

## Pollination in a patchily distributed lousewort is facilitated by presence of a co-flowering plant due to enhancement of quantity and quality of pollinator visits

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- **Background and Aims** Plants surrounded by individuals of other co-flowering species may suffer a reproductive cost from interspecific pollen transfer (IPT). However, differences in floral architecture may reduce or eliminate IPT.
- **Methods** A study was made of *Pedicularis densispica* (lousewort) and its common co-flowering species, *Astragalus pastori*, to compare reproductive and pollination success of lousewort plants from pure and mixed patches. Floral architecture and pollinator behaviour on flowers of the two plants were compared along with the composition of stigmatic pollen load of the louseworts. The extent of pollen limitation of plants from pure and mixed patches was also explored through supplemental pollination with self- and outcross pollen (PLs and PLx).
- **Key Results** Mixed patches attracted many more nectar-searching individuals of *Bombus richardsi*. These bumblebees moved frequently between flowers of the two species. However, they pollinated *P. densispica* with their dorsum and *A. pastori* with their abdomen. This difference in handling almost completely eliminated IPT. Lousewort plants from mixed patches yielded more seeds, and seeds of higher mass and germinability, than those from pure patches. Moreover, louseworts from mixed patches had lower PLs and PLx compared with those from pure patches.
- **Conclusions** Differences in floral architecture induced differences in pollinator behaviour that minimized IPT, such that co-flowering plants significantly enhanced quantity and quality of pollinator visits for the lousewort plants in patchy habitat. These findings add to our understanding of the mechanisms of pollination facilitation.

**Key words:** *Astragalus pastori*, bumble-bee, geitonogamous mating, interspecific pollen transfer, lousewort, mechanical isolation, *Pedicularis densispica*, pollen limitation, pollination, pollinator behaviour.

### INTRODUCTION

The reproduction of flowering plants can be influenced by the presence of co-flowering species. Co-flowering plants can compete for limited pollinator visits (Waser, 1978; Campbell and Motten, 1985; Chittka and Schürkens, 2001; Hochkirch *et al.*, 2012) and/or interfere with a focal species through interspecific pollen transfer (IPT) (Waser, 1978; Feinsinger *et al.*, 1991; Bjerknes *et al.*, 2007; Morales and Traveset, 2008; Mitchell *et al.*, 2009; Flanagan *et al.*, 2010). Alternatively, co-flowering plants can facilitate pollination of a focal species (Thomson, 1981, 1982; Lavery, 1992; Johnson *et al.*, 2003; Moeller, 2004; Ghazoul, 2006; Toräng *et al.*, 2006; Liao *et al.*, 2011). Evidence of facilitation has been largely limited to systems where plant species providing no pollinator rewards benefit from close proximity to rewarding (Lavery, 1992; Roy, 1994, 1996; Johnson *et al.*, 2003) or congeneric species with morphologically similar displays (Moeller, 2004; Toräng *et al.*, 2006). However, Ghazoul (2006) demonstrated that species-rich habitats consisting of species with morphologically distinct flowers could also facilitate pollination (see also Liao *et al.*, 2011).

For facilitation to occur, co-flowering species must enhance visitation to a focal species enough to outweigh any reproductive cost of IPT (Moeller, 2004; Morales and Traveset, 2008; Wirth *et al.*, 2011). This is most likely to occur if co-flowering

species either aid in the attraction of pollinators that they do not themselves use, or if they use a different part of the pollinator's body to transfer their pollen. The latter possibility is referred to as mechanical isolation (Grant, 1994a, b; Yang *et al.*, 2007) and may stem from differences in the floral architecture of co-flowering species and consequently in how shared pollinators handle them.

Here, we describe a study of an endemic Chinese lousewort, *Pedicularis densispica*, and a co-flowering species, *Astragalus pastori*. Although the two species share pollinators where they co-occur, their floral architectures are distinct and we hypothesized that this would lead to substantial mechanical isolation between them. Specifically, we asked the following questions: (1) Does *A. pastori* help to attract more pollinators and eventually enhance quantity of pollinator visits for the lousewort? (2) Does *A. pastori* increase movement of pollinators between flowers of different individuals and in turn reduce geitonogamous visits? (3) Does mechanical isolation play a role in reducing IPT?

### MATERIALS AND METHODS

#### *Study species and site*

*Pedicularis densispica* Franch. ex Maxim. (Scrophulariaceae) is an endemic annual lousewort of the Yunnan Himalaya, flowering

from June until September in meadows, mountain slopes and forest edges. The flowers have a short tube and no corolla beak, which represents the ancestral structure in genus *Pedicularis* (Li, 1951). The anthers lie in the upper lip (galea) and the pollen grains disperse from a downward cleft, while the stigma protrudes from the tip of the galea and points in a downward direction (Fig. 1A). The stigmatic surface is small and smooth, and so could receive pollen of congeners (Yang et al., 2002). Individual flowers last longer than 8 d and secrete nectar constantly (Yang et al., 2002). A plant usually has 1–3 inflorescences each comprising 6–30 whorls of 3–4 flowers. There is no autonomous pollination and pollinators are needed for reproduction. Plants often suffer pollen limitation (PL) in small populations, and selfing leads to inbreeding depression in seed number, mass and germinability (Li, 2003; Yang, 2004).

*Astragalus pastori* H.T.Tsai & T.T.Yu (Leguminosae) is a milk vetch endemic to south-west China and is commonly present on any bare land in north-west Yunnan. It habitually co-occurs with *P. densispica*. Anthers are concealed in the keel and the upward-pointing stigma protrudes slightly from the tip of the keel (Fig. 1B). Flowers of *A. pastori* emerge later and the blooming period is shorter than that of the lousewort. Thus, anthesis of the two species overlaps almost completely.

Field investigations were conducted in Shangeri-La county (27°52'N, 99°40'E), north-west Yunnan Province, China, from 2001 to 2003 and 2009 to 2011. We worked in three regions, labelled S (27°75'N, 99°77'E), R (27°52'N, 99°40'E) and M (27°85'N, 99°64'E). Region S is a 3-km × 3-km mountain slope located in Wufengshan, R is along Pulacuo road measuring 100 m × 8 km in area while M covers an area of 1 km × 3 km in Yila meadow. Each study year we randomly selected the experimental patches at the beginning of the field season. In each region in each study year we chose an equal number of 'pure' patches, maintained at exactly three lousewort individuals by removing surplus individuals and any other plants, and 'mixed' patches, maintained at exactly three lousewort individuals and three milk vetch individuals. The area occupied by each patch was less than 2 m × 2 m. We removed any flowering plants within 8 m of each patch. The distances between any patches sampled within a year were greater than 50 m. The whorls in lousewort inflorescences that co-flowered with *A. pastori* were marked.

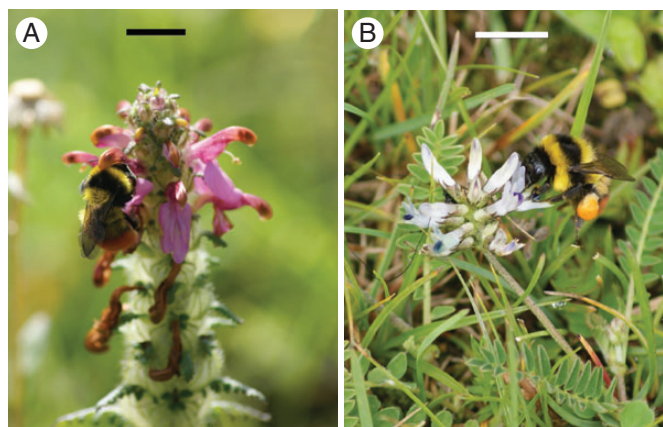


FIG. 1. *Bombus richardsi* visiting (A) *P. densispica* and (B) *A. pastori* flowers; and floral architecture of the species. Scale bars, 1 cm.

### Pollinator behaviour

We observed pollination from 1000 to 1500 h on fine days from 13 July to 27 July in 2010 and from 17 July to 30 July in 2011, spending at least 100 total person-hours in each region. Three pure and three mixed patches were used in each region in each year except for in region R in 2010 where two pure and two mixed patches were used. We quantified the faunal composition of pollinators visiting louseworts in both the pure and the mixed patches as the relative frequency of flower visits by each insect species during a 30-min observation period, making at least 30 observation periods per patch. We noted pollination frequency to evaluate differences of pollinator abundance for lousewort plants in pure and mixed patches, which was estimated as the cumulative times for any pollinators visiting at least one lousewort flower in a patch over an observation period. To estimate geitonogamous visits, we recorded the numbers of visited flowers within an inflorescence during single foraging bouts. We also recorded the total number of lousewort flowers visited by all pollinators over an observation period within each patch.

To detect flower constancy of pollinators in *P. densispica* in mixed patches, each observed visit in a patch during an entire foraging bout was distinguished either as an interspecific visit or as a constant visit on lousewort flowers. Flower constancy for a particular pollinator type refers to the proportion of constant visits summed over all observation periods in the three regions. During interspecific visits of certain pollinators, we noted how the insect landed on the flower, the specific body parts to which pollen attached, how the insect deposited pollen on the stigma and its grooming behaviour during or after a visit.

### Estimation of IPT

Stigmatic pollen loads on lousewort stigmas were used to estimate the extent of IPT and pollination efficiency. For estimation of pollination efficiency, more than 50 wilting flowers from pure and mixed patches in each region were harvested and fixed in FAA solution. Pistils were dissected and observed under a fluorescence microscope (NikonE-600) after treatment with 8 M NaOH for 24 h, followed by 0.1 % aniline blue dye. To evaluate the potential for IPT we harvested at least 50 individual lousewort flowers in mixed patches in each region that were visited by bumble-bees that had also visited *A. pastori* flowers. Stigmatic pollen contents were analysed as described above, and pollen of the two species was easily distinguished. We also harvested at least 50 flowers from pure patches within each region to estimate the potential for inter-patch IPT.

### Reproductive success

We used seed production per capsule, seed mass and seed germinability to evaluate reproductive success of lousewort. At least 50 fruits derived from flowers that had co-flowered with milk vetch were harvested from each of 92 patches in total: 34 from region S (6 in 2002, 4 in 2003, 4 in 2009, 10 in 2010 and 10 in 2011); 22 from region R (4 in 2002, 4 in 2009, 8 in 2010 and 6 in 2011); and 36 from region M (4 in 2001, 6 in 2002, 4 in 2003, 10 in 2010 and 12 in 2011). Equal numbers of pure and mixed patches were sampled in any year within a region. We also weighed at least 30 batches of 100 seeds each from each

region in 2011 to assess mean seed mass in pure and mixed patches. Seeds from ten of these batches per region were stored at  $-18^{\circ}\text{C}$  for 1 month to break dormancy, then placed on filter papers in Petri dishes and kept in a growth chamber at 14 h light/ $25^{\circ}\text{C}$  and 10 h dark/ $10^{\circ}\text{C}$ . The number of germinated seeds was determined daily for approx. 3 weeks until no more seeds germinated.

#### Estimation of PL

To ascertain the extent of PL, we added self pollen and outcross pollen to flowers from all regions in 2010 to 2011. All hand-pollinations were performed using a fine brush twice a day on a given flower until the flower wilted. Only one flower within a whorl received additional pollen, whereas another flower within the whorl served as a control, receiving only natural pollination. Although conducting supplemental hand-pollinations at the flower level could lead to PL overestimation due to resource reallocation (Ashman *et al.*, 2004), our approach may be useful to compare patch types as long as the overestimation is of the same magnitude for all individuals (de Jong *et al.*, 2005; Jakobsson *et al.*, 2009). Within each region, at least 20 flowers from each type of patch were used for each treatment in each year. We define PLs (self PL) and PLx (outcross PL) indices after Eckert *et al.* (2010, box 1, p. 39) as:

$$\text{PLs} = (F_{\text{IS}} - F_{\text{IN}})/F_{\text{IN}}$$

$$\text{PLx} = (F_{\text{IX}} - F_{\text{IN}})/F_{\text{IN}}$$

where  $F_{\text{IS}}$  and  $F_{\text{IX}}$  indicate seed production per capsule under supplemental hand pollination with self and outcross pollen grains, while  $F_{\text{IN}}$  represents control. By using these two different PL indices we aimed to distinguish whether the plants suffered quantity PL (PLs), quality PL (PLx) or both.

#### Data analyses

We used general linear mixed models (GLMMs) to detect the influences of patch type and region on pollination and seed production. Year was a random effect for all parameters collected over multiple years. Additionally, we tested the effects of patch type and year on seed production by GLMM within each region. Analysis of pollination frequency, flowers visited per inflorescence within a foraging bout, flowers visited per

observation period and seed production were based on the patch unit. Each value for a certain patch was averaged over all observations from the patch. We used a general linear model (GLM) to test the effects of patch type and region on seed mass and germinability. We also investigated the effects of pollen source (self and outcross), patch type, region and year on extent of PL using GLMM. To fulfil requirements of GLMM (GLM), all data were analysed for normality of variance prior to analysis. Percentage data (e.g. seed germinability) were arcsine transformed. Within each region, average values of PLs and PLx from pure and mixed patches were tested by Tukey HSD test (homogeneous variances) or Games–Howell test (unhomogeneous variances) for multiple comparisons when one-way ANOVA revealed significance. The extent of IPT (ratio of ‘mixed’ stigmas containing some heterospecific pollen to ‘pure’ stigmas lacking it) and frequency composition of the pollinator fauna were analysed by Pearson’s chi-squared test to detect differences between pure and mixed patches. All statistical analyses were carried out in SPSS 18.0 and at a 5 % significance level.

## RESULTS

#### Pollinator behaviour

We observed four species of bumble-bees as pollinators of the lousewort (Table 1). The composition of the pollinator fauna did not differ between pure and mixed patches in the three regions. However, different pollinator types appeared to exhibit varying preferences for the pure and mixed patches (Table 1). The most frequent pollinators for both pure and mixed patches, nectar-feeding *Bombus richardsi* workers, visited lousewort plants with higher frequency in mixed (162 visits out of 346 total visits) than in pure patches (109 visits out of 310 total visits) ( $\chi^2 = 9.17$ ,  $P = 0.002$ , Table 1).

Patch type significantly affected pollination frequency, flowers visited per observation period, stigmatic pollen loads and flowers visited within an inflorescence on a single foraging bout (Table 2). Within each region, lousewort plants from mixed patches attracted more pollinators, had more flowers to be visited and had higher stigmatic pollen loads (Fig. 2A–C). The higher quantity of pollinator visits is mainly attributable to *Bombus richardsi* (Table 1). On the other hand, a pollinator in a mixed patch moved more frequently between individual

TABLE 1. Characteristics of pollinators visiting *P. densisipica* in the study system; flower constancy and frequency composition are combined from all regions and observation times

Pollinator	Rewards	Foraging behaviour	Flower constancy in mixed patch (%) <sup>†</sup>	Frequency composition (%) <sup>‡</sup>	
				Pure patch	Mixed patch
<i>Bombus richardsi</i> queens	Nectar and pollen	Licking nectar	61.3	9.5 <sup>a</sup>	8.6 <sup>a</sup>
<i>B. richardsi</i> workers	Nectar and pollen	Licking nectar	56.8	35.2 <sup>a</sup>	46.8 <sup>b**</sup>
<i>B. yunnanicola</i> workers	Pollen	Scraping pollen	95.3	29.0 <sup>a</sup>	24.9 <sup>a</sup>
<i>B. atrocinctus</i> queens	Pollen	Buzz-pollination	100	22.9 <sup>a</sup>	15.6 <sup>b*</sup>
<i>B. mirus</i> workers	Nectar and pollen	Licking nectar	92.3	3.5 <sup>a</sup>	3.8 <sup>a</sup>

<sup>†</sup>Flower constancy indicates the percentage of constant visits for lousewort among all visits for a certain pollinator type visiting mixed patches.

<sup>‡</sup>Frequency composition is the percentage of visits for a certain pollinator out of total visits by all pollinators recorded in pure and mixed patches, respectively. Sites with the same letter are not statistically different under Pearson’s chi-squared test: \* $P < 0.05$ , \*\* $P < 0.01$ .



TABLE 2. General linear mixed model of the effects of patch type, region and year on parameters of pollination and seed production of *P. densispica*

Source	Pollination frequency (individuals per 30min)			Flowers visited per observation period (30 min)			Stigmatic pollen load			Flowers visited per inflorescence within a foraging bout			Seed production per capsule							
	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P				
Patch type	1	4.38	4268.44	0.010	1	32.17	556.93	0.027	1	1013.27	172.11	0.048	1	22.59	18726.85	0.005	1	216.14	1093.85	0.001
Region	2	0.41	30.52	0.032	2	7.63	242.54	0.004	2	318.50	8.02	0.111	2	1.81	118.93	0.008	2	13.27	54.61	0.001
Year	1	0.00	0.06	0.837	1	0.09	—*	—*	1	6.86	0.45	0.563	1	0.02	—*	—*	1	0.01	0.05	0.952
Region × year	2	0.01	7.53	0.117	2	0.03	0.32	0.760	2	4.87	14.98	0.063	2	0.02	0.71	0.584	4	0.25	1.25	0.416
Patch type × year	1	0.00	0.53	0.532	1	0.06	0.58	0.525	1	81.97	2.28	0.270	1	0.00	0.05	0.836	2	0.20	1.02	0.436
Patch type × region	2	0.23	128.29	0.008	2	0.11	1.10	0.477	2	2.19	0.82	0.550	2	0.63	29.39	0.033	2	1.15	5.89	0.060
Patch type × region × year	2	0.00	0.06	0.941	2	0.10	0.39	0.683	2	3.20	0.14	0.867	2	0.02	0.13	0.881	4	0.20	1.62	0.182
Error	22	0.03			22	0.26			298	22.45			22	0.17			54	0.12		
Total	34				34				310				34				72			

Data for seed production per capsule are from 2002, 2010 and 2011; other data are from 2010 and 2011. Stigmatic pollen load was analysed at the level of an individual flower while all other analyses are based on values averaged at the patch level.

\*The missing value is due to the model being unable to compute the error degrees of freedom using Satterthwaite's method because of the very small mean square for this random factor.

plants and hence visited fewer flowers within an inflorescence in a foraging bout (Fig. 2D).

#### Estimation of IPT

Flower constancy of each *Bombus* species that visited mixed patches was different (Table 1). *Bombus richardsi* workers and queens, mainly searching for nectar, moved frequently between flowers of *P. densispica* and *A. pastori*, while pollen-collecting bumble-bees exhibited very high flower constancy. *Bombus yunnanicola* workers scraped pollen in a downward position and accomplished sternotribic pollination while *B. atrocinctus* queens collected pollen by buzzing flowers. The indiscriminate *B. richardsi* exhibited similar handling skills on *P. densispica* and *A. pastori*: they landed on and clung to the lower lip and licked nectar in an upward position. During this behaviour, pollen grains of *P. densispica* were deposited on the bee's dorsum and transferred to the stigma from its prodorsum (Fig. 1A), whereas pollen grains of *A. pastori* were deposited on the abdomen and transferred to the stigma protruding from the tip of the keel (Fig. 1B). In addition, we observed a conspicuous dorsum grooming habit of *B. richardsi* when visiting *P. densispica* flowers but not those of *A. pastori*. The extent of IPT did not differ between pure and mixed patches for any of the three regions (Table 3).

#### Reproductive success

For each region, plants from mixed patches yielded significantly more seeds than those from pure patches (Fig. 2E), as well as seeds of greater mass and germinability (Fig. 2F, G). Patch type and region were the main factors affecting overall seed production per capsule (Table 2), as well as seed production within each region (Table 4), seed mass and seed germinability (Table 5).

#### Estimation of PL

Lousewort plants from pure patches had higher values of both PLs and PLx than those from mixed patches, and PLx was much higher than PLs for plants in all patches and regions (Fig. 3). The extent of PL was significantly affected by pollen source and patch type (Table 6). Within each region, PLs and PLx of the plants from pure patches were significantly higher than those from mixed patches (S:  $F_{3,172} = 72.78$ ,  $P < 0.001$ ; R:  $F_{3,160} = 27.82$ ,  $P < 0.001$ ; M:  $F_{3,148} = 35.83$ ,  $P < 0.001$ ; see also Fig. 3).

## DISCUSSION

Reproduction of *P. densispica* appears to be strongly facilitated by co-flowering with *A. pastori*, regardless of region or year. Facilitation occurred in terms both of the quantity of pollinator visits, and associated increase in seed number, and in the quality of visits, and associated increase in seed mass and germinability.

#### Enhancement of quantity of pollinator visits

Flowers of *P. densispica* easily suffer PL due to their relatively smooth and small stigma surfaces (Yang et al., 2002). For full fertilization of ovules to take place, flowers require multiple

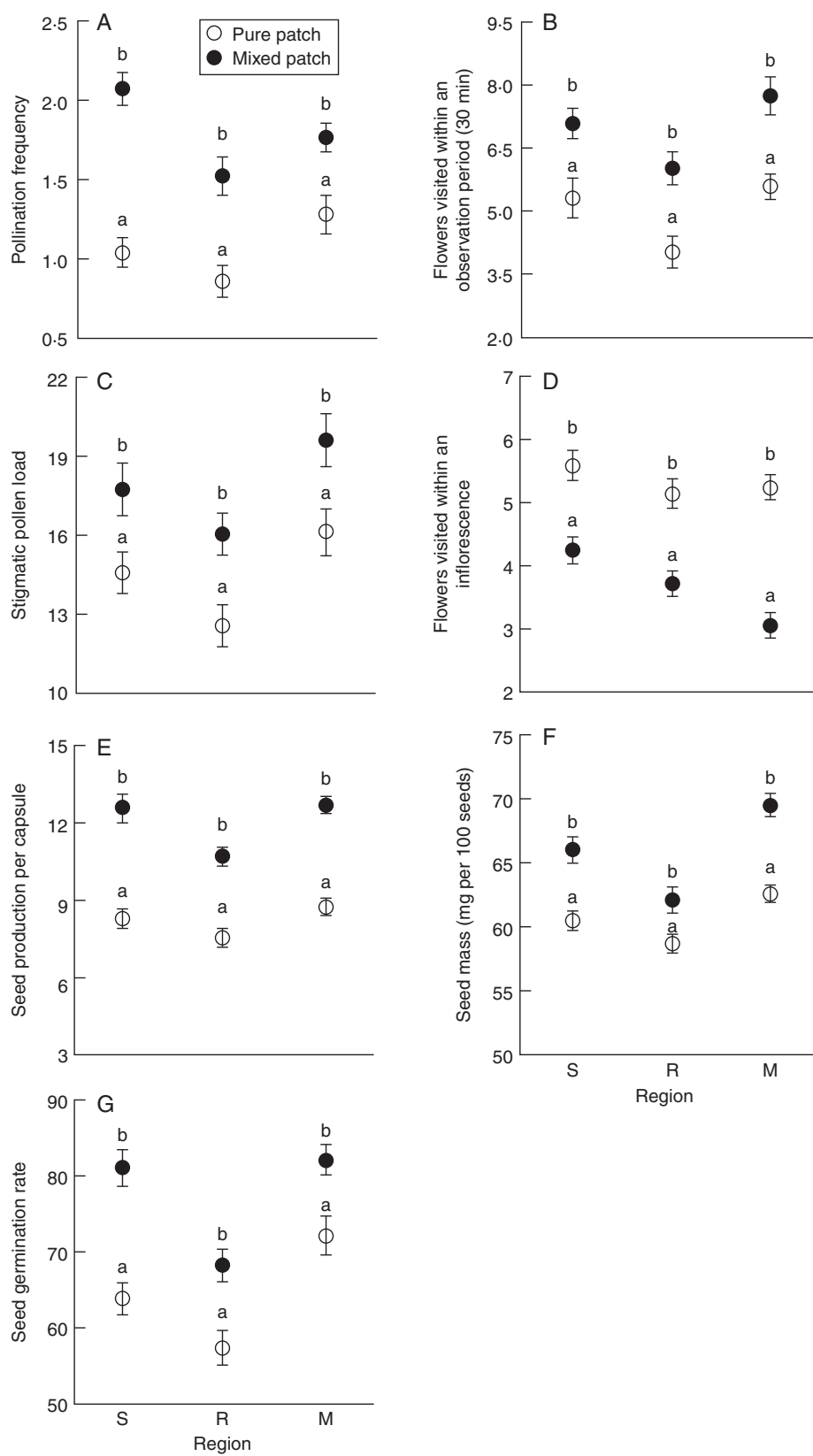


FIG. 2. Differences in parameters of pollination and reproductive success between lousewort plants from pure and mixed patches in each region (mean  $\pm$  2s.e.). Within each region, sites with different lower-case letters are significantly different.

visits by pollinators (Li, 2003; Yang, 2004). In comparison with pure patches, pollinators visited mixed patches at a higher frequency, so that flowers in those patches tended to receive more pollen and experience less PL. This conclusion is supported by our findings that both PLs and PLx are more severe for plants in pure than in mixed patches.

Co-flowering plant species may collectively attract more pollinators due to a combined display size (Schemske, 1981; Rathcke, 1983; Roy, 1994) or complementary attraction (Rathcke, 1988; Moeller, 2004). Either possibility could apply to our system, in which floral architecture differed strongly between the two species. Ghazoul (2006) also suggested that facilitation of pollinator attraction might occur when co-flowering species provide both nectar and pollen in complementary fashion. This possibility does not seem to apply here, as most pollinators searched for nectar in flowers of both species.

#### Enhancement of quality of pollinator visits

Previous studies showed that outcrossed *P. densispica* seeds have higher mass and germinability than those derived from

TABLE 3. Comparison of the extent of interspecific pollen transfer (IPT) between pure and mixed patches in the three studied regions (Pearson's chi-squared test)

Region	Pure patch		Mixed patch		$\chi^2$	P-value
	Pure stigmas	Mixed stigmas	Pure stigmas	Mixed stigmas		
S	76	11	69	9	0.05	0.828
R	56	8	66	11	0.10	0.757
M	47	5	51	8	0.42	0.519

selfing (Li, 2003). We found that PLx is much lower for plants in mixed than in pure patches. This is consistent with our finding that plants from mixed patches produced seeds with greater mass and germinability. Taken together, these results suggest that lousewort flowers receive more outcross pollen in mixed patches.

Any differences in outcrossing between plants from pure and mixed patches are most likely caused by differences in pollinator behaviour. The fact that bumble-bees move frequently between different kinds of flowers and visit fewer flowers within an inflorescence in mixed patches should reduce geitonogamous selfing, and this in turn would explain the observed differences in quality of pollinator visits.

#### Avoidance of IPT

Even if a focal plant receives more visits and experiences lower geitonogamy in the presence of a co-flowering plant species, this does not automatically mean that the interaction is facilitative, as IPT may still occur and may result in pollen loss or interference and thus limit reproductive success (Morales and Traveset, 2008). IPT has certainly been demonstrated to be an important potential mechanism of competition in co-flowering systems (Waser and Fugate, 1986; Galen and Gregory, 1989; Mitchell *et al.*, 2009). In short, the achievement of facilitative pollination is dependent strongly on the extent of IPT.

In our system, differences in floral architecture lead to strong mechanical isolation between *P. densispica* and *A. pastori*. Stigmas of *P. densispica* pick pollen grains from the dorsum of the bumble-bees, where anthers of *A. pastori* have no chance of depositing their pollen. This difference almost completely eliminates IPT while also reducing conspecific pollen loss.

TABLE 4. General linear mixed model of the effects of patch type and year on seed production per capsule within regions

Source	S region				R region				M region			
	d.f.	MS	F	P	d.f.	MS	F	P	d.f.	MS	F	P
Patch type	1	139.15	226.54	<0.001	1	48.49	986.70	<0.001	1	122.18	651.74	<0.001
Year	4	0.34	0.51	0.738	3	0.04	0.92	0.526	4	0.03	0.17	0.943
Patch type $\times$ year	4	0.68	5.15	0.004	3	0.04	0.23	0.872	4	0.20	1.97	0.128
Error	24	0.13			14	0.18			26	0.10		
Total	34				22				36			

Data were collected in 2002, 2003 and 2009–2011 for region S; in 2002 and 2009–2011 for region R; and in 2001–2003, 2010 and 2011 for region M. Values for analysis were averaged at the patch level.

TABLE 5. General linear model of the effects of patch type and region on seed mass (mg per 100 seeds) and percentage seed germination rate (arcsine transformed data)

Source	Seed mass				Seed germination rate			
	d.f.	MS	F	P	d.f.	MS	F	P
Patch type	1	1269.36	127.80	<0.001	1	0.46	39.72	<0.001
Region	2	487.44	49.08	<0.001	2	0.22	19.25	<0.001
Patch type $\times$ region	2	48.87	4.92	0.008	2	0.01	0.88	0.418
Error	174	9.93			90	0.01		
Total	180				96			

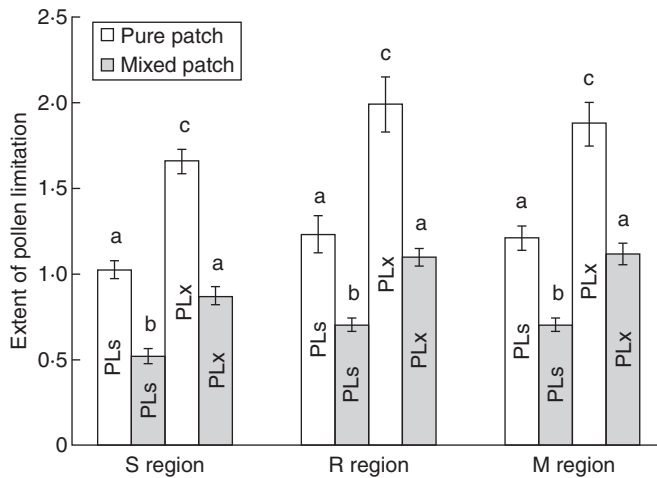


FIG. 3. Differences in extent of self and outcross pollen limitation (PLs vs. PLX) between lousewort plants from pure and mixed patches of the three regions (mean  $\pm$  2s.e.). Within each region, sites with different lower-case letters are significantly different.

TABLE 6. General linear mixed model of the effects of pollen source, patch type, region and year on extent of pollen limitation (PL)

Source	d.f.	MS	F	P
Pollen source (PLs and PLX)	1	36.09	505.94	0.028
Patch type	1	54.75	1347.34	0.017
Region	2	2.85	16.40	0.057
Year	1	0.00	0.16	0.994
Pollen source $\times$ patch type	1	2.94	171.63	0.049
Pollen source $\times$ region	2	0.08	0.73	0.577
Patch type $\times$ region	2	0.08	0.47	0.680
Patch type $\times$ year	1	0.04	0.24	0.669
Pollen source $\times$ year	1	.071	0.60	0.506
Region $\times$ year	2	0.17	0.67	0.565
Patch type $\times$ region $\times$ year	2	0.16	42.86	0.023
Patch type $\times$ pollen source $\times$ year	1	0.02	4.38	0.158
Pollen source $\times$ region $\times$ patch type	2	0.02	6.33	0.137
Pollen source $\times$ region $\times$ year	2	0.11	28.39	0.034
Patch type $\times$ pollen source $\times$ year $\times$ region	2	0.00	0.01	0.986
Error	476	0.27		
Total	500			

Floral divergence among sympatric species may be widespread as an evolutionary and ecological consequence of natural selection to reduce IPT (e.g. Waser, 1978; Morales and Traveset, 2008), although we do not claim that our study system necessarily has been structured in this way.

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