolitan University, Hachioji 192-0397, Japan (Tomimatsu).

Address correspondence to M. Ohara at the address above, or e-mail: ohara@ees.hokudai.ac.jp.

Abstract

Spatial genetic structure (SGS) within populations was analyzed for the long-lived understory perennial herb *Trillium camschatcense* using allozyme loci. We used $F$ statistics to compare SGS between 2 life-history stages, juveniles (J) and reproductives (R), as well as between 2 populations, continuous and fragmented, with different habitat conditions. In the continuous population, significant SGS was detected in both stages but the extent was greatly reduced with the progress of the stage (J, $F_S = 0.0475$; R, $F_S = 0.0053$). We inferred that limited seed dispersal and subsequent random loss of individuals from the family patches are responsible for the J and R stage structures, respectively. The fragmented population differed in the patterns of SGS; significant structure was detected in the R stage, but not in the J stage (J, $F_S = 0.0021$; R, $F_S = 0.0165$) despite significant positive inbreeding coefficients (J, $F_{IS} = 0.251$). The observed differences in the J-stage structures between populations may be explained by habitat fragmentation effects because reduced recruitment in the fragmented population prevents the development of maternal sibling cohort. Such comparative analysis between populations and life-history stages can be useful to understand the different underlying causes of SGS.

Spatial genetic structure (SGS) within plant populations is determined by many processes, including gene flow and local selection at different life-history stages (Hedrick 1986; Ennos 1994; Latta et al. 1998; Kalisz et al. 2001). In the case of long-lived perennial plant species, SGS of juveniles primarily reflects their parental structure and the patterns and distances of both pollen and seed dispersal. This structure can be modified in subsequent life-history stages by postdispersal selection or mortality of individuals. Therefore, analyses of SGS over the life cycle can infer the roles of these demographic and genetic processes in the formation and maintenance of the within-population genetic structure (Kalisz et al. 2001; Chung et al. 2003). For example, some studies have found that significant structure in juveniles is greatly reduced in reproductive adults, suggesting that limited dispersal and the subsequent random loss of individuals from the family patches are responsible for the juvenile and adult structures, respectively (e.g., Epperson and Alvarez-Buylla 1997; Parker et al. 2001; Chung et al. 2003). Conversely, the opposite trend was also observed in some species, and the increasing SGS has been attributed to local selection during recruitment or historical events (Tonsor et al. 1993; Ueno et al. 2000; Kalisz et al. 2001).

Moreover, the extent of SGS may vary among populations under different habitat conditions, and such interpopulation variation can also improve our understanding of the relationship between environmental factors and life-history factors. In particular, habitat alteration due to human activities affects many demographic and genetic processes including the breeding system (e.g., Rajimann et al. 1994) and recruitment (e.g., Tomimatsu and Ohara 2002; Ward and Johnson 2005), which are likely reflected in the patterns of genetic structuring. Several studies have revealed habitat-associated variations of SGS, for example, between fragmented and continuous populations of *Acer saccharum* (Young and Merriam 1994) and between old-growth and logged forests of *Pinus strobus* (Epperson and Chung 2001); however, few studies have examined the differences in SGS of different life-history stages both among and within populations (but see Epperson and Chung 2001; Cruse-Sanders and Hamrick 2004).

In this study, we analyzed SGS of 2 life-history stages in 2 populations of the long-lived understory herb, *Trillium camscottense* Ker Gawler (Trilliaceae or Melanthiaceae). First, we compared SGS between juveniles and reproductives in a large, continuous population. Although the species has an outcrossing mating system in our populations (Ohara et al. 1996), the dispersal distance of seeds by ants has been reported to be short, only 0.60 m on the average (Ohara and...
This study was conducted in 2 populations, Hiroo and Kiyokawa, in the Tokachi plain of eastern Hokkaido, Japan. In this study, we selected 2 distinct populations of 14 populations used in our previous studies (Tomimatsu and Ohara 2002, 2003a, 2004), one large continuous (Hiroo) and one small fragmented (Kiyokawa). The Hiroo population (42°19′N, 143°20′E; hereafter “continuous”) grows in a large, continuous forest (~300,000 m²), whereas the Kiyokawa population (42°45′N, 143°7′E; hereafter “fragmented”) grows in a small, fragmented forest (~7700 m²), fully surrounded by agricultural fields. The estimated habitat size and the number of reproductive plants are 50,000 m² and 126,000 for the continuous and 7700 m² and 102 for the fragmented population, respectively. Our previous study demonstrated that the fragmented population showed a significant inbreeding coefficient (FIS = 0.129), possibly due to biparental inbreeding (Tomimatsu and Ohara 2003a). Considering the size of habitats and the development history of the region, habitat conditions must be much more altered in the fragmented population. Thus, when interpreting the data, we assume that environmental conditions that gave rise to SGS in the reproductive and juvenile stages are similar in the continuous population, but they are quite different in the fragmented population owing to the fragmentation events.

### Materials and Methods

#### Study Species and Populations

*Trillium camtschatense* is a diploid (2n = 10), nonclonal perennial herb that commonly occurs in the understory of broad-leaved deciduous forests of Hokkaido, Japan. Vegetative individuals of *T. camtschatense* comprise 2 morphologically distinct forms: 1-leaf and 3-leaves stages. It takes more than 10 years for seedlings to become flowering plants. Reproductive have one or several flowers, and seed production results from obligatory outcrossing by insect pollination in our study area (Ohara et al. 1996). After reaching flowering, the plants can live for at least 25 years with continuous flowering every year (Ohara et al. 2006). The flowers are visited by a wide range of insects, primarily beetles (e.g., Nitidulidae and Melandryidae) and flies (e.g., Bibionidae and Scathophagidae; Tomimatsu and Ohara 2003b), and the fruits contain ~80 seeds on average (Tomimatsu and Ohara 2002). As each seed has a soft, juicy elaiosome, the seeds are attractive to ants, which consequently contribute to seed dispersal (Ohara and Higashi 1987).

Leaf materials were transported on ice to the laboratory and kept at ~80 °C until electrophoresis. Approximately 70 mg of leaf tissue from R plants was homogenized in 0.9 ml of extraction buffer made up of 0.1 M Tris–HCl (pH 8.0), 0.2 g/ml glycerol, 63 mg/ml Tween 80, 8 mM dithiothreitol, 0.50% (v/v) β-mercaptoethanol, 0.40% (w/v) β-nicotiamide.
adenine dinucleotide, 0.45% (w/v) β-nicotiamide adenine dinucleotide phosphate, 0.3% (w/v) bovine serum albumin, and 7% (w/v) polyvinylpolypyrrolidone (modified from Shiraishi 1988). As the leaves of J plants were small, we homogenized 2 mg of leaf tissue in 0.25 ml of extraction buffer. After the homogenates were centrifuged (15,000 rpm for 15 min at 4 °C), 10 μl of the resulting supernatant was used for vertical polyacrylamide gel electrophoresis. We tested 14 enzymes first, and then examined 6 enzyme systems that consistently showed clear and interpretable banding patterns: aspartate aminotransferase (AAT, 2 loci [EC 2.6.1.1]), leucine aminopeptidase (LAP, 1 locus [EC 3.4.11.1]), glutamate dehydrogenase (GDH, 1 locus [EC 1.4.1.2]), malate dehydrogenase (MDH, 1 locus [EC 1.1.1.37]), sorbitol dehydrogenase (SODH, 1 locus [EC 1.1.1.14]), and alcohol dehydrogenase (ADH, 2 loci [EC 1.1.1.1]). On the basis of the banding patterns, we assumed Mendelian inheritance of all loci.

Data Analysis

Standard measures of genetic diversity, including the percentage of polymorphic loci (P), the mean number of alleles per locus (A) corrected for differences in sample size (i.e., rarefied; Leberg 2002), the observed heterozygosity (H_o), and the expected heterozygosity (H_e) were calculated for each life-history stage in each population. Inbreeding coefficient (F_IS) was calculated as estimates of Weir and Cockerham (1984) using the computer program FSTAT version 2.9.3.2 (Goudet 2002), and the levels of significance were obtained by randomization-based procedures (for details, see Goudet 2002). To investigate fine-scale SGS, we conducted spatial autocorrelation analyses with kinship coefficients (Loiselle et al. 1995) using SPAGeDi version 1.2 (Hardy and Vekemans 2002). Mean multilocus kinship coefficients (F_0) were computed for the following distance classes: 0.2, 0.3, 0.4, 0.5, 0.75, 1, 2, 4, and 8 (upper-bound distance in meters) and were plotted against the logarithm of the geographic distance (d). Standard errors for the kinship coefficients were estimated using a jackknife procedure over the loci. The linear regression slope (b) can be a good estimator of the extent of SGS (Vekemans and Hardy 2004). We tested the significance of b against the null hypothesis H_0: b = 0 (i.e., the overall absence of SGS) by comparing the observed values with those obtained after 1000 random permutations of individuals among positions. We also quantified SGS by θp statistics, calculated as −b/(1 − F(1)) where F(1) is the mean kinship coefficient in the first distance class (see Vekemans and Hardy 2004 for details). The θp statistics could be considered robust in our sampling scheme because F(0) tended to decrease linearly with ln(d).

Results

Genetic Diversity and Levels of Inbreeding

Seven of the 8 loci resolved were polymorphic in at least one of the 2 populations examined: 6 (AAT [2 loci], ADH, SODH, LAP, GDH) and 4 (AAT [2 loci], ADH, LAP) loci were polymorphic in the continuous and fragmented populations, respectively; MDH was monomorphic. In both stages, the continuous population had higher genetic diversity (P, A, H_o, and H_e) than the fragmented population (Table 1). For example, when the differences in sample sizes were adjusted, the mean number of alleles per locus (A) was 1.875 for R and 2.227 for J stages in the continuous population and 1.620 for R and 1.484 for J stages in the fragmented population. In the continuous population, inbreeding coefficients were nearly equal to zero (R, F_IS = 0.072; J, F_IS = 0.079). In contrast, the fragmented population showed high inbreeding coefficients in both stages (R, F_IS = 0.189; J, F_IS = 0.251). Randomization tests detected a significant

Figure 1. Spatial distribution of Trillium camschatcense individuals within the study plots. Individuals of different stage classes are represented by different symbols in the maps. Maps are shown for (A) continuous and (B) fragmented populations.
deficit of heterozygosity relative to Hardy–Weinberg expectations only in the J stage.

Spatial Genetic Structure

The slopes ($b$) of linear regressions between the kinship coefficient and the logarithm of the geographic distance were found to be significantly negative ($P < 0.05$) in all but the J stage in the fragmented population (Table 1; Figure 2). In the continuous population, the $\hat{\psi}$ statistics showed that the J stage was much more genetically structured than the R stage ($J$, $\hat{\psi} = 0.0475$; $R$, $\hat{\psi} = 0.0053$). In contrast, the fragmented population exhibited significant SGS at the R stage ($\hat{\psi} = 0.0165$), but not in the J stage ($\hat{\psi} = 0.0021$). When the strength

<table>
<thead>
<tr>
<th>Population</th>
<th>Genetic diversity and inbreeding coefficient</th>
<th>Genetic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$</td>
<td>$A$</td>
</tr>
<tr>
<td>Continuous Reproducives (R)</td>
<td>0.750</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.750</td>
<td>2.22</td>
</tr>
<tr>
<td>Juveniles (J)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmented Reproducives (R)</td>
<td>0.500</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.375</td>
<td>1.48</td>
</tr>
<tr>
<td>Juveniles (J)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$P$, percent polymorphic loci; $A$, average number of alleles per locus corrected for differences in sample size; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity (gene diversity); $F_{IS}$, estimates of Weir and Cockerham (1984) of inbreeding coefficient; $\hat{\psi}$, estimates of Vekemans and Hardy (2004) of SGS; $b$, slope of the regression of kinship coefficients on the logarithm of geographic distance (*$P < 0.05$; **$P < 0.01$); $F_{(1)}$, the value of $F_{(1)}$ on the first distance class.

Figure 2. Correlograms (solid lines) resulting from spatial autocorrelation analyses based on allozyme loci for 2 study populations of *Trillium camschatcense*. Least-squares linear regressions are indicated by dotted lines. Vertical bars show standard errors. Distance classes are 0.2, 0.3, 0.4, 0.5, 0.75, 1, 2, 4, and 8 m (upper-bound distance).
of SGS of each life-history stage was compared between populations, the R stage in the fragmented population was slightly more structured than that in the continuous population (continuous, $Sp = 0.0053$; fragmented, $Sp = 0.0165$). In contrast, the J stage exhibited about 20-fold stronger structure in the continuous population than in the fragmented population (continuous, $Sp = 0.0475$; fragmented, $Sp = 0.0021$).

**Discussion**

This study indicates how fine-scale SGS of *Trillium camtschatense* differs across 2 life-history stages and 2 populations under contrasting habitat conditions. In addition, the fragmented population exhibited lower allelic diversity and higher inbreeding coefficients than the continuous population (Table 1). These results are consistent with our previous study (Tomimatsu and Ohara 2003a). The fragmented population would have experienced a stochastic loss of alleles at the time of fragmentation and biparental inbreeding due to localized pollen transfer.

In the continuous population, as expected, we found significant SGS in juveniles (Table 1; Figure 2A). This structure could develop as a result of limited pollen and seed dispersal (Wright 1946; Slatkin 1985). Although the distance of pollen dispersal would not be far, the value of inbreeding coefficient ($F_{is}$) indicates that mating within this population was essentially random (Table 1). Therefore, the results suggest that restricted seed dispersal is mainly responsible for SGS in juveniles. In fact, seeds that fell on the ground were rarely transported by ants even after 72 h and many remained around the maternal plants (dispersal frequency 11.7%; Yamagishi H., unpublished data). By contrast, the reproductive stage showed a weak structure relative to the juvenile stage (Table 1; Figure 2A). The reduction pattern of SGS with the progress of the stage could occur when juveniles experience genetically random mortality that occurs in a density-dependent manner. Several previous studies also demonstrated the same pattern, which was attributed to random mortality during recruitment (Hamrick et al. 1993; Epperson and Alvarez-Buylla 1997; Chung et al. 2003). It should be noted, however, that the opposite trend was observed in a related species of the same genus, *Trillium grandiflorum* (Kalisz et al. 2001); significant SGS was detected in reproductive but not in juvenile stages, and it was suggested that the increase in SGS between the stages could arise from local selection resulting from microenvironmental conditions or from specific historical events.

The fragmented population differs in the extent and pattern of SGS from the continuous population; namely, juveniles did not show significant SGS despite relatively high levels of inbreeding coefficients (Table 1; Figure 2B). Given our previous knowledge on the effects of habitat fragmentation, juvenile stage SGS could either increase due to biparental inbreeding or decrease due to reduced recruitment. Our results suggest that the low recruitment may be responsible for the absence of SGS. In this population, seedling recruitment is much more restricted than in the continuous population probably because of reduced seed production, microclimatic edge effects, and biparental inbreeding (Tomimatsu and Ohara 2002, 2004). Actually, the frequency of juveniles was only 2.5 times that of reproductives in the fragmented population (cf., 9.5 times that of the continuous population; Figure 1). This suggests that only a few maternal siblings exist among juveniles, and thus the low recruitment could explain the absence of genetic structure. Interestingly, a related species, *T. grandiflorum*, also did not show significant SGS at the juvenile stage (Kalisz et al. 2001). The number of seeds produced per reproductive plant is small in *T. grandiflorum* (~16 seeds; Kalisz et al. 1999), so that the resulting low recruitment, in conjunction with a moderate level of seed dispersal, would have a homogenizing effect on the genetic structure (Kalisz et al. 2001). Although the difference between $Sp$ statistics was not tested in our study, the reproductive stage SGS in the fragmented population was slightly stronger with lower adult density than that in the continuous population (Table 1; Figure 2). This result is consistent with theoretical and empirical evidence that density is a major determinant of SGS because it affects the strength of local genetic drift and the level of gene flow distance (Heywood 1991; Vekemans and Hardy 2004). We are not sure, however, whether this difference in SGS is significant because $F_{is}$ in these populations are similar (Table 1) and $F_{is}$ in the short distance classes had large variance in the fragmented population (Figure 2B).

Some limitations and caveats should be considered before we draw any conclusions from this study. First, we examined only a single plot within each population. Although we carefully established the plots to reflect typical environmental conditions and demographic structures of populations, 2 or more replicates are needed to draw more corroborative evidence. Second, the accuracy of $Sp$ statistics critically depends on $F_{is}$ in the first several distance classes, particularly $F_{is}$ (Vekemans and Hardy 2004). For example, $F_{is}$ in the first 4 distance classes had large standard errors in the continuous population (Figure 2A) and this could partly be caused by how we defined distance classes. We recalculated $F_{is}$ and $Sp$ statistics under different sets of distance classes, but these calculations gave essentially the same interpretation of our data (results not shown). Finally, in the fragmented population, we do not infer the causes of creating the strong pattern of structure observed in the reproductives from the pattern in the juveniles. Considering the long life-span of *T. camtschatense* (>50 years) and the population history, many reproductive individuals may still have survived fragmentation. Therefore, the existent structure in this reproductive population was likely to develop under habitat conditions before or immediately after habitat fragmentation, whereas the structure in the juveniles was established recently, long after the fragmentation events.

In summary, under a high level of recruitment, SGS appears at the juvenile stage probably because of limited seed dispersal around maternal plants, and subsequent random loss of individuals from family patches is a plausible explanation for the loss of SGS from reproductive to juvenile stages. However, under low recruitment due to habitat fragmentation, juveniles do not show a significant genetic structure possibly because only few maternal siblings exist. Although
replicates of plots or populations may be needed to confirm our conclusions, such comparative analysis between populations as well as between life-history stages can be useful to understand the different potential causes of the SGS.

Funding

The Akiyama Memorial Foundation; the Foundation for Earth Environment; the Ministry of Education, Culture, Sports, Science; and Technology for the 21st Century Center of Excellence Program (E-01) and the Japan Society for the Promotion of Science for Scientific Research (Nos. 16370007 and 18405010).

Acknowledgments

We are grateful to K. Nemoto for assistance in the field and Hiroo town office for offering the use of their facility. We also thank M. T. Kimura and G. Kudo for helpful advice.

References


Received May 6, 2006
Accepted April 2, 2007

Corresponding Editor: David Wagner