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Ex situ Conservation Studies of *Primula sieboldii* (Primulaceae) in the Tsukuba Botanical Garden: I. Seed Production by Open Pollination

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田中法生*・福田陽子**：筑波実験植物園におけるサクラソウの自生地外保全に関する研究：1. 自然交配による種子生産

The ideal strategy for conserving rare species is *in situ* conservation, protecting the natural habitat as a whole. However, if a native population is very small, fragmented or isolated, and if its habitat may be destroyed in the near future, then *ex situ* conservation can be an effective method. Millar and Libby (1991) suggested that *ex situ* collections also serve an essential role in filling gaps and extending the range of genetic variation that is protected, and as a source of germplasm to damaged habitats. Kennedy (1987) and Robinson (1992) stated that *in situ* and *ex situ* conservation strategies are complementary approaches.

In *ex situ* conservation, attention must be paid to conserving the genetic diversity of species or populations that require cross-fertilization for seed production. Cross-fertilization in entomophilous species normally results only from pollination by insects. Although hand pollination may be useful in some species, to obtain seeds by hand pollination, a mixture of pollen grains derived from several pollen donors must be used (Domínguez *et al.* 1997; Mitchell and Marshall 1998), or hand pollination results in fewer seeds (Hiei 1997; Fukuda 1998).

Threatened species, *Primula sieboldii* E. Morren is heterostylous, which is thought to be adaptive and to facilitate intermorph pollination by insect pollinators (Darwin 1877; Piper and Charlesworth 1986; Barrett and Glover 1985). Therefore, seed production requires insect pollinators. Under severe pollinator limitation, fewer seeds are produced than under high pollinator activity (Washitani *et al.* 1994, 1995). Washitani's (1996) simulation study predicted that under severe pollinator limitation, the thrum morph will disappear from a population, even with high inbreeding depression. Undoubtedly, low pollinator activity is not suitable for maintaining genetic diversity in *ex situ* conservation. It has been suggested that the most useful pollinators are *Bombus diversus tersatus* Smith in Hokkaido and B. *diversus diversus* Smith in Honshu (Washitani *et al.* 1995). The activities of bumblebees in Tsukuba Botanical Garden, however, have never been investigated. Therefore, an assessment of seed production in the garden must clarify how often useful pollinators visit the populations. These data will give us a guide to *ex situ* conservation in the garden.

To obtain basal data for the *ex situ* conservation of *P. sieboldii* in the Tsukuba Botanical Garden, this study investigated the seed production of three populations of *P. sieboldii* in the garden, with open pollination. We compare these results with data for natural populations, assess seed production, and

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Fig. 1. General view and *Primula sieboldii* of three study sites: ST (A, B), CE (C, D) and MO (E, F). Arrows show the location of *P. sieboldii* in each site. All photographs were taken in May 1999 except for E photo taken in August 1999.

make some assumptions on the level of bumblebee pollination. Finally, we discuss how to accomplish *ex situ* conservation in the garden with open pollination.

Study sites

Primula sieboldii populations were investigated at three sites in the Tsukuba Botanical Garden (Amakubo 4-1-1, Tsukuba, 305-0005, Japan) (Fig. 1). One population was along the stream through the Sandy and Gravelly Section (High Altitude) (ST), where there is gravel and soil on the riverbank, and shrubs provide some shade. The second was in the Central Circle (CE), a marsh with sand and soil, where there is no shade. The third was in the Montane Grassland Section (High Altitude) (MO), a marsh with gravel, where shrubs and herbs provide shade.

Materials and Methods

Populations ST and CE were introduced in April 1999 from seedlings derived from a natural population in Monbetsu, Hokkaido. Population MO was introduced from a natural population in Tajimagahara, Urawa, Saitama, in May 1991. The three populations contain about 135 (ST), 165 (CE), and 50 (MO) individuals, respectively.

Plants in each location were identified as the pin or thrum morph, and labeled with plastic tags. The numbers of open flowers, fruited ovaries, and mature seeds in each ovary were counted for all the plants labeled. A fruited ovary is a fruit containing more than one seed. We calculated the seed set and fruit set from these data. Fruit set is the number of fruited ovaries per flower, and seed set is the number of mature seeds per flower. Differences in the seed and fruit set between morphs and sites were tested using the Mann-Whitney U-test or the Kruskal-Wallis test.

Table 1. Fruit sets for pin and thrum morphs of *Primula sieboldii* by open pollination in the three site of Tsukuba Botanical Garden.

	Pin		Thrum		Total	
Site	mean±SD (N)	range	mean±SD (N)	range	mean±SD (N)	range
ST	0.80±0.2(33)	0-1.0	0.66±0.4(150)	0-1.0	0.68±0.4(183)	0-1.0
CE	0.45±0.1(4)	0.29-0.5	0.25±0.2(39)	0-0.6	0.27±0.2(43)	0-0.6
MO	0.88±0.1(14)	0.8-0.91	0.86±0.2(39)	0.5-1.0	0.86±0.2(53)	0.5-1.0

Table 2. Seed sets for pin and thrum morphs of *Primula sieboldii* by open pollination in the three site of Tsukuba Botanical Garden.

	Pin		Thrum		Total	
Site	mean±SD (N)	range	mean±SD (N)	range	mean±SD (N)	range
ST	58.72±38.1(33)	0-118	28.25±32.4(141)	0-122	34.03±35.5(174)	0-1.0
CE	17.33±40.5(6)	0-100	27.20±28.5(6)	0-102	24.92±31.1(26)	0-0.6
MO	27.15±19.4(13)	5-69	0.86±0.2(39)	0-85	36.98±25.8(50)	0.5-1.0

Results

We observed mature *P. sieboldii* fruits and seeds at all three sites. The range for fruit set in the pin and thrum morphs was from 0 to 1.0. The range for seed set for the pin morph was from 0 to 118 and for thrum was from 0 to 122. The average values for fruit set and seed set are shown in Tables 1 and 2. The mean values of the fruit set for pin and thrum differed between the sites (Kruskal-Wallis test, $p<0.01$, $p<0.001$), and the seed set also differed (Kruskal-Wallis test, $p<0.01$, $p<0.05$). These values were lower in site CE. The mean overall fruit set and seed set was significantly greater for the pin than for the thrum morph (Mann-Whitney U-test, $p<0.001$, $p<0.01$). At site ST, the fruit and seed sets for pin were significantly greater than for thrum (Mann-Whitney U-test, $p=0.001$, $p<0.001$), while at the other sites, the differences between morphs were not significant.

Discussion

In this study, we observed high fruit set and seed set. These were much higher values than those for the Tajimagahara nature reserve population, where low pollinator activity and low fruit and seed sets are reported (pin: 0.20 ± 0.021 fruit set and 7.64 ± 18.59 seed set, thrum: 0.09 ± 0.13 and 20.9 ± 8.86) (Washitani *et al.* 1994), while they are similar to those of a Hokkaido population, where high pollinator activity and high fruit and seed sets are reported (pin: 0.53 ± 0.366 and 27.12 ± 22.92, thrum: 0.67 ± 0.299, 30.6 ± 22.35) (Washitani *et al.* 1995). Therefore, *P. sieboldii* seed production in the garden was assessed as high, and as similar to that of a natural population with high pollinator activity. Since Washitani *et al.* (1994) reported that little or no fruit or seed sets were observed after self-pollination in either morph, it is reasonable to suppose that some pollinators frequently visited *P.*

Fig. 2. Claw marks (arrows) made by bumblebees on the petals of the flowers of *Primula sieboldii* in the MO site.

sieboldii at the three sites. Claw marks were observed on some flowers (Fig. 2). Washitani *et al.* (1995) stated that claw marks, which result from the bumblebee's habit of clinging to the flower while visiting, seem to be a useful indicator of the level of pollinator service provided by bumblebees. Judging from the above, we think that bumblebees frequently pollinated *Primula sieboldii* in the garden. Further investigations are necessary to obtain sufficient evidence, as we did not observe any bumblebees visiting *P. sieboldii* during our study. However, bumblebees were seen visiting other flowering plants in the garden. Between 9:00 and 12:00 on 18, 22, and 26 May, we observed a total of 29 *Bombus ardens* Smith on *Weigela decora* (Nakai) Nakai, *Rosa hirtula* (Regel) Nakai, *Weigela hortensis* (Sieb. et Zucc.) K. Koch, *Rhododendron obtusum* (Lindl.) Planchon var. *kaempferi* (Planchon) Wilson, *Rosa rugosa* Thunb., *Stylax japonica* Sieb. et Zucc., *Rhaphiolepis indica* (L.) Lindl. ex Ker var. *umbellata* (Thunb. ex Murry) Ohashi, and *Rosa multiflora* Thunb., and a single *Bombus diversus* Smith on *Iris pseudacorus* L. *B. diversus* is considered to be a useful pollinator of *P. sieboldii*, because the length of its proboscis is similar to that of the corolla tube, whereas *B. ardens* is not suitable as a pollinator because of its short proboscis (Inoue and Kato 1990; Washitani *et al.* 1995).

Fruit and seed set in the CE population were lower than in the ST and MO populations. This may have been because there are few flowers around site CE, and few bumblebees were observed there.

This study showed that it is possible to produce seeds by open pollination in the Tsukuba Botanical Garden. Some issues surrounding how to accomplish *ex situ* conservation in the garden using pollinators remain to be resolved. First, a pollinator species that is useful for seed production of *P. sieboldii* should be identified. Second, the activity of this pollinator must be maintained continuously during the flowering season and over a period of several years. Since the environment of the farms and residences around the garden is not stable, pollinators that breed and live in the garden are desirable. *B. diversus* is a eusocial insect, and to survive a colony needs a continuous supply of energy in the form of nectar and pollen from flowers with a sequential flowering phenology (Heinrich 1976). Therefore, we must investigate ways to sustain a working colony, perhaps by supplying a nectar substitute when flowers are unavailable, or by providing a suitable building site for a colony.

There are many other issues that need to be addressed, including seed germination, the selection of planting sites, the proportion and spatial distribution of both morphs, and hybridization between different populations or species that are in isolated natural habitats.

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Summary

The fruit and seed sets of *Primula sieboldii* resulting from open pollination were investigated in three populations in the Tsukuba Botanical Garden, to obtain basal data for *ex situ* conservation of *P. sieboldii*. The numbers of fruit and seed sets were much higher than those seen in natural populations in the Tajimagahara nature reserve, where there is low pollinator activity and low fruit and seed set

values, but were similar to numbers seen in a Hokkaido population with high pollinator activity and high fruit and seed sets. *P. sieboldii* seed production in the garden was assessed as high, and is similar to that of a natural population with sufficient pollinating service. Although we did not observe bumblebees visiting *P. sieboldii*, two bumblebee species, *Bombus ardens* and *B. diversus*, were seen pollinating several other plant species in the garden, and they probably also pollinated the *P. sieboldii.*

摘　要

筑波実験植物園において，サクラソウの遺伝的多様性を維持できる自生地外保全のための基礎データを得るために，園内3カ所のサクラソウ個体群の自然交配による結果率，種子生産量を調査した。これらを自生地2カ所のデータと比較したところ，送粉者が制限され種子生産量が低いと報告されている田島ヶ原の個体群よりも多く，送粉者が多く種子生産量が高いと報告されている北海道の個体群と同程度であることが示された。園内での種子生産は，送粉者の豊富な自生地と同様の良好な状態と評価できる。また，園内において何らかのマルハナバチ類が頻繁にサクラソウを訪花したことが推測された。今回，サクラソウを訪花するマルハナバチ類は確認できなかったが，園内の他の植物に訪花する2種類のマルハナバチ，コマルハナバチとトラマルハナバチが観察された。

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