

## Spatial Variation in Female Fertility Related to Interactions with Flower Consumers and Pathogens in a Forest Metapopulation of *Primula sieboldii*

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**Abstract.** Antagonistic biological interactions with flower consumers and pathogens may influence reproductive success of flowering plants, affecting population dynamics and natural selection for floral traits. However, ecological and evolutionary consequences of the interactions may depend on both spatial and temporal patterns of the interactions. In a forest metapopulation of *Primula sieboldii* E. Morren, an endangered clonal plant species, we measured between-subpopulation patterns of seed sets and interactions with an influential flower consumer, a rove beetle, *Eusphalerum bosatsu* Watanabe, and a specific smut fungal pathogen, *Urocystis tranzschelina* (Lavrov) Zundel (Ustilaginales), for three years. Mean female fertility (seed set per flower) for individual subpopulations fluctuated moderately among years but was highly variable within each year among the five subpopulations studied. In two subpopulations, the impact of *Eusphalerum* beetle, was sufficiently large to result in almost complete failure in seed production over eight years including the three study and five previous preliminary observation years. In the two other subpopulations, seed set failure was caused by infection by the smut fungus. Infected capsules which constitute 10–30% of the capsules produced in the subpopulations were filled with ustilospores instead of seeds. In the subpopulation that escaped flower damage by *Eusphalerum* beetles and smut fungal infection, seed sets of both pin and thrum flowers were much higher than in the other subpopulations. The spatial restriction of individual antagonistic agents to a part of subpopulations suggest that dispersal of the agents, as well as the mode of spatial subdivision of the plant population would be important for determining the overall effects of antagonistic interactions on plant performances at the metapopulation level.

**Key words:** *Eusphalerum*, herbivore, pathogen, seed set, spatial pattern, spatial subdivision, subpopulation, *Urocystis*.

### Introduction

Antagonistic biological agents such as flower, fruit and seed consumers and pathogens may profoundly affect reproductive success of plants and influence plant population dynamics (Inouye et al. 1980; Louda 1982a, 1982b, 1989; Louda and Potvin 1995; Alexander and Antonovics 1988; Burdon et al. 1989) and natural selection for a range of floral traits including flowering phenology (Breedlove and Ehrlich 1968; Zimmerman 1980; Augspurger 1981; Gross and Werner 1983; Bertness et al. 1987; Dieringer

1991). Biological agents fluctuate in time and space. Temporal fluctuations among multiple agents, which result in variable selection on timing of flowering, are considered to be among the major factors maintaining within-population variation in flowering phenology (Schemske and Horvitz 1989; Ollerton and Lack 1992; Gomez 1993). Concerning the spatial patterns and consequences of antagonistic biological interactions in natural habitats, negative impacts of flower and seed consumers have been demonstrated to be responsible for the spatial distribution of certain plant species due to spatially differential reproductive success (Louda 1982a, 1982b; Bertness et al. 1987).

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Even within a relatively homogeneous habitat, con-specific plants often grow separately in isolated patches, of which size and distribution mainly reflect the spatial patterns of vegetative expansion and recruitment from seeds. Such spatial subdivision may influence population dynamics through spatially variable biological interactions as well as spatially heterogeneous abiotic factors such as light, moisture and soil fertility. Importance of the spatial subdivision for the dynamics and biodiversity of communities has been stressed depending on various mechanisms including prevention of the eradication of food or host species through to complete exploitation or competitive exclusion (Levin 1974; Hanski 1983; Hastings 1990; Tilman 1994).

Concerning abundance patterns of herbivores, the resource concentration hypothesis (Root 1973) predicts that specialist insects will favor dense patches of host plants because they will find and breed on dense patches more easily than in patches with few plants. Alternatively, poor dispersal of consumers is likely to result in more heterogeneous patterns of interactions (Harrison and Thomas 1991). The resource concentration hypothesis has hardly been tested for natural plant populations (Howe and Westley 1988) and few empirical data have been reported on how dispersal ability of the herbivores and the spatial structure of the plant population will influence spatial patterns of the plant-consumer interaction (Cappuccino 1987; Sollbreck and Sillern-Tullberg, 1986).

Disease spread is a function of both ecological and genetic factors, and in a plant population it may be strongly affected by the spatial and genetic structure of the population and the mode of transmission of pathogens (Burdon et al. 1989; Thrall and Jarosz 1994). Dispersal and transmission of the pathogen may be efficient if there are suitable vectors such as pollinators as in the case of anther smut disease of *Silene* (Alexander 1990), but the degree and pattern of the spatial subdivision of the population should affect disease spread crucially. In our previous studies (Washitani et al. 1991, 1994a, 1994b), we found that the seed set of *Primula sieboldii* E. Morren, an endangered heterostylous plant, greatly varies among habitats according to among-habitat variation in pollinator availability. At a smaller spatial scale, there is also a great variation of female fertility among isolated patches or subpopulations within a forest population (metapopulation) free from pollinator limitation (Takahashi et al. 1994), suggesting the importance of other sources of the variation at this spatial scale. Spatial subdivision of the population and antagonistic biological interactions are suspected as the factors responsible for the latter mode of the variation.

Therefore, to understand the roles played in fertility variability by antagonistic biological factors and spatial subdivision of the plant population, we investigated

between-subpopulation patterns of female fertility and antagonistic biological interactions with flower consumers and pathogens. We also examined the within-subpopulation distribution and dispersal of the influential consumer *Eusphalerum bosatsu* Watanabe to obtain some insights into the mechanisms involved in the generation of spatial patterns of interaction.

## Plant and study site

*Primula sieboldii* E. Morren is a clonally growing herb that occurs in a range of moist habitats throughout Japan. Like many other *Primula* species, *P. sieboldii* is distylous (Richards 1986), and like most heterostylous species, it requires insects for legitimate pollination between mutually compatible morphs, i.e., pin and thrum morphs (Ganders 1979). In recent years it has declined rapidly to be included in the national 'red list' as a 'vulnerable' category.

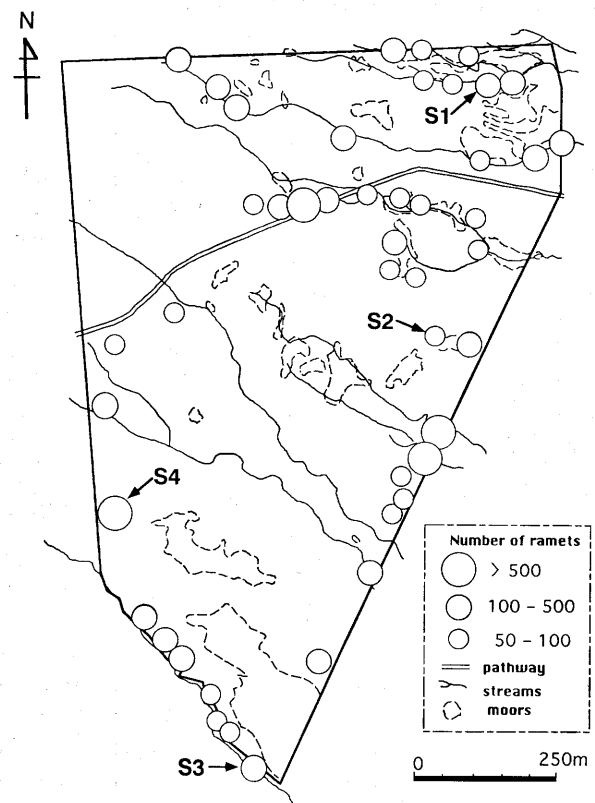


Fig. 1. Spatial distribution of subpopulations (circles) constituting a metapopulation of *P. sieboldii* in the moist forest habitat of the University Forest of the University of Tsukuba. Circle size represents the size (ramet number) of the subpopulation. Labels in bold show the subpopulations chosen for the study.

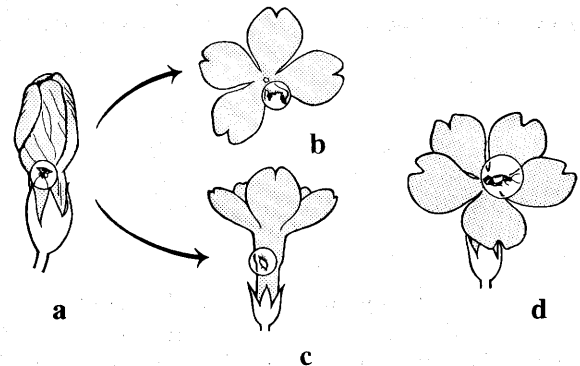
**Table 1.** Summary of the status of the subpopulations of *P. sieboldii* studied.

Subpopulation	Occupied area (m <sup>2</sup> )	No. of genets	No. of flowering ramets in 1993	Density of fl. ramets in 1993 (m <sup>-2</sup> )
S1	200	112	424	2.12
S2	200	37	65	0.33
S3	100	84	256	2.56
S4	600	113	555	0.93
S5	20	23	454	22.7

We have studied one population of *P. sieboldii* in the University Forest of Tsukuba University (79.47 ha) located at an altitude of 1350m on the flank of Mt. Yatsugatake (35°57'N, 138°28'E) in Nagano Prefecture, Central Japan. Ten-year averages for annual mean air temperature and yearly total precipitation are 6.9°C and 1400 mm, respectively. Snow falls during the winter season from November to April, with the maximum snow cover depth being less than 50 centimeters. *P. sieboldii* grows on the moist floor of forest dominated by *Quercus mongolica* ssp. *crispula* (Blume) Menitsky. The distribution of the species within this habitat is patchy, and there are dozens of isolated subpopulations, each composed of several to hundreds of ramets (Fig. 1). Since individual genets (clones) contain a small number of flowering ramets, mostly below 10, only floral morphs (pin or thrum), but not genets, were distinguished for individual flowering ramets in the present study.

Four subpopulations (S1, S2, S3, and S4 in the map shown in Fig 1) and one (S5) located outside of the University forest at a distance of several km from the University Forest were chosen for the study. The present status of the individual subpopulations are summarized in Table 1. Within the individual subpopulations, no anisoplethy was recognized for the numbers of the ramets of pin and thrum morphs.

In the forest, the species flowers in late May immediately before the seasonal regeneration of oak tree canopy. Various types of insects visit the flowers of *P. sieboldii*, including queen bumble bees of *Bombus diversus* Smith, sylphids such as *Rhigia laevigata* Loew and various butterflies such as *Polygonia c-aureum* L. (Washitani et al. 1991). Preliminary observations over several years preceding the present investigation suggested that a rove beetle, *Eusphalerum bosatsu* Watanabe, is an influential consumer of the flowers of *P. sieboldii*. Infection by a smut fungus *Urocystis tranzschelina* (Lavrov) Zundel (Ustilaginales), which is a specialist parasite of *P. sieboldii* (Kakishima et al. 1995), was also found in some subpopulations. This smut fungus causes systemic infection of *P. sieboldii* and produces sori in its ovaries. However,



**Fig. 2.** Schematic representation for the types of symptoms of flower damage by *E. bosatsu*. Arrows show time elapses with flower opening. In most flowers with any of these symptoms shown within the circles, stigmas and/or anthers were damaged. a, the presence of the rove beetle(s) in the pre-opened flower with a hole the beetles opened at the time of invasion; b, loss of a part of corolla; c, the presence of the holes on the corolla of an opened flower that the beetle(s) had made; d, the presence of the beetle(s) within the opened flower.

details of the life histories of these biological agents have not yet been studied.

## Methods

### *Antagonistic biological interactions and female fertility*

At the peak of flowering (late May) in 1991 and 1992, all the flowers in the subpopulations were inspected, and any symptoms of herbivory on individual flowers were recorded: (1) presence or absence of consumer insects (mostly *Eusphalerum* beetles) within the flower, and (2) visible damages to any part of the corolla (Fig 2). Since the reproductive organs, stigmas and/or anthers tended to be damaged in flowers with such symptoms, flowers with any of these symptoms at inspection were regarded as damaged by the consumers. In 1991, only three subpopulations, S1, S2 and S5 were censused, while all the five subpopulations were examined in 1992.

In 1993, all the inflorescences of the five subpopulations were inspected every three to four days during the flowering season and newly opened or closed flowers were marked with small strips of vinyl chloride tapes at their pedicels. At the same time, any symptoms of herbivory on the flowers were also recorded in a manner similar to that used in previous years. In late July of each year, the infructescences with newly matured capsules were harvested. The number of vestigial flowers in the individual infructescences and matured seeds in individual capsules were counted and recorded, along with the

presence or absence of damage to capsules or seeds by herbivores or pathogens. Female fertility, i.e., seed set per flower, for individual flowering ramets of both pin and thrum morphs, and the proportion of the capsules infected by smut fungi were calculated from these data.

### Abundance, distribution and dispersal of *Eusphalerum*

The abundance and distribution of the flower consuming beetle *Eusphalerum*, were studied in subpopulation S1. In the mid-flowering season of 1992, 35 unopened and 35 newly opened flowers were randomly sampled from the S1 subpopulation and the number and sex of the *Eusphalerum* beetles and the number of the mating pairs within individual flowers were censused after the corolla tubes were torn open. Sex was determined by the shape of the abdomen which is markedly thicker in females. Similar data were collected in 1993 from 189 randomly

chosen flowers. Also in the 1993 season, 46 *Eusphalerum* beetles were collected from the flowers of the subpopulation S1, each insect was uniquely marked with paint on the elytra, and released on the same flower from which it had been collected. In the next day and for two days after the release, all the flowers of the subpopulation were checked for the presence or absence of the marked *Eusphalerum* beetles. At the height of the flowering in 1994, a similar experiment was performed with 60 collected *Eusphalerum* beetles.

### Statistics

Significance of differences in seed set per flowers between years, between morphs, and among subpopulations and the effects of the interactions between years, morphs and subpopulations were tested by two-way ANOVA (Sokal and Rohlf 1981) with flowers as an error term using PROC GLM in the SAS/STAT statistical package (SAS Institute

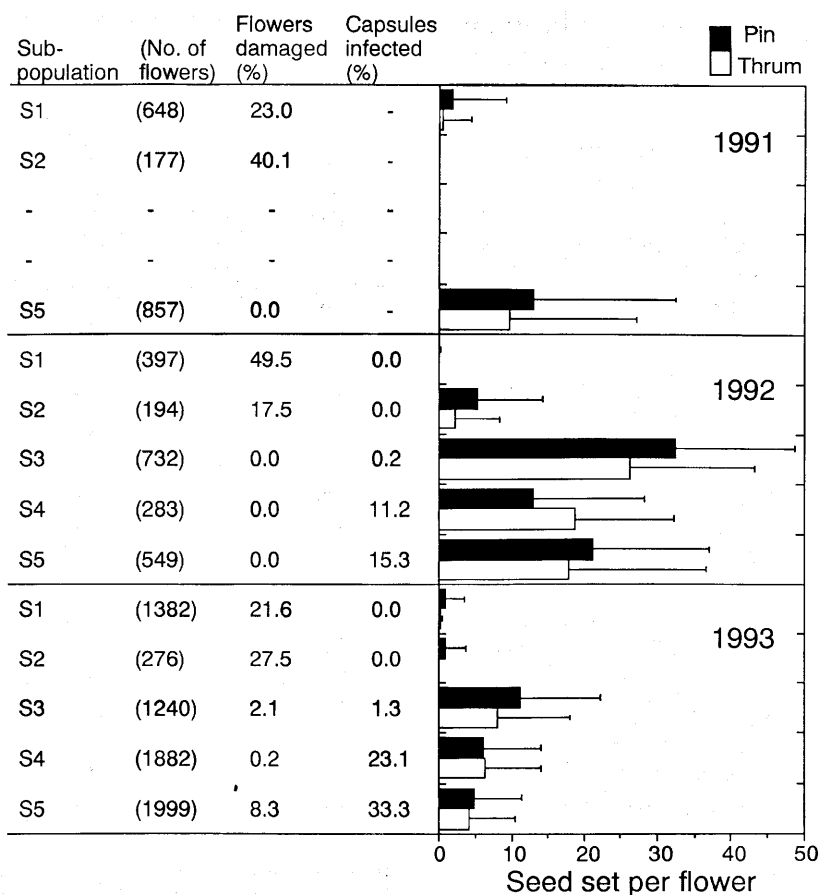


Fig. 3. Between-subpopulation patterns of flower damage by consumer insects, infection by smut fungi, *U. tranzschelina*, and female fertilities of pin and thrum morphs of *P. sieboldii*. Almost all the flower damage in subpopulations S1 and S2 could be ascribed to *E. bosatsu*, but that in S3, was due to an unidentified lepidopteran larva. In the right part, seed set per flower of pin (shaded bar) and thrum (open bar) morphs are shown for individual subpopulations. Each error bar shows 1 SD. In 1992, only subpopulations S1, S2 and S5 were investigated, and - indicates no measurement.

**Table 2.** Results of two-way ANOVA of the effects of year, subpopulation, and morph on the female fertility (seed set per flower) of the flowers of *P. sieboldii* in the five subpopulations of the forest metapopulation in the years 1992 and 1993.

Source	df	SS	MS	F-value	P-value
Year	1	133776	133776	502	0.0001
Subpop.	4	332443	83111	312	0.0001
Morph	1	1806	1806	7	0.0092
Year × Subpop.	4	98055	24514	92	0.0001
Year × Morph	1	82	82	0	0.5797
Subpop. × Morph	4	5134	1283	5	0.0007
Residual	8613	2293169	266		

1988). Type III sums of squares were utilized to account for unequal sample sizes. Paired comparisons for the means were made by Scheffe's method (Scheffe 1959).

## Results

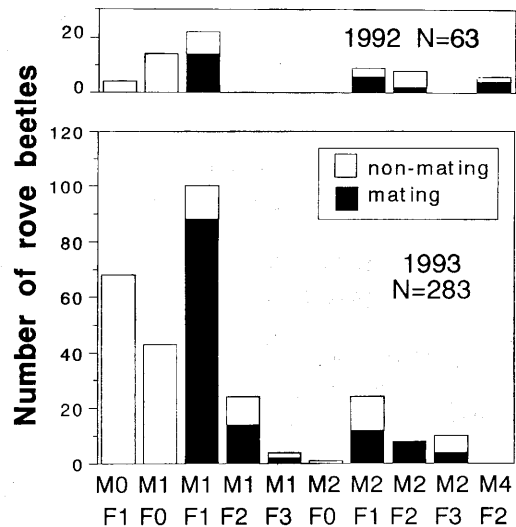
### *Biological interactions and female fertility patterns*

Three years data on the percentages of flowers damaged and the percentages of capsules infected by smut fungi for the individual subpopulations of *P. sieboldii* are shown in Fig. 3, together with the female fertility patterns for these years. Although there was significant yearly fluctuation in female fertility, there were large spatial variations between the subpopulations within each year (Table 2, Fig. 3). Between-subpopulation trends for both antagonistic biological interactions and fertilities were relatively constant over the years investigated, although there was a significant year × subpopulation interaction in seed set per flower which was caused by relatively low seed set recorded in the S5 population in 1994.

There was a significant but small difference in the fertility between the floral morphs (Table 2), with the pin morph setting slightly more seeds than the thrum morph in all the subpopulations except for S4 (Fig. 3). However, the between-morph variance was much smaller than between-subpopulation and between-year variances (Table 2).

In S1 and S2 subpopulations, relatively high percentages of flowers were damaged by *Eusphalerum* beetles in all the three years. In these subpopulations, female fertility was consistently low or negligible during these years. In S1, subpopulation female fertility (seeds/flower, mean ± SD) was  $0.06 \pm 0.39$  in 1992 and  $0.45 \pm 4.25$  in 1993 and that of S2 were  $4.59 \pm 21.33$  in 1992 and  $0.44 \pm 4.75$  in 1993.

Occurrences of flower consumers other than *Eusphalerum* beetles were negligible, but in 1993, infre-



**Fig. 4.** Distributions of the numbers of *E. bosatsu* beetles which were found co-occurring within individual flowers of *Primula sieboldii* in S1 subpopulation in various combinations of sexes (M, male; F, female; numerical, the number of the beetles of the each sex in the combination) given at the abscissa.

quent herbivory caused by larvae of unidentified Lepidoptera was observed in S3 and S4, where no activities of *Eusphalerum* beetles were detected.

In S4 and S5 subpopulations, in which flowers bore no signs of rove beetle attack, some seed set failure was caused by the infection by the smut fungi, *U. tranzschelina*, in considerable percentages of capsules (Fig. 3). Infected capsules were filled with black fungal spores and contained no seeds. In S4, percentages of infected capsules were 11.2 and 23.1% in 1992 and 1993, respectively. In a more dense subpopulation S5, these percentages were 15.3 and 33.3%. All the capsules of the infected ramets were filled with the ustulospores. In S4, subpopulation female fertility (seeds/flower, mean ± SD) was  $18.46 \pm 24.72$  in 1992 and  $6.13 \pm 15.95$  in 1993 and that of S5 was  $21.32 \pm 24.37$  in 1992 and  $4.42 \pm 12.96$  in 1993. In the subpopulation S3 which was almost free from both influences by rove beetles and smut fungi, the female fertility ( $30.5 \pm 24.2$  in 1992 and  $9.69 \pm 21.2$  in 1993) was significantly higher than in any other subpopulations ( $P < 0.01$  in Scheffe's tests).

### *Abundance, distribution and dispersal of Eusphalerum beetles*

In the censuses of *Eusphalerum* beetles in *P. sieboldii* flowers of the S1 subpopulation at the height of the flowering season, 63 and 283 beetles were found in 1992 and 1993, respectively. The frequency distribution of numbers of male and female beetles co-occurring within the same flowers and those in mating pairs are shown in

Fig. 4. The numbers of the insects occurring together with the opposite sex in the same flowers were larger than those of singles for both sexes. Although the between-year comparison is somewhat difficult due to the different sampling methods, ('opened and unopened flowers' in 1992 but only 'open flowers' in 1993) similar patterns were observed for the occurrences of the beetles in the both the years.

In 1992, a total of 39 males and 24 females were found in 70 randomly sampled flowers. Seventy one % of them occurred together with insects of the opposite sex, and 26 insects (40% of the total) were found in mating pairs.

In 1993, 128 males and 155 females were found in a total of 189 flowers. Seventy five % insects were found together with insects of opposite sex in the same flower, and mating was observed with 173 insects (46% of the total).

The *Eusphalerum* beetles were rarely observed in flowers other than those of *P. sieboldii*. In early spring of the year 1993 a few were found in the flowers of *Lonicera gracilipes* Miquel.

In the capture-release-recapture experiment, eight *Eusphalerum* beetles (17%) among the 46 released were recaptured from the flowers of the subpopulation. Seven beetles among the recaptured were found in the flowers of the same genet, and the remaining one, within a flower at a distance of 2 m from the release position. Similarly, in another trial with 60 beetles in the following year, seven beetles among the recaptured eight beetles were found within the same clonal patch of *P. sieboldii*, and one beetle, in a flower at the distance of 3.6 m from the release position.

## Discussion

Ecological processes occurring at different spatial scales may be fundamentally different in features and outcomes (Wiens 1989). Concerning the female fertility pattern of *P. sieboldii*, at a larger spatial scale, i.e., between habitats, pollinator availability is mostly responsible for the fertility variation (Washitani et al. 1991, 1994b), while at a smaller scale, between subpopulations of a forest metapopulation, antagonistic interactions are likely to be the major factors creating variation in seed set. Female fertility can vary among genets due to genetic differences in physiological compatibility traits (Washitani 1994). However, such an among-genet variance should never be a major factor for the among-subpopulation variation revealed in the present study, since a large number of genets were sampled for each subpopulation.

In the subpopulations S1 and S2, the impact of the flower consumer *Eusphalerum* beetle was shown to be sufficiently large to result in almost complete failure in seed

production over years. Including the preliminary observation period, the subpopulations S1 and S2 had harbored *Eusphalerum* beetles at high densities and exhibited poor seed outputs, at least over eight years (Washitani unpublished observation). This most influential flower consumer beetle utilizes the flowers of *P. sieboldii* as not only food but also for shelters and mating places. The size and tubular morphology of the flowers of *P. sieboldii* are likely to be quite suitable for shelters and mating places for this species.

The abundance patterns of non-pollinating insects as well as pollinators can strongly depend on the phenology of the plants (Goldburg 1987). In *P. sieboldii*, both early spring flowering phenology and tubular corolla morphology are supposed to have evolved and been maintained under the selective pressures exerted by queen bumble bees, which are the most important pollinators for *P. sieboldii* (Washitani et al. 1994b). The same phenological and morphological traits which facilitate effective pollination may enhance the antagonistic biological interactions with the potent flower consumer.

The resource concentration hypothesis (Root 1973) on the abundance of consumer insects assumes that population densities of specialist insects should be higher in areas with a high resource concentration. In addition to the spatial distribution pattern of the host plants, ability and mode of natural dispersion of the insect as well as its enemies are thought to be among the important factors to stabilize spatial distribution of herbivorous insects (Capuccino 1987). The temporally-stable spatially variable pattern observed for the occurrence of the *Eusphalerum* in the *P. sieboldii* subpopulations suggests the importance of the dispersal ability of the beetle and patchy distribution of the plant species in determining the spatial pattern. Such a spatio-temporal pattern is contrasted to that of the milkweed-tephritid fly system, in which temporal fluctuation far surpasses the spatial variation among plant patches (Solbreck and Sillen-Tullberg 1986).

The impacts of the flower consumer *Eusphalerum* beetles and those of the specific pathogen *U. tranzschelina* on *P. sieboldii* seem to be mutually exclusive at the subpopulation level, since no infection of the pathogen was found in the subpopulations harboring the rove beetles. Since the smut fungus produces sori in the ovaries of *P. sieboldii*, flower damage by the beetle, especially damage to the ovaries, should make the plants less susceptible to the smut.

In the disease system of *Trientalis europaea* L.-*Urocystis trientalis*, in which the infection is systemic, seed capsule production did not differ between healthy and diseased plants (Wennstrom and Ericson 1990). However, no seeds were formed in the ramets of *P. sieboldii* infected by *U. tranzschelina*. Contribution of the dispersion of the ustilospores filling the capsules in the

disease transmission should be studied in a future study focusing on the life history of the fungi. Nevertheless, the spatial restriction of the disease within two subpopulations suggests the endemic nature of the disease with rare transmission between the subpopulations.

In the subpopulations that escaped both flower damage by *Eusphalerum* beetles and smut fungal infection, seed sets of both pins and thrums were quite high. These fertility patterns are very different from the pattern for isolated nature-reserve populations in which female fertility is generally low but significantly lower in the thrum morph than in the pin morph (Washitani et al. 1994a).

We cannot yet answer definitely whether the flower consumer and/or the pathogen critically affect the long-term population dynamics of *P. sieboldii*, since the present study concerned only relatively short-term effects of antagonistic agents. Generally, demographic susceptibility to inflorescence-feeding insects is considered to be relatively low in long-lived perennials, since they have a buffering system for short-term variation in current seed supply (Louda and Potvin 1995). Whether the impacts of antagonistic biological interactions observed in the present study, i.e., almost complete failure of sexual reproduction in some subpopulation over several years, will result in decline and/or extinction of the subpopulations, will depend on both the time scale of persistence of the interactions and the relative importance of seed output for the genet recruitment compared to the other processes constituting 'reproduction by seeds'.

As generally suggested, subdivided metapopulations may be buffered from the negative effects of environmental variation (Goodman 1987; Quinn and Hasting 1987). Spatial structure can stabilize host-parasitoid and predator-prey interactions (Hassell et al. 1991; Pacala et al. 1990). Spatially restricted patterns for the antagonistic interactions with *Eusphalerum* beetles and *Urocystis* smut fungus suggest that spatial subdivision of the present *P. sieboldii* population would serve to prevent the antagonistic interactions from prevailing over the whole population, even if their influences are crucial at the subpopulation level. Not only the intensity of the negative influences, but also the dispersal ability of the agents as well as the mode of spatial subdivision of a plant metapopulation would be important in determining the overall effects of the antagonistic interaction on the plant performance at the metapopulation level.

*Eusphalerum* beetles and smut fungi are often found in the flowers or capsules of *P. sieboldii* in other habitats including those of different geographical ranges (Washitani and Nishihiro personal observation). General occurrence of these antagonistic biological agents might suggest the presence of coexistence mechanisms for these antagonistic agents and *P. sieboldii*. It would be among such mechanisms that the negative impacts are largely local

and restricted mainly within the subpopulations.

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