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<th>著者</th>
<th>森田 耕子, 本多 豊, 化目窝 達男, 横橋 友博</th>
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<td>適応多様性に基づく保全単位の再考</td>
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Reconsideration for conservation units of wild *Primula sieboldii* in Japan based on adaptive diversity and molecular genetic diversity

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**Summary**

*Primula sieboldii* E. Morren is a perennial clonal herb that is widely distributed in Japan, but in danger of extinction in the wild. In a previous study, we revealed the genetic diversity of the species using chloroplast and nuclear DNA and used this information to define conservation units. However, we lacked information on adaptive genetic diversity, which is important for long-term survival and, thus, for the definition of conservation units. In order to identify adaptive traits that showed adaptive differentiation among populations, we studied the genetic variation in six quantitative traits within and among populations for 3 years in a common garden using 110 genets from five natural populations from three regions of Japan. The number of days to bud initiation was adaptive quantitative trait for which the degree of genetic differentiation among populations (Q<sub>ST</sub>) was considerably larger than that in eight microsatellite markers (F<sub>ST</sub>). The relationship between this trait and environmental factors revealed that the number of days to bud initiation was negatively correlated, with the mean temperature during the growing period at each habitat. This suggests that adaptive differentiation in the delay before bud initiation was caused by selective pressure resulting from temperature differences among habitats. Our results suggest that based on adaptive diversity and neutral genetic diversity, the Saitama population represents a new conservation unit.

1. **Introduction**

The extent of genetic diversity that is maintained among and within remnant wild populations plays a key role in understanding the adaptive evolution of a species in response to fluctuating environments. Such knowledge can therefore support the conservation of endangered species (Frankham *et al*., 2002). Similarly adaptive genetic diversity within and among local populations is particularly important when conservation procedures such as translocation of plant materials between sites or the restoration of local populations is promoted (McKay & Latta, 2002). The term ‘Restoration’ is commonly used for the maintenance of the genetic structure, i.e. the hierarchical structure of genetic variation, in a species and many researchers used different approaches for the implication of restoration efforts in endangered species (e.g. Gordon & Rice, 1998). In this context, a ‘conservation unit’ is a group of populations of a species whose genetic structure differs from that of other groups of populations (i.e. differs from other conservation units).

Genetic variation is mainly evaluated using quantitative traits and molecular markers. The evaluation of quantitative traits in common-garden studies began more than 60 years ago (Clausen *et al*., 1940). The utilization of molecular markers in such studies has become increasingly common during the last several decades (Haig, 1998), because it is easier to evaluate genetic variation using molecular markers. The relationships between quantitative and molecular genetic variation within and among populations have

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been tested by many researchers (Karhu et al., 1996; Merilä & Crnokrak, 2001; Reed & Frankham, 2001; Bekassy et al., 2003; Carvajal-Rodriguez et al., 2005), but no significant relationship was found between the two parameters in many cases. As a result, because molecular markers do not reflect the differentiation among populations in their adaptive traits, it has become necessary to directly evaluate the genetic variation of these populations based on quantitative traits in conservation efforts (Storfer, 1996; Mckay et al., 2001; Holderegger et al., 2006).

Population genetic structure is often quantified using Wright’s \( F_{ST} \) parameter in combination with the analogous measure for quantitative traits, \( Q_{ST} \) (Spitze, 1993). When \( Q_{ST} \) is compared directly with \( F_{ST} \), there are three possible patterns (Merilä & Crnokrak, 2001; McKay & Latta, 2002): (1) when \( Q_{ST} > F_{ST} \), populations have become differentiated as a result of directional natural selection that acts on different phenotypes among the populations; (2) when \( Q_{ST} = F_{ST} \), differentiation among populations has arisen mainly through genetic drift; or (3) when \( Q_{ST} < F_{ST} \), stabilizing selection has favoured the maintenance of the same phenotype in different populations. Thus, by comparing \( Q_{ST} \) with \( F_{ST} \), it becomes possible to identify adaptive traits that show adaptive differentiation among populations as a result of directional selection.

Understanding adaptive differentiation by comparisons of \( Q_{ST} \) with \( F_{ST} \) has become increasingly popular in the last 5 years (Leinonen et al., 2008). Despite wide recognition of the importance of evaluating both adaptive and molecular genetic diversity, few conservation units have been defined for use in conservation efforts that address both adaptive and molecular genetic variation (de Guía & Saitoh, 2007). Many studies using molecular markers have assigned conservation priority to populations that are considered to represent conservation units (e.g. Moritz, 1994), but Crandall et al. (2000) and Fraser & Bernatchez (2001) emphasized that conservation units should be defined by considering both adaptive and molecular genetic variation. Although increasing numbers of studies have defined conservation units based on both parameters (e.g. Denoël, 2007; Cano et al., 2008), few of these studies have focused on plants (see, de Guía & Saitoh, 2007).

**Primula sieboldii** E. Morren is a perennial clonal herb that occupies a range of moist habitats, from the understory of deciduous forests to well-managed grasslands. The species is distributed in Japan, on the Korean Peninsula, in northern China, and in eastern Siberia (Yamazaki, 1993). However, overexploitation and destruction of its habitats and horticultural collection are threatening wild populations with extinction, and the species has become endangered in Japan; originally classified in the lower rank of ‘vulnerable’, the species was reclassified to ‘near threatened’ in 2007 (Iwatsuki, 2008).

The genetic diversity of *P. sieboldii* has been evaluated using cpDNA (Honjo et al., 2004) and microsatellite markers (Honjo et al., 2009) to define conservation units in Japan. These studies revealed that genetic differentiation among populations corresponded to the geographic distance between populations, and that genetic variation within populations correlated with population size. On this basis, we defined four conservation units.

In our previous research on quantitative traits, we investigated the genetic diversity in the petal shape and area of *P. sieboldii* in a common garden and revealed that genetic variation was mostly maintained within wild populations, i.e. low differentiation among populations (Yoshida et al., 2008). These traits were not under directional selection and appeared to be basically selection-neutral. However, there is less information on adaptive traits, and understanding adaptive genetic diversity is required for more precise definition of conservation units for *P. sieboldii*.

In the present study, we evaluated genetic variation of six quantitative traits within and among wild populations of *P. sieboldii* to obtain new information that would help us understand the adaptive traits and genetic structure of this species. By comparing \( Q_{ST} \) with \( F_{ST} \), calculated using eight microsatellite (simple sequence repeat (SSR)) markers, we estimated the cause of genetic differentiation among the populations and identified potentially adaptive traits of *P. sieboldii*. In addition, we investigated the relationships between quantitative genetic variation and five ecological factors in order to detect the potential causes of any differentiation among populations. Ultimately, we attempted to use this new knowledge to redefine the conservation units for *P. sieboldii* by considering both adaptive and molecular genetic variation.

### 2. Materials and methods

#### (i) Plant species and sampling populations

Each genet is composed of various numbers of physiologically independent ramets, which propagate clonally by means of short rhizomes. In early spring, these rhizomes bud and subsequently flower from April to June. After July, new rhizomes for next year are generated from a mother ramet below the ground.

In 2000, we collected 110 genets of *P. sieboldii* from five wild populations in three regions of Japan (Table 1): Hokkaido (a lowland area in northern Japan), Saitama (a lowland area in central Japan) and Nagano (a highland area in central Japan). In the Hokkaido and Nagano 1 populations, we randomly
sampled 26 and 27 genets, respectively, from a population that contained more than 100 genets. In the Nagano 2 and Nagano 3 populations, we sampled nearly all genets (26 and 13, respectively). For the Saitama population, we used 18 genets that had been grown from seeds obtained from a natural population in Saitama region in a previous experiment (Washitani & Kabaya, 1988). These 18 genets were from several different mothers and originated from the region surrounding Saitama population from the results of both an assignment test based on nuclear DNA markers and cpDNA (Honjo et al., 2004, 2008; Honjo, 2005).

After these samples were obtained, the ramets were acclimatized in Tsukuba (a lowland area of central Japan) for 3 years. Each genet used in the present study had a unique SSR genotype (Honjo et al., 2009).

In this study, we selected three regions (Hokkaido, Saitama and Nagano) with sample sites distributed over a wide range of altitudes (from 10 to 1120 m asl) and latitudes (from 35°50’N to 42°31’N) (Table 1). We selected three Nagano populations to investigate whether adaptive differentiation has occurred on a small geographical scale.

Meteorological data for each habitat were obtained from the Japan Meteorological Agency Web site (http://www.jma.go.jp/jma/indexe.html) (Table 1). Mean temperatures from February to June (the growing period of *P. sieboldii*) were obtained using data from 1979 to 2000. Because the Nagano 2 and Nagano 3 sites are so close to each other, they had the same mean temperature.

(ii) Common-garden experiment

From 2003 to 2005, we grew 425 to 450 ramets from the 110 genets in a greenhouse at the University of Tsukuba (140°10’E, 36°11’N) to evaluate genetic variation in quantitative traits. Clonally propagated shoots were planted into 12-cm pots at a depth of 1 cm below the soil surface in Metro-Mix 350 (Hyponex Japan, Corp.) in January. The pots were assigned randomly to their positions in the greenhouse. Four ramets from each genet were used as replications, when available; otherwise, we used two or three ramets.

We measured six quantitative traits: budding date, first flowering date, number of flowers, number of leaves, and the lengths of the peduncle at the start and end of flowering. Because the heights of the plants surrounding *P. sieboldii* in the habitats increased greatly from early spring to early summer, and the environmental conditions of the habitats between the start and end of the flowering period also changed greatly, we measured the two lengths of the peduncle. We measured all traits for each ramet in each of the 3 years of the study period. We scored the date when

| Population Location | Latitude | Longitude | Altitude | No. of genets in population | Average temperature (°C) February | March | April | May | June |
|---------------------|----------|-----------|----------|-----------------------------|----------------------------------|------|------|-----|------|-----|
| Hokkaido Southern   | 42°31’N  | 142°01’E  | >300 m   | 140                         | -5.1                             | 4.2  | 4    | 0.7 | 0    |
| Saitama Central     | 35°50’N  | 139°30’E  | >940 m   | >300                        | 28                               | 21   | 18   | 16  | 15   |
| Nagano 1 Central    | 38°50’N  | 138°38’E  | 940 m    | 26                          | 26                               | 16   | 15   | 13  | 12   |
| Nagano 2 Central    | 36°20’N  | 138°31’E  | 940 m    | 26                          | 26                               | 16   | 15   | 13  | 12   |
| Nagano 3 Central    | 36°21’N  | 138°21’E  | >1120 m  | 16                          | 16                               | 15   | 15   | 13  | 12   |

Average temperatures represent means from 1979 to 2000 obtained from the Japan Meteorological Agency Web site (http://www.jma.go.jp/jma/indexe.html).
a plant attained a height of 1 cm above the soil surface as the budding date. To ensure that we did not miss budding or first flowering, we surveyed the plants daily at the start of each growing season. Before analysis, we converted the budding date and the first flowering into the number of days to bud initiation and flowering from the first budding date observed for the entire population of ramets. We measured the lengths of the peduncle on the first flowering day and at the end of the flowering period, i.e. the date when all flowers had abscised. We recorded the number of flowers and leaves at the end of the flowering period.

(iii) DNA extraction and genotyping of microsatellite markers

We extracted the genomic DNA of each plant from frozen leaves using a modified Cetyl trimethyl ammonium bromide (CTAB) method (Murray & Thompson, 1980). We have already developed many microsatellite markers for this species (Ueno et al., 2003), but we have no information on linkage between these markers and quantitative traits. We determined the genotypes of the 110 genets using the eight pairs of microsatellite PCR primers (Table 2) used by Honjo et al. (2009). The PCR conditions for each primer followed the protocol described by Honjo et al. (2009). The PCR products were analysed using a 3100 Genetic Analyzer and the GeneScan software (Applied Biosystems).

3. Data analysis

(i) Evaluation of quantitative traits

We used factorial analysis to examine the effects of population, year, the population × year interaction and genet on the investigated traits using the values for each ramet. We performed restricted maximum likelihood (REML) tests to estimate the contribution of variance among populations, among genets within a population and among ramets within a genet to the total phenotypic variance for each investigated trait. The variation among ramets was assumed to represent environmental variation, because each ramet of a given genet has an identical genotype. Thus, the variation among genets and populations was considered to represent genetic variation, and we calculated broad-sense heritability (\( h^2 \)) by dividing the genotypic variance by the total variance (Falconer, 1981). We used the Tukey–Kramer multiple-comparison test (with \( P < 0.05 \) considered significant) to detect significant differences in the average values among pairs of populations for all traits. All statistical tests were performed using the JMP 6.0 statistical software (SAS Institute Inc.).

(ii) Genetic variation within populations

The genetic variation within populations in quantitative traits, i.e. the variation among genets \( V_g \) was calculated as

\[
V_g = \frac{\text{MS genets} - \text{MS ramets}}{r},
\]

where MS represents the mean square. To represent the number of ramets per genet, we used \( r \), the effective number of replicates:

\[
r = \left( \frac{\sum a r_i - \sum a r_i^2 / \sum a r_i}{(a-1)} \right)
\]

where \( a \) is the number of genets and \( r_i \) is the number of replicates of genet \( i \).

To evaluate the genetic variation for the eight microsatellite markers, we used the FSTAT 2.9.3 software (Goudet, 2001) to calculate unbiased estimates of gene diversity averaged over all loci (\( H_e \); Nei & Roychoudhury, 1974); the expected number of alleles observed in 13 genets from each population,
Table 3. Number of genets and years analysed for each trait in this study

<table>
<thead>
<tr>
<th>Traits</th>
<th>Number of genets</th>
<th>Number of years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to bud initiation</td>
<td>104</td>
<td>3</td>
</tr>
<tr>
<td>Days to flowering</td>
<td>93</td>
<td>2</td>
</tr>
<tr>
<td>Length of peduncle at the start of flowering</td>
<td>91</td>
<td>2</td>
</tr>
<tr>
<td>Length of peduncle at the end of flowering</td>
<td>88</td>
<td>2</td>
</tr>
<tr>
<td>Number of flowers</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>92</td>
<td>3</td>
</tr>
</tbody>
</table>

which represents the smallest sample size ($n_{th}$, allelic richness).

(iii) Genetic variation among populations

We estimated the degree of population differentiation for each of the investigated traits using $Q_{ST}$ (Spitze, 1993), which was calculated as

$$Q_{ST} = \frac{\sigma_{gb}^2}{\sigma_{gb}^2 + 2\sigma_{gw}^2}$$

where $\sigma_{gb}^2$ is the between-population variance component and $\sigma_{gw}^2$ is the within-population, i.e. among genets variance component taken from the nested ANOVA using the JMP software.

The degree of population differentiation in molecular markers ($F_{ST}$; Weir & Cockerham, 1984) was estimated using the genotype data for the 110 genets based on the eight pairs of microsatellite primers. Furthermore, we compared the pairwise $Q_{ST}$ and $F_{ST}$ values to provide insights into the potential mechanisms responsible for genetic differentiation among the populations. We tested for correlations between each pair of $Q_{ST}$ and $F_{ST}$ values using the Mantel test (Mantel, 1967). We calculated $F_{ST}$ and the pairwise $F_{ST}$ and carried out the Mantel test using the FSTAT 2.9.3 software.

(iv) Relationships between pairwise $Q_{ST}$ and ecological factors

To explore the potential causes of differentiation among populations, we investigated the relationships between five ecological factors and the traits that revealed to have differentiated among the populations by directional selection from the relationship between $Q_{ST}$ and $F_{ST}$. We investigated between four environmental factors (temperature, altitude, latitude and longitude) and the average values of these traits per population, respectively. We also investigated the relationship between geographical distance and the pairwise $Q_{ST}$ for these traits. These relationships were quantified using Spearman’s rank-correlation coefficient, calculated using the JMP software.

4. Results

Because few genets flowered in 2005, we have only analysed the number of days to flowering, the number of flowers, and the lengths of the peduncle at the start and end of flowering using the 2003 and 2004 data. Table 3 shows the number of genets and years that we actually analysed.

(i) Evaluation of quantitative traits

All traits differed significantly among genets ($P < 0.01$; Table 4). This suggests that all the quantitative traits were determined by genetic factors. All traits differed significantly among years, with large $F$-values (Table 4), indicating that quantitative traits were greatly influenced by variations in environmental effects among the years. In 2004, the number of days to bud initiation was longer, the length of the peduncle at the start of flowering was longer, the length of the peduncle at the end of flowering was shorter and the number of leaves was smaller than in 2003; this was also true in 2005.

The number of days to bud initiation, number of days to flowering, number of flowers and number of leaves differed significantly among populations, but the two lengths of the peduncle did not ($P < 0.01$; Table 4). The number of days to bud initiation showed the largest $F$-value of the six traits (Table 4), and the Saitama population produced buds consistently and significantly earlier than the other populations in all 3 years, whereas Nagano 2 tended to produce buds later than the other four populations, with the difference significant in 2003 (Table 5). The number of days to flowering tended to differ between years in each population (Table 5). For the number of flowers, the differences among populations were smaller in 2004 than in 2003, and there were fewer significant differences. The genets from the Saitama and the Nagano1 populations tended to have a large number of leaves in all 3 years (Table 5).

We found a significant population × year interaction for three traits (Table 4): the number of days to bud initiation and flowering (both $P < 0.01$) and the length of the peduncle at the end of flowering ($P < 0.05$).

Table 6 presents the broad-sense heritability values for the six traits. The heritabilities of all traits were largest in 2003, with one exception: the value for the number of leaves was largest in 2005. In each year, the number of days to bud initiation consistently showed the smallest variance among the ramets and the largest heritability (61.2–74.6), whereas the
number of leaves showed the biggest variance among ramets and the smallest heritability (32.4–40.1).

(ii) Genetic variation in quantitative traits within and among populations

Of the total variance in the number of days to bud initiation, an average of about 45% was partitioned among the populations across the 3 years, and the variance among populations was larger than that among genets (Table 6). For the other five traits, the variance among genets was larger than that among populations. This reveals a high degree of genetic variation within the populations.

The degree of genetic differentiation among populations ($Q_{ST}$) ranged widely, from 0.004 to

<table>
<thead>
<tr>
<th>Table 4. Results of the factorial analysis for the six investigated traits</th>
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<td><strong>Factors</strong></td>
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<td>-------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Population</td>
</tr>
<tr>
<td>Year</td>
</tr>
<tr>
<td>Population × Year</td>
</tr>
<tr>
<td>Genet</td>
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</tbody>
</table>

*P < 0.05, **P < 0.01.

<table>
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<tr>
<th>Table 5. Mean values and genetic variation ($V_g$) for each trait within the five populations from 2003 to 2005</th>
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<tbody>
<tr>
<td><strong>Days to bud initiation</strong></td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Mean $V_g$</td>
</tr>
<tr>
<td>Hokkaido</td>
</tr>
<tr>
<td>Saitama</td>
</tr>
<tr>
<td>Nagano 1</td>
</tr>
<tr>
<td>Nagano 2</td>
</tr>
<tr>
<td>Nagano 3</td>
</tr>
<tr>
<td>Mean $V_g$</td>
</tr>
<tr>
<td>Hokkaido</td>
</tr>
<tr>
<td>Saitama</td>
</tr>
<tr>
<td>Nagano 1</td>
</tr>
<tr>
<td>Nagano 2</td>
</tr>
<tr>
<td>Nagano 3</td>
</tr>
</tbody>
</table>

Means within a year followed by different letters differ significantly among populations ($P < 0.05$).
The QST values for the number of days to bud initiation were particularly large (0.400–0.623), indicating that this trait was considerably differentiated among the populations. Of the other five traits, QST ranged from 0.004 for the length of the peduncle at the end of flowering in 2004 to 0.296 for the number of leaves in 2005, and these QST values were clearly smaller than those for the number of days to bud initiation.

(iii) Molecular versus quantitative genetic variation

According to our SSR analysis, each locus was polymorphic, with 6–24 alleles per locus (Table 2), and we detected a total of 96 alleles over the eight loci. Mean He ranged from 0.604 to 0.751, mean n13a on ranged from 4.03 to 6.63.

The FST value calculated using the data for the eight SSR markers was 0.172 (two-sided 95% confidence interval, 0.115–0.232). The average QST values for the number of days to bud initiation and the number of leaves were considerably and slightly larger than FST, respectively (Fig. 1), whereas the average QST values for the lengths of the peduncle at the start and end of flowering were smaller than FST. The average QST for days to flowering and the numbers of flowers was approximately equal to FST.

Pairwise QST and pairwise FST were not significantly correlated for any parameter. However, the pairwise QST value for the number of days to bud initiation differed greatly between the Saitama population and the other four populations in 2004 and 2005 (Fig. 2). These results suggest that the Saitama population differed considerably from the other populations. While the pairwise QST value for the number of leaves ranged widely from 0 to 1 and Saitama population differed from each population expect Nagano 1 population in 2005, this difference is not yet pronounced as the days to bud initiation (Fig. 2).

Table 6. Proportions (%) of the three variance components (among populations, genets, and ramets) calculated by means of REML, and the broad-sense heritability (h2) values for the investigated traits from 2003 to 2005. QST represents the degree of genetic differentiation among populations

<table>
<thead>
<tr>
<th></th>
<th>Days to bud initiation</th>
<th>Days to flowering</th>
<th>Length of peduncle at the start of flowering</th>
<th>Length of peduncle at the end of flowering</th>
<th>Number of flowers</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>42.6</td>
<td>44.3</td>
<td>47.0</td>
<td>17.6</td>
<td>16.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Genet</td>
<td>32.0</td>
<td>21.0</td>
<td>14.2</td>
<td>45.5</td>
<td>39.7</td>
<td>53.8</td>
</tr>
<tr>
<td>Ramet</td>
<td>25.3</td>
<td>34.6</td>
<td>38.8</td>
<td>36.9</td>
<td>43.4</td>
<td>42.9</td>
</tr>
<tr>
<td>h2</td>
<td>0.07</td>
<td>0.61</td>
<td>0.56</td>
<td>0.63</td>
<td>0.61</td>
<td>0.71</td>
</tr>
<tr>
<td>QST</td>
<td>0.400</td>
<td>0.513</td>
<td>0.623</td>
<td>0.162</td>
<td>0.175</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Variance values may not add to 100% due to rounding errors.

Fig. 1. QST values varies for all traits in 2003, 2004 and 2005, and the FST value evaluated using the data for the eight microsatellite markers in Table 2. The grey lines represent the two-sided 95% confidence interval for FST (mean = 0.172, range = 0.115–0.232). Traits: DB, days to bud initiation; DF, days to flowering; LPS, length of the peduncle at the start of flowering; LPE, length of the peduncle at the end of flowering; NF, number of flowers; NL, number of leaves.

(iv) Relationships between quantitative traits and ecological factors

Because the average QST values for the number of days to bud initiation and the number of leaves were larger than FST (Fig. 1), we examined the relationship between these parameters and the five ecological factors. The mean temperature of each month, from February to June, were significantly negatively correlated with the number of days to bud initiation in 2004 and 2005, and they were significantly positively correlated with the number of leaves in all 3 years (Fig. 3), suggesting that the differences in mean values of these parameters among populations correspond to differences in the mean temperature of each habitat. Latitude, longitude and altitude were not significantly correlated with the mean values of these two parameters, and geographical distance was not significantly correlated with the pairwise QST for these two traits.
Days to bud initiation

Number of leaves

Fig. 2. Relationship between the pairwise $Q_{ST}$ and pairwise $F_{ST}$ values for days to bud initiation and the number of leaves. The data points surrounded by a dotted border represent the genetic distance between the Saitama population and the other four populations.

Days to bud initiation

Number of leaves

Fig. 3. The relationship between the mean temperature and the mean number of days to bud initiation and the mean number of leaves in 2003, 2004 and 2005. Because the relationships at each month were same patterns, only mean February temperature was shown. The data points surrounded by a dotted border represent the data of Saitama population. *$P<0.05$, **$P<0.01$. 
5. Discussion

$H_e$ and $n_{aa}$ of five populations were compared to those of wild populations evaluated by Honjo et al. (2009), and genetic variation in the five populations is reflected in the mean variation that *P. sieboldii* have maintained in Japan.

(i) What traits were adaptive in *P. sieboldii*?

The lengths of the peduncle at the start and end of flowering did not differ significantly among populations (Table 4), although the length of the peduncle at the end of flowering varied among the years. The mean $Q_{ST}$ was smaller than $F_{ST}$ for both parameters (Fig. 1), suggesting that the length of the peduncle has not undergone directional selection and maintained mostly genetic variation within populations. However, the numbers of days to bud initiation, days to flowering, flowers, and leaves differed significantly among the populations (Table 4). The numbers of days to flowering and flowers appear to have become differentiated among populations mainly as a result of genetic drift because $Q_{ST}$ was nearly equal to $F_{ST}$ for these parameters (Fig. 1). Because high genetic diversity of these traits is maintained within the wild populations, $Q_{ST}$ values were relatively low (Table 6). The mean $Q_{ST}$ values for the number of days to bud initiation and the number of leaves were both larger than the corresponding $F_{ST}$. However, we do not necessarily conclude that the number of leaves is an adaptive trait from the result that this trait showed the smallest heritability in all years and is susceptible to environmental variation. Thus, the days to bud initiation is likely to be involved in adaptive differentiation among the populations as a result of directional selection.

The mean numbers of days to bud initiation in 2004 and 2005 were negatively correlated with the mean temperatures at each site (Fig. 3; $P<0.05$), indicating that the mean temperature of the Saitama population, which appears to have differed from the other populations (Fig. 2), is considerably warmer (by more than 5 °C) than that of the other populations (Table 1). Thus, the winter temperature appears to be involved in determining the earliness of the budding date in *P. sieboldii*. In the Nagano region, which is located at the highest altitudes of the five study areas and showed correspondingly low temperatures, sudden late frost is often observed until the middle of May. Because *P. sieboldii* usually buds in early spring, genets with early budding are susceptible to frost injury and selection would favour genets with late budding in all except the Saitama population, where sudden late frosts are uncommon. In out-crossing forest trees such as Scots pine, *Populus* spp. and Douglas-fir, it has often been reported that traits related to budding, such as bud set and bud flush dates, are adaptive traits that are closely related to winter temperatures (Hurme et al., 1997; Frewen et al., 2000; Howe et al., 2003).

Positive relationships between bud flush and cold hardiness have been reported, and their quantitative trait loci (QTLs) were detected at the same position as a result of pleiotropy or strong linkage (Howe et al., 2003). Winter chilling, spring temperatures and frost are factors capable of controlling the timing of bud flush (Jermstad et al., 2003). Thus, low temperatures in early spring appear to act as a selective pressure that selects genets with late budding in the three Nagano populations and the Hokkaido population. In contrast, genets with early budding appear to have been selected in the Saitama population. Frewen et al. (2000) noted that trees in which bud flush occurs too late in the spring have a shortened growing season that reduces their competitive ability and growth potential. Thus, temperature appears to be a selective factor involved in local adaptation, and phenotypic fixation of the budding date has occurred as a result of the selection of optimal genotypes in each habitat.

(ii) Defining conservation units for *P. sieboldii* based on adaptive and neutral genetic markers

Honjo et al. (2009) defined four *P. sieboldii* conservation units (Hokkaido, northern Honshu, central Honshu and western Japan) using eight microsatellite markers and cpDNA. Based on those results, the Saitama population and the three Nagano populations in the present study belonged to the same conservation unit, i.e. central Honshu, and only the Hokkaido population belonged to a different conservation unit. However, our budding date results suggest that the Saitama population differed considerably from the other populations. Therefore, the Saitama population should represent different conservation units. Among these Nagano populations, Nagano 2 differed significantly in the budding date from Nagano 1 and Nagano 3 in 2003 (Table 5). Therefore, further study is required to exploit the genetic basis of such differences that may be because of difference in the conservation unit.

As a result, the five populations in the present study could be divided into three conservation units previously proposed by Honjo et al. (2009) as well as at least one new conservation unit based on our analysis of both their adaptive traits and molecular markers. These results clearly demonstrate the importance of defining conservation units based on adaptive diversity, not just molecular genetic diversity.

Hypothetically speaking, if transplantation of genets is necessary to maintain remnant populations of *P. sieboldii*, it should be guided by a careful consideration of the genetic composition of the genets in
terms of the budding date, which represents an important adaptation to environmental conditions. The introduction of foreign genotypes that have adapted to a different environment can potentially disrupt the genetic architecture of native populations, which is optimal for long-term existence of these populations in their native habitat. This phenomenon has been called ‘outbreeding depression’ and has frequently been reported for some species (Keller et al., 2000; Montalvo & Ellstrand, 2001). For *P. sieboldii*, the temperature during the budding stage appears to be a strong selection force for the budding date. Therefore, it should be possible to roughly estimate the timing of budding using the mean February temperature at a genet’s native habitat. Wild genets or populations used for transplantation between regions should be selected based on the temperature during the budding stage, not based solely on geographical distance.

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References


Keller, M., Kollmann, J. & Edwards, P. J. (2000). Genetic introgression from distant provenances reduces fitness in...


