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Interspecific pollinator movements and heterospecific incompatibility: comparisons between *Phyllodoce caerulea* and *Phyllodoce aleutica* along snowmelt gradients

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ABSTRACT

Relationships between interspecific pollinator movements and heterospecific incompatibility were investigated in two co-flowering alpine-snowbed shrubs, *Phyllodoce caerulea* and *Phyllodoce aleutica*, among plots arranged along snowmelt gradients. Bumblebees, the predominant pollinator of these species, preferred *P. caerulea* in early- to middle-snowmelt plots but *P. aleutica* in late-snowmelt plots. This switching of preference reflected the changes in the relative floral densities of these species along the snowmelt gradients. The frequency of bumblebee movements from *P. aleutica* to *P. caerulea* flowers increased at late-snowmelt plots, whereas that from *P. caerulea* to *P. aleutica* flowers decreased at late-snowmelt plots. Thus, the risk of heterospecific pollen receipt increased in *P. caerulea* but decreased in *P. aleutica* from early- to late-snowmelt habitats. *Phyllodoce caerulea* showed similar seed-set rates among conspecific, heterospecific and species-mixed pollination treatments throughout the plots, indicating that there is no mechanism to avoid hybridization at *P. aleutica* pollen. On the other hand, *P. aleutica* pollinated with *P. caerulea* pollen showed a very low seed set in comparison with that pollinated with conspecific pollen in early- and middle-snowmelt plots. However, the seed set of *P. aleutica* did not differ among the treatments at late-snowmelt plots. Therefore, *P. aleutica* showed heterospecific incompatibility with *P. caerulea* pollen only in the early- to middle-snowmelt populations. Changes in interspecific bumblebee movements might be responsible for the variation in heterospecific incompatibility among the *P. aleutica* populations.

Keywords: alpine-snowbed plants, bumblebee, heterospecific incompatibility, interspecific pollinator movement, reciprocal pollination, reproductive isolation.

INTRODUCTION

Pollinators using multiple plant species generate interspecific pollen transfer between co-flowering species (Waser, 1978a,b; Rathcke, 1983; Campbell and Motten, 1985; Feinsinger *et al.*, 1988; Caruso, 1999).

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Interspecific pollen transfer may decrease not only male fitness through pollen wastage (Waser, 1978a; Campbell, 1985) but also female fitness through ovule wastage caused by clogging of the stigma with heterospecific pollen, seed abortion due to heterospecific fertilization, and the production of hybrids with low viability (Waser and Fugate, 1986; Galen and Gregory, 1989; Murphy and Aarssen, 1995; Caruso and Alfaro, 2000). Many plants have mechanisms of reproductive isolation to prevent hybridization (Carney *et al.*, 1994; Rieseberg *et al.*, 1995; Arnold, 1997; Hauser *et al.*, 1997). Heterospecific incompatibility is a form of post-pollination reproductive isolation (Arnold, 1997). Interspecific pollinator movements between related plant species may trigger heterospecific incompatibility if the negative effects of heterospecific pollen receipt are serious enough. Some recent studies have shown a relationship between the hybridization process and unidirectional patterns of interspecific pollinator movements (e.g. Leebens-Mack and Milligan, 1998; Wesselingh and Arnold, 2000; Campbell *et al.*, 2002). It is probable that a species at high risk of receiving pollen from related species tends to evolve heterospecific incompatibility (Wolf *et al.*, 2001).

Non-random foraging among plant species is common in many taxa of pollinators (Waser, 1986; Chittka *et al.*, 1999; Jones, 2001). Bumblebees in particular exhibit a flexible preference for plant species in response to foraging efficiency, and tend to visit a specific attractive plant species during a sequence of foraging (Heinrich, 1976, 1979; Heinrich *et al.*, 1977). Thus, less attractive plant species suffer from a low visiting fidelity of pollinators in addition to low visitation frequency (Heinrich *et al.*, 1977; but see Meléndez-Ackerman *et al.*, 1997), resulting in a high risk of heterospecific pollen receipt (Heinrich, 1979; Kunin, 1993; Jones, 2001). We assume that a less attractive species among related species sharing the same pollinators may have stronger heterospecific incompatibility for reproductive isolation. If this is the case, the extent of heterospecific incompatibility of single species may vary among populations in which competition for pollination success differs.

In alpine snowbeds, the flowering of single species occurs sequentially in response to the timing of snowmelt among neighbouring populations along snowmelt gradients (Galen and Stanton, 1991; Kudo, 1991). Two alpine-shrubs, *Phyllodoce caerulea* and *Phyllodoce aleutica*, are ideal plants for studying the evolution of reproductive isolation. Both species commonly grow in snowbeds over a wide range of snowmelt gradients in the Taisetsu Mountains, northern Japan (Kudo, 1991). The flowering of these species occurs at the same time within an area, and the two species share bumblebees as predominant pollinators. These species are important nèctar resources for bumblebees in the Taisetsu Mountains due to their high flower density and large nectar production in comparison with other snowbed plants. In our previous study, bumblebee preference switched between the species along snowmelt gradients (Kasagi and Kudo, 2003); bumblebees preferred *P. caerulea* to *P. aleutica* at early- to middle-snowmelt plots but *P. aleutica* at late-snowmelt plots, reflecting the changes in relative attractiveness between the species. In such a situation, one would expect the pattern of bumblebee movements between the *Phyllodoce* species to vary along snowmelt gradients, with the result that the frequency of heterospecific pollination should vary between neighbouring populations. Consequently, the extent of heterospecific incompatibility in each species may vary along snowmelt gradients within a local area. To test this prediction, we measured the frequency of interspecific bumblebee movements and seed-set ability of these species in a reciprocal pollination experiment along snowmelt gradients.

MATERIALS AND METHODS

Research site

Three snowbeds were selected as research sites near Lake Hisago (HIS), Mt. Goshiki (GOS) and Mt. Pon-kaun (PON), between 1700 and 1880 m above sea level, in the central part of the Taisetsu Mountains, northern Japan (peak altitude: 2290 m; 44°33′N, 142°53′E). The sites were 3.5–6 km away from each other. Three plots were established along the snowmelt (E-plot: early-snowmelt plot; M-plot: middle-snowmelt plot; L-plot: late-snowmelt plot) at each site. The dimensions of the plots were approximately 20 × 20 m to 50 × 50 m. The three plots at each site were located 100–700 m from each other. Annual mean temperature at 1700 m above sea level near Lake Hisago is −0.9°C, and summer temperature and precipitation, from June to August, is 11.8°C and 740 mm, respectively. The snow usually melts in mid-June at E-plots, early July at M-plots and mid- to late July at L-plots, although actual snowmelt times are highly variable among years. These sites are usually covered with snow again by early to mid-October.

The plants

Phyllodoce caerulea (L.) Babington and *Phyllodoce aleutica* (Spreng.) A. Heller are evergreen ericaceous shrubs. They form dense patches and plant height is usually less than 30 cm. *Phyllodoce caerulea* is distributed throughout the subarctic and middle-latitude alpine regions. *Phyllodoce aleutica* is distributed only around the north pacific region (Hultén, 1968). They are common and co-exist widely at snowbeds in the Taisetsu Mountains. However, the distribution mode of *P. caerulea* occurs at places of earlier snowmelt than that of *P. aleutica* (Kudo and Ito, 1992).

Each inflorescence of both species has 1–10 flowers (generally 3–7) with a bell-shaped corolla. Although the shape and size of corolla are almost the same between the species, floral colour is clearly discriminable – that is, pink in *P. caerulea* and whitish-yellow in *P. aleutica* (Kasagi and Kudo, 2001). Flowering of both *Phyllodoce* species occurs simultaneously about 2 weeks after snowmelt in the Taisetsu Mountains (Kudo, 1991). Normal peak flowering seasons are mid-July at E-plots, late July to early August at M-plots and mid-August at L-plots. Peak flowering of both species continues within a week at each plot. Both *Phyllodoce* species are mainly pollinated by bumblebees, and they monopolize bumblebees from other co-flowering species, such as *Sieversia pentapetala, Peucedanum multivittatum* and *Veronica stelleri*, due to their high flower density and large amount of nectar. *Phyllodoce caerulea* is an obligate outcrosser with low self-compatibility. The mating system of *P. aleutica* changes along the snowmelt gradient: the species is self-compatible at early- to middle-snowmelt populations but self-incompatible at late-snowmelt populations in the Taisetsu Mountains (Kasagi, 2002).

Bumblebee behaviour

The foraging pattern of bumblebees on both *Phyllodoce* species was observed at every plot (E-, M- and L-plots) of each site (HIS, GOS and PON) in 1999 and 2000, although no observation was made at the E-plot of PON in 2000 because of bad weather. The observations were conducted at peak flowering at all plots. We recorded the sequential foraging of individual bumblebees on inflorescences of both *Phyllodoce* species up to a maximum of 50 sequential visits to inflorescences. Because both *Phyllodoce* species form dense creeping stems, discrimination of individual plants is usually difficult. Thus, we recorded bumblebee movements between inflorescences of these species.

To compare the probability of heterospecific pollen transfer by bumblebees among plots, we conducted a log-likelihood-ratio test (Sokal and Rohlf, 1995) for the interspecific movements between the plots (between E- and M-plot, between M- and L-plot, and between L- and E-plot) at each site (HIS, GOS and PON) for each *Phyllodoce* species. All inter-inflorescence movements by multiple bumblebees (33–72 per plot) were pooled within plots in each year. Then, we classified the pooled data into four patterns: pattern 1 was conspecific movements to *P. caerulea* (i.e. movement from a *P. caerulea* inflorescence to a *P. caerulea* inflorescence); pattern 2 was interspecific movements to *P. caerulea* (i.e. from *P. aleutica* to *P. caerulea*); pattern 3 was conspecific movements to *P. aleutica* (i.e. from *P. aleutica* to *P. aleutica*); and pattern 4 was interspecific movements to *P. aleutica* (i.e. from *P. caerulea* to *P. aleutica*). Under the null hypothesis that the probability of interspecific movements does not differ between the plots, the log-likelihood-ratio test was performed by calculating the expected frequencies of interspecific movements for each species (see the Appendix for a detailed procedure of the analysis). In this analysis, three study sites were treated as independent tests.

Pollination experiment

To quantity heterospecific incompatibility, an artificial pollination experiment was conducted at every plot (E-, M- and L-plots) near Lake Hisago (HIS) in 1999 and 2003 and near Mt. Goshiki (GOS) in 2003. We selected 20 individuals of each *Phyllodoce* species as recipients at each plot. Three inflorescences on each recipient plant were assigned for the treatments of conspecific, heterospecific and species-mixed pollination, respectively. All but one of the flower buds were removed from each inflorescence to reduce the heterogeneity of resources for seed production. We covered the target buds with fine-meshed nylon nets to exclude insect visitation, and emasculated all (five) stamens just before the anther dehiscence. After the flowers had opened, we conducted pollination treatments by contacting the dehisced anthers of donor flowers to stigmas by hand. The pollen-donor flowers were collected from different plants growing more than 5 m from each recipient plant. The conspecific-pollination treatment was conducted by covering the stigma surface completely with pollen from four conspecific plants. The heterospecific-pollination treatment was conducted using pollen from four heterospecific plants. The recipient flowers of species-mixed pollination were pollinated with pollen from two conspecific and two heterospecific plants, in the order heterospecific, conspecific, heterospecific, conspecific pollen. The fine-meshed nylon nets were removed soon after anthesis. All fruits for the pollination experiment were harvested just before dehiscence. Then, mature seeds and aborted (developed but inmature seeds) or unfertilized ovules were enumerated for all fruits under a microscope in the laboratory. Seed set was defined as the proportion of ovules that developed into mature seeds.

Seed set was compared using three-way repeated-measures analysis of variance (ANOVA) after arcsine-square-root transformation factored by species, plot (E-, M- and L-plot) and pollination treatment (conspecific, heterospecific and species-mixed pollination) as repeated measures within plants for each site and year. A significant interaction between species and treatment would be expected if heterospecific incompatibility varies between the species. In such cases, we analysed each species separately by two-way repeated-measures ANOVA factored by plot and pollination treatment. When a significant difference was detected for treatment or the plot × treatment interaction, we conducted a Tukey-Kramer test for multiple comparisons among treatments at each plot.

RESULTS

Bumblebee behaviour

Visitation of two bumblebee species, *Bombus hypocrita sapporoensis* Cockerell and *Bombus beaticola moshkarareppus* Sakagami et Ishikawa, was observed on both *Phyllodoce* species in this study. *Bombus hypocrita* represented 87–100% of visitations at E-plots, 71–91% of visitations at M-plots and 67–86% of visitations at L-plots throughout all sites (HIS, GOS and PON) and years (1999 and 2000). At the E-plots of all sites, overwintered queens and workers of *B. hypocrita* were common. Workers of *B. hypocrita* were still the most common visitors at M- and L-plots. We observed visitation patterns of 33–72 bumblebees per plot for every site and year. In total, 861 bouts of sequential visits were recorded throughout the study. The mean number of inflorescences visited per bout was 33–42 within plots. The visitation frequency on *P. caerulea* inflorescences (patterns 1 and 2) decreased at L-plots at each site in each year, while that on *P. aleutica* inflorescences (patterns 3 and 4) increased at L-plots (Table 1). The ratio of bumblebee visits on the inflorescences of *P. caerulea* to

Table 1. Frequencies of bumblebee visits on *Phyllodoce caerulea* inflorescences by conspecific (pattern 1) and interspecific transition (pattern 2), and on *P. aleutica* inflorescences by conspecific (pattern 3) and interspecific transition (pattern 4), at each plot (E, M, L) of every site (HIS, GOS, PON) in each year (1999 and 2000)

		1999			2000		
		HIS	GOS	PON	HIS	GOS	PON
E-plot	Pattern 1	2097	1682	1222	1537	1082	no data
	Pattern 2	38	19	25	30	53	no data
	Pattern 3	263	181	152	238	338	no data
	Pattern 4	40	18	26	28	56	no data
M-plot	Pattern 1	1642	1076	954	1374	1355	1129
	Pattern 2	72	72	30	34	44	34
	Pattern 3	556	658	329	354	488	288
	Pattern 4	71	81	33	35	47	35
L-plot	Pattern 1	183	241	84	278	216	210
	Pattern 2	34	46	15	38	26	23
	Pattern 3	2346	1931	1086	1669	1863	1492
	Pattern 4	34	46	41	38	37	44

Note: Data for 33–72 bumblebees are pooled at each plot in each year.
Abbreviations: HIS = Lake Hisago, GOS = Mt. Goshiki, PON = Mt. Pon-kaun.

total visits on the inflorescence of both *Phyllodoce* species (pattern 1 + 2/all patterns) was 0.74–0.90 at E-plots, 0.61–0.78 at M-plots and 0.08–0.16 at L-plots throughout the sites and years. Thus, bumblebee preference switched from *P. caerulea* to *P. aleutica* at the late-snowmelt habitats.

The relative frequency of interspecific movements versus the total movements to *P. caerulea* inflorescences (pattern 2/pattern 1 + 2) tended to increase at later snowmelt plots at each site in each year (Fig. 1). The proportion of interspecific movements to *P. caerulea* inflorescences was 1–5% at E-plots, 2–6% at M-plots and 10–16% at L-plots throughout the sites and years. The log-likelihood-ratio tests indicated significant differences in the probability of interspecific movements to *P. caerulea* inflorescences between E- and L-plots ($P < 0.001$ at each site in each year but $P < 0.01$ in GOS 2000; Table A1), and between M- and L-plots ($P < 0.001$ at each site in each year). Significant differences between E- and M-plots were detected near Lake Hisago (HIS) in 1999 ($P < 0.001$) and near Mt. Goshiki (GOS) in both years ($P < 0.001$ in 1999 and $P < 0.05$ in 2000), but not near Lake Hisago (HIS) in 2000 or Mt. Pon-kaun (PON) in 1999 ($P > 0.05$).

In contrast, the frequency of interspecific movements versus the total movements to *P. aleutica* inflorescences (pattern 3/pattern 3 + 4) tended to decrease at later snowmelt plots (Fig. 1). The proportion of interspecific movements to *P. aleutica* inflorescences was

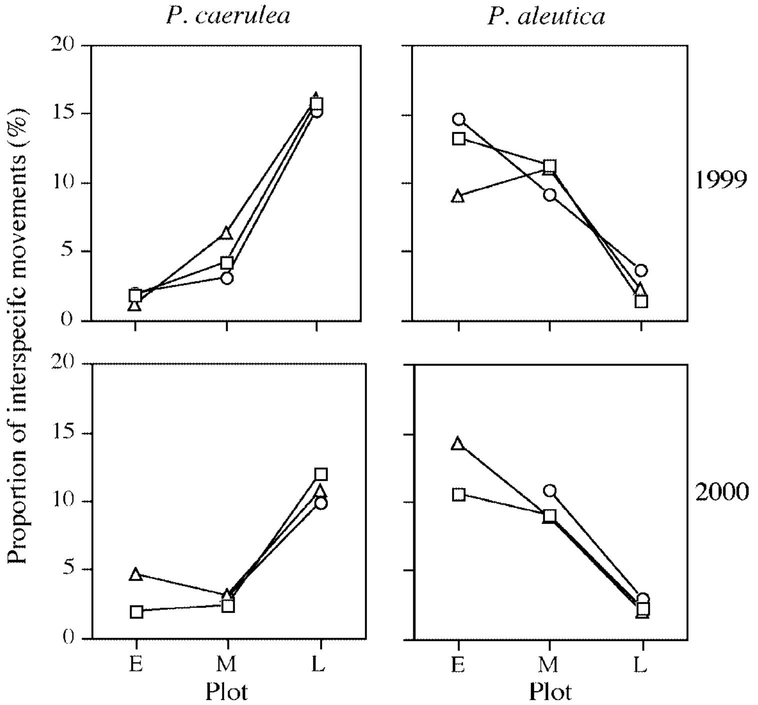

Fig. 1. Proportions of inflorescences that received heterospecific bumblebee movements at each plot (E-, M- and L-plot) near Lake Hisago (□), Mt. Goshiki (△) and Mt. Pon-kaun (○) in 1999 and 2000 for *P. caerulea* and *P. aleutica*. An observation was not conducted at the E-plot near Mt. Pon-kaun in 2000.

9–15% at E-plots, 9–11% at M-plots and 1–4% at L-plots throughout the sites and years. The log-likelihood-ratio tests indicated significant differences in the probability of interspecific movements to *P. aleutica* inflorescences between E- and L-plots, and between M- and L-plots, at each site in each year ($P < 0.001$; Table A2), while the probability did not differ between E- and M-plots throughout the sites and years ($P > 0.05$), except near Mt. Goshiki (GOS) in 2000 ($P < 0.01$).

Pollination experiments

The results of three-way repeated-measures analyses of variance revealed that there was a significant interaction between species and pollination experiment in each case ($F_{2,228} = 13.557$, $P < 0.0001$ in HIS 1999; $F_{2,212} = 6.202$, $P = 0.0024$ in HIS 2003; and $F_{2,204} = 4.863$, $P = 0.0086$ in GOS 2003). This means that *P. caerulea* and *P. aleutica* have different levels of heterospecific incompatibility. Thus, we performed two-way repeated-measures analyses of variance for each species separately. The seed sets of *P. caerulea* differed among the plots but did not differ among the pollination treatments near Lake Hisago (HIS) in 1999 and near Mt. Goshiki (GOS) in 2003, using two-way repeated-measures analyses of variance (Table 2 and Fig. 2). A similar trend was obtained near Lake Hisago (HIS) in 2003, but seed set did not differ significantly. No significant interaction between plot and treatment was detected with any ANOVA, indicating that *P. caerulea* and *P. aleutica* pollen were compatible throughout the plots.

Table 2. Results of two-way repeated-measures analyses of variance of seed set factored by plot (E-, M- and L-plot) and pollination treatment (conspecific, heterospecific and species-mixed pollination) for each species (*Phyllodoce caerulea* and *P. aleutica*)

	P. caerulea		*P. aleutica*	
	d.f.	*F*-value	d.f.	*F*-value
HIS in 1999				
Plot (P)	2, 57	4.333*	2, 57	5.765**
Treatment (T)	2, 114	0.336	2, 114	37.985***
P × T	4, 114	0.245	4, 114	6.074**
HIS in 2003				
Plot (P)	2, 52	1.394	2, 54	5.765*
Treatment (T)	2, 104	0.298	2, 108	37.985***
P × T	4, 104	0.930	4, 108	6.074**
GOS in 2003				
Plot (P)	2, 54	9.556**	2, 48	25.005***
Treatment (T)	2, 108	0.355	2, 96	17.767***
P × T	4, 108	0.166	4, 96	5.311**

***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$.
Abbreviations: HIS = Lake Hisago, GOS = Mt. Goshiki.

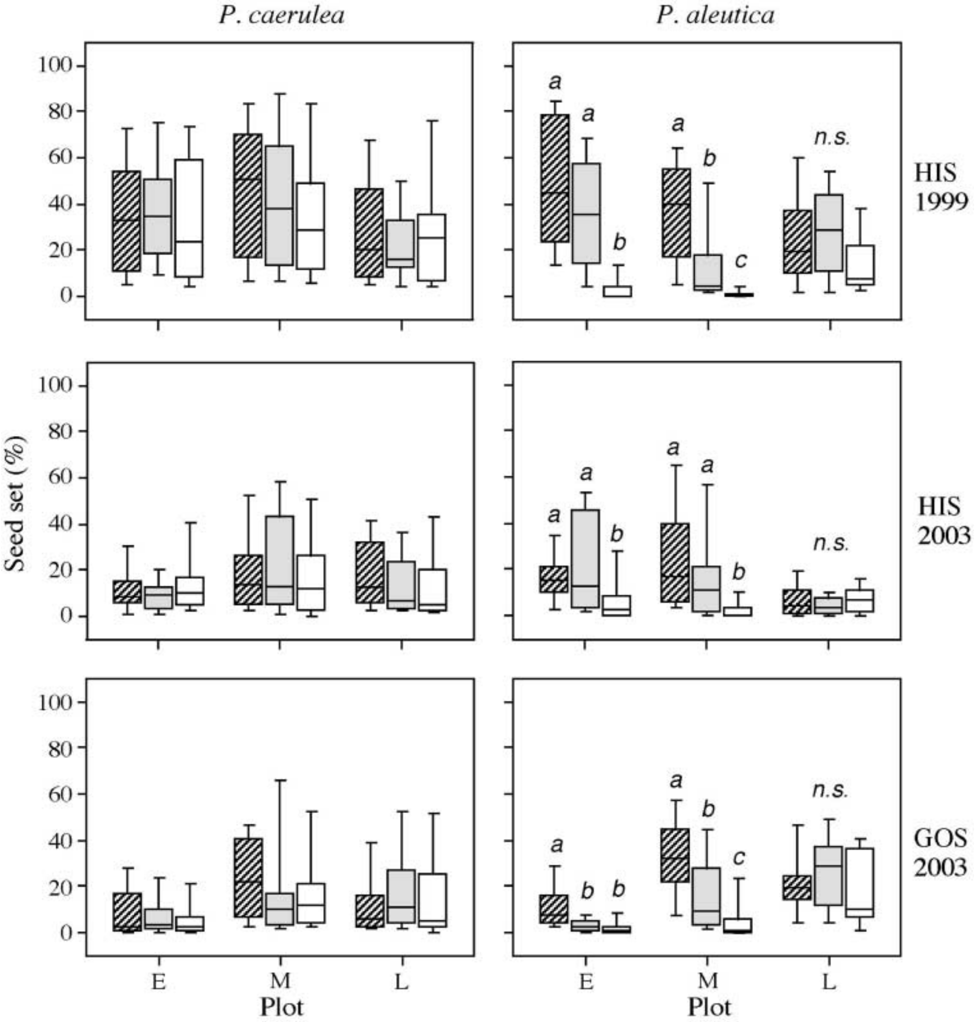

Fig. 2. Boxplots of seed set in *P. caerulea* and *P. aleutica* by conspecific pollination (hatched box), species-mixed pollination (shaded box), and heterospecific pollination (open box) at each plot near Lake Hisago (HIS) in 1999 and 2003 and Mt. Goshiki (GOS) in 2003. The top of a box represents the 75th percentile and the bottom the 25th percentile; a box thus contains the middle 50% of the values and the lines in the box represent the median. The top whisker ranges from the 75th to the 90th percentile and the bottom whisker from the 25th to the 10th percentile. Different letters indicate significant differences among treatments in each plot at each site according to a Tukey-Kramer test. Sample sizes are 18–20.

On the other hand, seed sets of *P. aleutica* differed significantly among the plots and among the treatments at every site in every year by the two-way repeated-measures analyses of variance (Table 2 and Fig. 2). The interaction between plot and treatment was significant for every ANOVA, indicating variations in heterospecific compatibility among the plots. Seed set by heterospecific pollination was lowest among the three pollination treatments at E- and M-plots for every site ($P < 0.05$, Tukey-Kramer test; Fig. 2), in which 35–75% of *P. aleutica* flowers entirely failed to set seeds following heterospecific pollination. This indicates significant incompatibility between *P. aleutica* and *P. caerulea* pollen in early- to middle-snowmelt populations. Mixed pollen from both species significantly decreased the seed sets of *P. aleutica* compared with conspecific pollination in the M-plot near Lake Hisago (HIS) in 1999 and in the E- and M-plots near Mt. Goshiki (GOS) in 2003 ($P < 0.05$ at every plot), indicating interference by the heterospecific pollen deposited on stigmas. A similar trend was observed in the E-plot near Lake Hisago (HIS) in 1999 and the M-plot near Lake Hisago in 2003 ($P > 0.05$). Interestingly, seed sets of *P. aleutica* at L-plots did not differ among pollination treatments throughout the sites and years ($P > 0.05$). The percentage of flowers that entirely failed to set seeds after heterospecific pollination ranged from 0 to 10%. This indicates a lack of heterospecific incompatibility in late-snowmelt populations of *P. aleutica*.

DISCUSSION

Phyllodoce caerulea was a major target species for bumblebees at E- and M-plots, but bumblebees switched preference to *P. aleutica* at L-plots. This switching of target species reflected the shift in relative attractiveness between the two *Phyllodoce* species along the snowmelt gradients – that is, intensive decreases in floral density and nectar production of *P. caerulea* at late-snowmelt habitats because of the susceptibility of this species to a short growing season (Kasagi and Kudo, 2003). The probability of interspecific bumblebee movements changed significantly along the snowmelt gradients in each species as expected, and this trend was consistent throughout the sites and years (Fig. 1). Kunin (1993) demonstrated that the local density of a plant population influenced the quality of pollinator service and that the frequency of conspecific pollen transfer decreased at low density resulting in low seed-set success for self-incompatible plants. A similar situation was expected in our research system. Because pollinator movements between species determine the pattern of interspecific pollen transfer (Waser and Fugate, 1986; Galen and Gregory, 1989; Campbell *et al.*, 1998), the risk of heterospecific pollen receipt should be higher in *P. caerulea* at late-snowmelt plots and in *P. aleutica* at early- to middle-snowmelt plots.

The frequent bumblebee movements from *P. caerulea* to *P. aleutica* might be a decisive factor in the heterospecific incompatibility in *P. aleutica* in the early- to middle-snowmelt populations. The species-mixed pollination in *P. aleutica* resulted in an intermediate seed set, indicating interference by *P. caerulea* pollen even when enough conspecific pollen was deposited on stigmas. Heterospecific pollen deposition may inhibit seed set through conspecific pollination due to stigma clogging (Caruso and Alfaro, 2000), a disturbance in pollen tube growth (Galen and Gregory, 1989) or a disturbance in fertilization (Thomson *et al.*, 1981). Although it is not clear which mechanism of interference worked in *P. aleutica*, such sensitivity to heterospecific pollen reflects a risk of receiving heterospecific pollen under natural conditions as reported in other species (e.g. Wolf *et al.*, 2001). The heterospecific incompatibility disappeared in the late-snowmelt populations probably because a mechanism of reproductive isolation is not crucial for *P. aleutica* due to the decreased risk of receiving pollen from *P. caerulea*. The abrupt change in the pattern of bumblebee movements between the *Phyllodoce* species along the snowmelt gradients might be a selective force causing the variation in heterospecific incompatibility in *P. aleutica*. In a previous study, we (Kasagi and Kudo, 2003) demonstrated that the selfing ability of *P. aleutica* was high at E- and M-plots, but very low at L-plots where bumblebees preferred *P. aleutica* to *P. caerulea*. Therefore, a low frequency of pollinator visits accompanied by a high risk of heterospecific pollen receipt might drive the evolution of both self-compatibility and heterospecific incompatibility of *P. aleutica* in early- and middle-snowmelt habitats.

On the other hand, *P. caerulea* seemed not to reject heterospecific (*P. aleutica*) pollen because heterospecific incompatibility was not detected throughout the plots in any sites and years. Furthermore, the seed set of *P. caerulea* after species-mixed pollination did not differ from that after conspecific pollination. When a mixture of conspecific and heterospecific pollen is deposited on stigmas, many plants selectively accept only the conspecific pollen (Carney *et al.*, 1994; but see also Alarcón and Campbell, 2000). Further studies with genetic approaches are necessary to clarify whether *P. caerulea* has selective mechanisms for conspecific pollen or not. In any case, *P. caerulea* has a low risk for hybridization in early- to middle-snowmelt populations because the proportion of *P. aleutica* pollen deposited on the *P. caerulea* stigma may be small due to a low frequency of bumblebee visits from *P. aleutica*.

Phyllodoce caerulea showed similar seed sets after heterospecific pollination to that following conspecific pollination at L-plots in spite of a high risk of heterospecific pollen receipt. Why didn't heterospecific incompatibility arise in *P. caerulea* at L-plots? *Phyllodoce caerulea* tended to dominate at earlier snowmelt habitats than *P. aleutica* (Kudo and Ito, 1992), and L-plots in this study correspond to the edge of the distribution of *P. caerulea* towards late-snowmelt habitats. Reproductive activity, such as number of flowers per stem and nectar production per flower, of *P. caerulea* decreased rapidly in late-snowmelt populations (Kasagi and Kudo, 2003). Sexual reproduction may not fully contribute to late-snowmelt populations of *P. caerulea*, and the populations may be partly maintained by occasional immigration from neighbouring earlier-snowmelt populations. In such a case, heterospecific incompatibility may not be selected strongly in late-snowmelt populations. Various *P. caerulea* × *aleutica* hybrids have been found in the Taisetsu Mountains, for example *P. caerulea* var. *yesoensis* Koidzumi and *P. aleutica* var. *marmorata* Toyokuni (Shimizu, 1982). Many of them inhabit late-snowmelt areas (personal observation). This is consistent with our results: the frequency of hybridization may increase at late-snowmelt plots because both *Phyllodoce* species lack heterospecific incompatibility.

Several studies have demonstrated the unilateral incompatibility between related species (Williams *et al.*, 1982; Stucky, 1985; Harder *et al.*, 1993). Such asymmetric incompatibility between species has often been considered to be related to a self-incompatibility system: self-incompatible species often resist the pollen-tube growth of self-compatible species, while self-compatible species do not show such a response to the pollen of self-incompatible species (Harrison and Darby, 1955; Lewis and Crowe, 1958; Arnold, 1997). On this view, heterospecific incompatibility is a epiphenomenon of self-incompatibility. In contrast, there are attempts to consider heterospecific incompatibility as an adaptive response separate from self-incompatibility (e.g. Hogenboom, 1975). In the present study, the mildly self-compatible *P. caerulea* lacked heterospecific incompatibility, and the highly self-compatible *P. aleutica* in early- to middle-snowmelt populations successfully rejected heterospecific pollen. This does not appear to be consistent with previous studies based on the epiphenomenon hypothesis. Although further studies of the physiological mechanism of heterospecific incompatibility are required, the results of this study predict the importance of pollinator behaviour in fashioning heterospecific incompatibility as a mechanism of reproductive isolation.

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APPENDIX: THE PROCEDURE OF THE LOG-LIKELIHOOD-RATIO TEST FOR INTERSPECIFIC BUMBLEBEE MOVEMENTS

When a certain movement to a specific species (*P. caerulea* or *P. aleutica*) is selected from all movements observed within a plot, the probability of interspecific movements is expressed as p and the probability of conspecific movements as $1 - p$. When the frequencies of interspecific movements (pattern 2 for *P. caerulea* or pattern 4 for *P. aleutica*) and all movements (pattern 1 + 2 for *P. caerulea* or pattern 3 + 4 for *P. aleutica*) are N_{int} and N, respectively, the maximum likelihood estimate of the probability of interspecific movements is

$$\hat{p} = \frac{N_{\text{int}}}{N}$$

The expected frequency of interspecific movements, i.e. likelihood (L), is expressed using a binomial probability as

$$L(p, N) = {}_{N}C_{N_{\text{int}}}\, p^{N_{\text{int}}} (1 - p)^{N - N_{\text{int}}}$$

The null hypothesis is that the probability of interspecific movements does not differ between the plots. For example, when the frequencies of interspecific movements at an E- and M-plot are N_{Eint} and N_{Mint}, respectively, the frequency of interspecific movements at a pooled plot ‘E + M’ is assumed to be

$$N_{\text{E+Mint}} = N_{\text{Eint}} + N_{\text{Mint}}$$

Then, the maximum likelihood estimate of the null hypothesis is

$$\hat{p}_{\text{E+M}} = \frac{N_{\text{E+Mint}}}{N_{\text{E}} + N_{\text{M}}}$$

where N_{E} and N_{M} are the frequencies of total movements at the E- and M-plot respectively. Consequently, the log-likelihood of interspecific movements is expressed as a one-parameter model using $\hat{p}_{\text{E+M}}$ (model E + M) as follows:

$$\begin{aligned}\ln L\,(\hat{p}_{\text{E+M}}, \text{E-plot}) &= \ln {}_{N_{\text{E}}}C_{N_{\text{Eint}}}\, \hat{p}_{\text{E+M}}{}^{N_{\text{Eint}}} (1 - \hat{p}_{\text{E+M}})^{N_{\text{E}} - N_{\text{Eint}}} \\ &= \sum_{k = N_{\text{Eint}} + 1}^{N_{\text{E}}} \ln k - \sum_{k=1}^{N_{\text{E}} - N_{\text{Eint}}} \ln k + N_{\text{Eint}} \ln \hat{p}_{\text{E+M}} + (N_{\text{E}} - N_{\text{Eint}}) \ln (1 - \hat{p}_{\text{E+M}})\end{aligned}$$

and

$$\ln L\,(\hat{p}_{\text{E+M}}, \text{M-plot}) = \sum_{k = N_{\text{Mint}} + 1}^{N_{\text{M}}} \ln k - \sum_{k=1}^{N_{\text{M}} - N_{\text{Mint}}} \ln k + N_{\text{Mint}} \ln \hat{p}_{\text{E+M}} + (N_{\text{M}} - N_{\text{Mint}}) \ln (1 - \hat{p}_{\text{E+M}})$$

at E- and M-plots, respectively.

The alternative hypothesis is that the probability of interspecific movements differs between the plots – that is, $\hat{p}_{\mathrm{E}}$ at the E-plot and $\hat{p}_{\mathrm{M}}$ at the M-plot – as follows:

$$\hat{p}_{\mathrm{E}} = \frac{N_{\mathrm{Eint}}}{N_{\mathrm{E}}}$$

$$\hat{p}_{\mathrm{M}} = \frac{N_{\mathrm{Mint}}}{N_{\mathrm{M}}}$$

Then, the log-likelihood at the E-plot can be calculated using $\hat{p}_{\mathrm{E}}$ (model E) as

$$\begin{aligned} \ln L\,(\hat{p}_{\mathrm{E}}, \text{E-plot}) &= \ln {}_{N_{\mathrm{E}}}C_{N_{\mathrm{Eint}}}\, \hat{p}_{\mathrm{E}}^{\,N_{\mathrm{Eint}}}\, (1 - \hat{p}_{\mathrm{E}})^{N_{\mathrm{E}} - N_{\mathrm{Eint}}} \\ &= \sum_{k = N_{\mathrm{Eint}} + 1}^{N_{\mathrm{E}}} \ln k - \sum_{k=1}^{N_{\mathrm{E}} - N_{\mathrm{Eint}}} \ln k + N_{\mathrm{Eint}} \ln \hat{p}_{\mathrm{E}} + (N_{\mathrm{E}} - N_{\mathrm{Eint}}) \ln (1 - \hat{p}_{\mathrm{E}}) \end{aligned}$$

Similarly, the log-likelihood at the M-plot can be calculated using $\hat{p}_{\mathrm{M}}$ (model M) as

$$\ln L\,(\hat{p}_{\mathrm{M}}, \text{M-plot}) = \sum_{k = N_{\mathrm{Mint}} + 1}^{N_{\mathrm{M}}} \ln k - \sum_{k=1}^{N_{\mathrm{M}} - N_{\mathrm{Mint}}} \ln k + N_{\mathrm{Mint}} \ln \hat{p}_{\mathrm{M}} + (N_{\mathrm{M}} - N_{\mathrm{Mint}}) \ln (1 - \hat{p}_{\mathrm{M}})$$

The goodness-of-fit of the model for the null hypothesis is evaluated by summing the log-likelihood for 'model E + M' at each plot – that is, $\ln L\,(\hat{p}_{\mathrm{E+M}}, \text{E-plot}) + \ln L\,(\hat{p}_{\mathrm{E+M}}, \text{M-plot})$. The goodness-of-fit of the models for the alternative hypothesis is evaluated by summing the log-likelihood for 'model E' and 'model M' – that is, $\ln L\,(\hat{p}_{\mathrm{E}}, \text{E-plot}) + \ln L\,(\hat{p}_{\mathrm{M}}, \text{M-plot})$. Then, the difference of log-likelihood values between the null hypothesis and alternative hypothesis is calculated as

$$\ln r = \ln L\,(\hat{p}_{\mathrm{E+M}}, \text{E-plot}) + \ln L\,(\hat{p}_{\mathrm{E+M}}, \text{M-plot}) - \ln L\,(\hat{p}_{\mathrm{E}}, \text{E-plot}) + \ln L\,(\hat{p}_{\mathrm{M}}, \text{M-plot})$$

With large sample size, the distribution of '$-2 \ln r$' can be approximated using the χ^2-distribution with one degree of freedom, which is the difference in the number of parameters between the null hypothesis (one-parameter model) and the alternative hypothesis (two-parameter model). The null hypothesis can be tested as to whether the value of '$-2 \ln r$' is larger than 3.84, 6.64 and 10.83, which correspond to the 95%, 99% and 99.9% points of the χ^2-distribution, respectively (d.f. = 1). We show the values of log-likelihood for every plot at each site (HIS, GOS, PON) in each year (1999 and 2000) and the results of the log-likelihood-ratio tests for *P. caerulea* (Table A1) and *P. aleutica* (Table A2).

Table A1. Log-likelihood of interspecific movements of bumblebees for every model and the results of log-likelihood-ratio tests on *Phyllodoce caerulea* (pattern 1 and 2) at each site (HIS, GOS, PON) in 1999 and 2000

		1999			2000		
		HIS	GOS	PON	HIS	GOS	PON
Log-likelihood							
E-plot	Model E	−2.81	−2.39	−2.56	−2.68	−2.99	no data
	Model E + M	−7.01	−19.19	−2.94	−2.73	−4.61	NA
	Model L + E	−8.65	−20.08	−4.44	−9.63	−3.64	NA
M-plot	Model M	−3.04	−3.59	−2.79	−2.70	−2.93	−2.70
	Model E + M	−7.53	−23.48	−4.08	−3.20	−3.28	NA
	Model M + L	−6.28	−4.28	−3.53	−8.56	−4.37	−4.57
L-plot	Model L	−2.60	−2.75	−2.51	−2.68	−2.49	−2.55
	Model M + L	−17.41	−12.03	−15.56	−19.11	−11.38	−11.55
	Model L + E	−31.62	−41.78	−20.56	−23.79	−7.02	NA
−2 ln *r* value							
E- and M-plot competition		17.38***	73.37***	3.33	1.10	3.94*	NA
M- and L-plot competition		36.09***	19.95***	27.57***	44.57***	20.66***	21.75***
L- and E-plot competition		69.73***	113.45***	39.88***	56.13***	10.36**	NA

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; by χ^2 test of d.f. = 1. NA, not available.
Note: Values of −2 ln *r* and significant levels of null hypothesis are shown.
Abbreviations: HIS = Lake Hisago, GOS = Mt. Goshiki, PON = Mt. Pon-kaun.

Table A2. Log-likelihood of interspecific movements of bumblebees for every model and the results of log-likelihood-ratio tests on *Phyllodoce aleutica* (pattern 3 and 4) at each site (HIS, GOS, PON) in 1999 and 2000

		1999			2000		
		HIS	GOS	PON	HIS	GOS	PON
Log-likelihood							
E-plot	Model E	−2.69	−2.32	−2.47	−2.56	−2.86	no data
	Model E + M	−2.92	−2.57	−3.61	−2.81	−4.67	NA
	Model L + E	−35.45	−10.79	−21.40	−18.02	−40.41	NA
M-plot	Model M	−2.99	−3.06	−2.62	−2.65	−2.80	−2.64
	Model E + M	−3.10	−3.12	−3.26	−2.83	−4.34	NA
	Model M + L	−39.46	−27.18	−14.58	−15.04	−23.16	−21.16
L-plot	Model L	−2.68	−2.82	−2.36	−2.73	−2.54	−2.52
	Model M + L	−21.92	−17.73	−9.31	−7.31	−13.33	−10.13
	Model L + E	−12.17	−4.23	−9.03	−7.06	−19.93	NA
−2 ln *r* value							
E- and M-plot competition		0.68	0.63	3.55	0.85	6.70**	NA
M- and L-plot competition		111.42***	78.06***	37.82***	33.95***	62.28***	52.25***
L- and E-plot competition		84.48***	19.74***	51.21***	39.58***	109.88***	NA

***$P < 0.001$; **$P < 0.01$; ns, $P > 0.05$ by χ^2 test of d.f. = 1. NA, not available.
Note: Values of −2 ln *r* and significant levels of null hypothesis are shown.
Abbreviations: HIS = Lake Hisago, GOS = Mt. Goshiki, PON = Mt. Pon-kaun.