

The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (Orobanchaceae) through variation in bumble-bee foraging behaviour

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• **Background and Aims** The presence of co-flowering species can alter pollinator foraging behaviour and, in turn, positively or negatively affect the reproductive success of the focal species. Such interactions were investigated between a focal species, *Pedicularis monbeigiana*, and a co-flowering species, *Vicia dichroantha*, which was mediated by behaviour alteration of the shared bumble-bee pollinator.

• **Methods** Floral display size and floral colour change of *P. monbeigiana* were compared between pure (*P. monbeigiana* only) and mixed (*P. monbeigiana* and *V. dichroantha*) plots in two populations. Pollinator visitation rates, interspecific floral switching and successive within-plant pollinator visits were recorded. In addition, supplemental pollination at plant level was performed, and the fruit set and seed set were analysed in pure and mixed plots with different densities of *P. monbeigiana*.

• **Key Results** Pollinator visitation rates were dramatically higher in mixed plots than in pure plots. The higher pollinator visitation rates were recorded in both low- and high-density plots. In particular, successive flower visits within an individual plant were significantly lower in mixed plots. Supplemental pollination significantly increased fruit set and seed set of individuals in pure plots, while it only marginally increased seed set per fruit of plants in mixed plots.

• **Conclusions** The presence of *V. dichroantha* can facilitate pollination and increase female reproductive success of *P. monbeigiana* via both quantity (mitigating pollinator limitation) and quality (reducing geitonogamy) effects. This study suggests that successive pollinator movements among flowers within a plant, as well as pollinator visitation rates and interspecific flower switching, may be important determinants of the direction and mechanisms of interaction between species.

Key words: Floral traits, foraging behaviour, pollination, facilitative interaction, pollen limitation, *Pedicularis monbeigiana*, Orobanchaceae, reproductive success, *Vicia dichroantha*, bumble-bee, *Bombus richardsi*.

INTRODUCTION

Interactions among sympatric plants may affect their growth, reproduction and survival, thereby regulating plant community composition and dynamics (Callaway and Walker, 1997; Brooker, 2006). Competition and facilitation of abiotic resources (e.g. light, water or nutrients) is the most common plant–plant interaction mechanism (Callaway, 1997; Brooker *et al.*, 2008). In addition, biotic agents such as parasites and herbivores can also influence interactive processes (Hambäck and Beckerman, 2003; Hatcher *et al.*, 2006). For co-flowering species, shared pollinators may play an important role in the interactions between plants, thus influencing plant reproductive success and mating systems (Rathcke, 1983; Bell *et al.*, 2005; Bjerknes *et al.*, 2007; Mitchell *et al.*, 2009a, b).

Numerous studies have demonstrated that competitive effects are a common occurrence in pollinator-mediated interactions among plants (Caruso, 2000; Brown *et al.*, 2002; reviewed in Mitchell *et al.*, 2009a). Furthermore, these competitive interactions can be caused by exploitative competition for limited pollination service (Chittka and Schürkens, 2001) or interference competition via interspecific pollen transfer

(Galen and Gregory, 1989; Morales and Traveset, 2008) which, in turn, can lower the reproductive success of focal species (Bjerknes *et al.*, 2007; Flanagan *et al.*, 2010). In contrast, facilitative effects could occur in flowering plant communities in terms of the presence of pollinator-sharing species increasing pollination services of focal species, consequently ameliorating their seed output (Laverty, 1992; Johnson *et al.*, 2003). Feldman *et al.* (2004) have used mathematical models to shed light on the ecological requirements for pollination facilitation to occur; compared with competitive interactions a smaller number of facilitative effects have been observed in the field (Moeller, 2004; Ghazoul, 2006; Molina-Montenegro *et al.*, 2008). Moreover, several studies have reported neutral effects of interaction of plants on pollination (Jones, 2004; Kaiser-Bunbury and Muller, 2009). There is a need for further experimental evaluation of the ways in which ecological conditions and characteristics of species determine the direction and mechanisms of interaction due to pollinator sharing.

The presence of co-flowering species may produce alterations in pollinator guilds and foraging behaviour in focal species and, subsequently, influence plant reproductive

fitness (Bell *et al.*, 2005; Jakobsson *et al.*, 2008; Lázaro *et al.*, 2009). To date, most experimental studies have examined variation in pollinator visitation rates of focal species in the presence and absence of a co-flowering species (Johnson *et al.*, 2003; Muñoz and Cavieres, 2008; Yang *et al.*, 2011). In addition, pollinator movements between focal and neighbouring species during a single foraging bout have been extensively studied owing to the fact that foreign pollen interference often leads to conspecific pollen loss, sterility and/or hybridization (Burgess *et al.*, 2008; Jakobsson *et al.*, 2008; Morales and Traveset, 2008). More recently, Flanagan *et al.* (2009) reported that grooming by pollinators as they forage on a co-flowering species may also reduce pollen export and seed set in focal species.

Under-explored influence of the presence of co-flowering species involves its effect on successive visitation of flowers within an individual. This foraging behaviour may cause within-plant pollen transfer (geitonogamy) (Barrett *et al.*, 1994; Karron *et al.*, 2009). Geitonogamous pollen transfer often results in strong female mating costs due to selfing and associated inbreeding depression (Goodwillie *et al.*, 2005) and male mating costs via pollen discounting (i.e. the loss in outcross siring success as a result of self-pollination) (Harder and Barrett, 1995). It is likely that the effects of co-flowering species on the floral traits of focal species will alter pollinator foraging behaviour, and hence affect the patterns of pollen movement, such as the transfer of heterospecific pollen between co-flowering species and female fitness. For example, Fishman and Wyatt (1999) demonstrated variation in the style length in *Arenaria uniflora* in response to the presence of the competitor species *Arenaria glabra*, and Caruso (2000) reported that the presence of co-flowering *Castilleja linariaefolia* had an effect on the corolla length and flower number of *Ipomopsis aggregata*. Floral colour functions as one of the signals to attract pollinators and, if colour changes during anthesis, this signal may affect the pollinator's behaviour, such as staying for a shorter time on mixed inflorescences including pre- and post-change flowers, hence reducing successive visits (Sun *et al.*, 2005; Ida and Kudo, 2010). Floral display size is also thought to have a large impact on the degree of within-plant self-pollen movement (Goulson *et al.*, 1998; Karron *et al.*, 2009). Given that the presence of co-flowering species can have striking effects on floral display size and floral colour change, it is not difficult to envision that co-flowering species may have indirect effects on the level of geitonogamy of focal species, and hence lead to variation in reproductive success and genetic variability with potentially important ecological and evolutionary consequences of interaction among plants. To our knowledge, few studies have focused on the impact at the community level of pollinator movements among flowers within an individual of a species and estimated the overall interaction effects.

In this study, we investigated the interaction between a focal species, *Pedicularis monbeigiana*, and a co-flowering species, *Vicia dichroantha*, sharing a bumble-bee pollinator, in two alpine-meadow populations. We focused our studies on two floral traits of *Pedicularis*, namely floral display size and floral colour change, which are essential for pollinator attraction and foraging behaviour. Pollinator visitation rates, interspecific floral switching and successive within-plant

pollinator visits were observed. *Pedicularis monbeigiana* individuals were subjected to hand-pollination treatment and the fruit set and seed set were analysed. Specifically, this study addressed the following questions. (a) Are the floral colour change and floral display size of *P. monbeigiana* affected by the presence or absence of *V. dichroantha*? (b) Does the presence of *V. dichroantha* alter the foraging behaviour of the pollinator of *P. monbeigiana* and possibly the patterns of pollen movement in the species? (c) Does the presence of *V. dichroantha* affect the female reproductive success of *P. monbeigiana*?

MATERIALS AND METHODS

Study species and sites

Pedicularis monbeigiana Bonati (Orobanchaceae), an alpine perennial herb, is restricted to north-western Yunnan and north-western Sichuan in China. Reproductive plants may be up to 60 cm high and produce up to 16 inflorescences, and each racemose inflorescence acropetally bears 20–50 homogamous, nectarless, hermaphroditic flowers. The corolla consists of an upper lip (beak), a trilobate lower lip and a shallow tube. Anthers dehisce and the stigma is receptive at the time of anthesis. At the time of flower opening, the beak is purple and the lower lip is white. By late anthesis the colour of the lower lip has changed to purple (Fig. 1A). Like many other *Pedicularis* species (Karrenberg and Jensen, 2000), *P. monbeigiana* is self-compatible and exhibits inbreeding depression (Sun *et al.*, 2005). The capsule contains brown seeds and dehiscence occurs in August. *Vicia dichroantha* Diels (Leguminosae) is an alpine perennial herb native to Yunnan Province in China. The plant occurs in grasslands and meadows. Individuals produce one to six racemes, typically 60 cm in height. Each raceme has 20–30 nectariferous yellow flowers (Fig. 1B). The two species are pollinated exclusively by *Bombus richardsi* (Reinig), which forages in an inverted (sternotribic) position on *P. monbeigiana*, and forages in an upright (nototribic) position on *V. dichroantha* (Fig. 1A, B).

Fieldwork was conducted during two consecutive reproductive seasons (June to August in 2009 and 2010) in two natural populations located at 3300 m a.s.l. in Shangri-La County, Yunnan Province in China (27°52'N, 99°40'E). The populations are located far from the villages and are free from human disturbance and cattle grazing. One population (population M) is located on a hillside meadow in the east of Shangri-la County. The other population (population V) is located in a grassland valley about 10 km north of the site of population M. At our study sites, *P. monbeigiana* and *V. dichroantha* frequently grow together in a patchy distribution (Fig. 1C). Blooming periods in *P. monbeigiana* (hereafter *Pedicularis*) and *V. dichroantha* (hereafter *Vicia*) overlap in July.

Floral display size and floral colour change

To compare floral display size and floral colour change in *Pedicularis* in the presence and absence of *Vicia*, six 5 × 5 m plots, >100 m apart, were selected in population M in 2009. In three of the plots, the *Pedicularis* individuals were

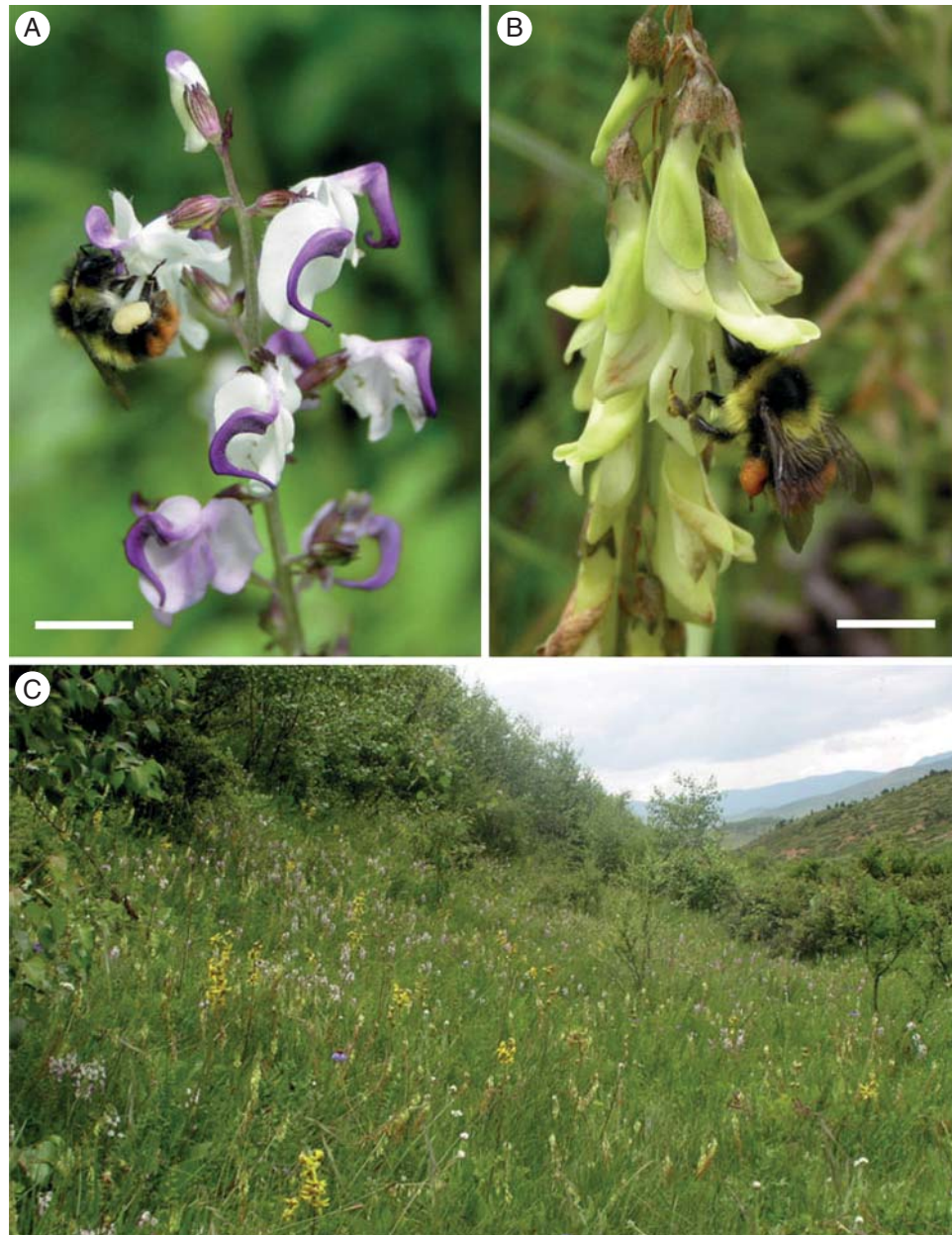


FIG. 1. Focal and co-flowering species and pollinator: (A) *Pedicularis monbeigiana* being pollinated by *Bombus richardsi*; (B) *Vicia dichroantha* being pollinated by *B. richardsi*; (C) *P. monbeigiana* and *V. dichroantha* growing in a natural population. Scale bars = 1 cm.

growing alone (>50 m away from any *Vicia* plants), while, in the other three mixed plots, *Pedicularis* individuals were associated (within 1 m) with three or four *Vicia* individuals. Three *Pedicularis* individuals per day were randomly chosen to count the number of white- and purple-phase flowers. The counts were repeated daily on eight sunny days during the peak of the flowering period. All treatments were performed in another six plots of population V in 2010.

Pollinator observation

To determine the effect of floral density on pollinator behaviour in *Pedicularis* in the presence or absence of *Vicia*, eight

5 × 5 m plots, 50 m apart, were assigned to two different flower-density categories in population M in 2009; i.e. four high flower-density plots with 30–40 flowering *Pedicularis* individuals, and four low flower-density plots with 5–15 flowering individuals. Individuals of each flowering density were further grouped into two types: two pure plots comprising *Pedicularis* individuals alone and two mixed plots comprising *Pedicularis* individuals associated with eight *Vicia* individuals. All the mixed plots had at least eight *Vicia* individuals at the beginning, and then extra *Vicia* individuals were removed to ensure equal numbers of *Vicia* individuals in dense and sparse plots. Flower visitors were recorded on six sunny days during the peak flowering season of *Pedicularis*

in July. Each day was divided into eight 20-min observation periods, beginning at 1000 h and finishing at 1400 h. During each observation period, the number of individual bumble-bee visitors to a single *Pedicularis* plant and the number of flowers visited by an individual bumble-bee within a *Pedicularis* individual was counted in each plot, and different plots were observed simultaneously by different observers. The number of interspecific flower visits was counted from each of the mixed plots. All pollinator observations were performed in another eight plots of population V on six sunny days in July 2010.

Pollen deposition on the stigma

To determine the number of pollen grains deposited on stigmas of *Pedicularis*, ten flowers were randomly collected from ten different individuals at a late phase of anthesis in each pure and mixed plot at 1700 h on each day of pollinator observation in both populations. Flowers were fixed immediately in formalin–acetic acid–alcohol (FAA) solution, and stored separately in 5-mL centrifugal tubes. The pistils were dissected carefully from flowers and gently squeezed under a cover glass in the laboratory. Later, the number of pollen grains from each stigma was counted using a microscope (Nikon E-600).

Pollination experiments and female reproductive success

The effects of the presence of *Vicia* on female reproductive success in *Pedicularis* were investigated in the same plots as the pollinator observation plots. Ten *Pedicularis* individuals in each plot were randomly subjected to hand-supplemented pollination, the other ten individuals served as a control in each plot. All pollination treatments were manipulated in another eight plots of population V in 2010. For hand-supplemented pollination, fresh anthers were collected from 20 flowers obtained from five non-focal individuals occurring approx. 100 m away from the study plot. Pollen was mixed and then transferred to recipient stigmas by gently rubbing with a fine paintbrush. To minimize any bias of resource allocation that might influence the evaluation of pollination limitation (Wesselingh, 2007), supplemental hand-pollinations were performed at the whole plant level at 2-d intervals equal to anthesis through the flowering period per plant, rather than at the level of flower or inflorescence. After 4 weeks, mature fruits on each study plant were collected, and the numbers of filled seeds, aborted seeds and unfertilized ovules were counted. A few of the fruits that had been damaged by seed predators were disregarded. Two components of female reproductive success for each plant were measured: (1) fruit set (ratio of fruits to flowers per plant) and (2) seed set per fruit as given by filled seeds/(filled seeds + aborted seeds + unfertilized ovules).

Data analysis

To ensure interspersed experimental units, and avoid pseudoreplication, a randomized complete block design was used (Hurlbert, 1984). This applies to all field experiments. Plots and individuals were in all cases treated as random

factors. Floral display size, the proportion of purple-phase flowers per plant and pollen deposition on the stigma were analysed by ANOVA (Pro MIXED), with plot type (pure or mixed plot) and population as fixed effects. Pollinator visitation rates were calculated as the average number of pollinator visits per plant and per hour, and were analysed by ANOVA (Pro MIXED), with plot type, population and plot density as fixed effects. Because the successive flower visits within *Pedicularis* individuals were a small integer thus violating the normal distribution assumption of ANOVA, this variable was analysed using a generalized linear model (Proc GLIMMIX) with Poisson errors, a log-link function and type III significance tests (Agresti, 1996). The fruit set and seed set were analysed using MANOVA (Pro MIXED), the pollination treatment, plot type, plot density, population and their interactions as fixed effects. Because fruit set and seed set may be intercorrelated, a MANOVA was used initially to control for Type I error (Rencher, 1995). A significant MANOVA was followed by univariate ANOVA, using the Bonferroni method for multiple comparisons. To achieve a normal distribution, the proportion of purple-phase flowers per plant, fruit set and seed set were arcsine square-root transformed, and floral display size and pollen deposition on stigma was log-transformed. All statistical analyses were performed with SAS statistical software (SAS Institute Inc., version 9.2).

RESULTS

Floral display size and floral colour change

The floral display size of *Pedicularis* in pure plots was higher than that in mixed plots ($F_{1,284} = 64.31$, $P < 0.001$; Fig. 2). However the differences in floral display size between populations were not statistically significant ($F_{1,284} = 0.07$, $P = 0.79$). The proportion of purple-phase flowers per plant in mixed plots was higher than that in pure plots ($F_{1,284} = 237.19$, $P < 0.001$; Fig. 2). The differences in the proportion of purple-phase flowers per plant between populations were not statistically significant ($F_{1,284} = 1.73$, $P = 0.18$).

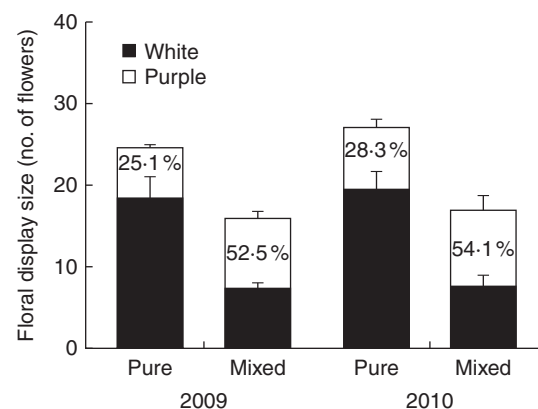


FIG. 2. Floral display size per individual of *P. monbeigiana* (mean \pm s.e.) and the proportion of purple-phase flowers per individual of *P. monbeigiana* (percentages shown) in pure and mixed plots in 2009 and 2010.

Pollinator behaviour

During 768 observation periods, both *Pedicularis* and *Vicia* were exclusively pollinated by *Bombus richardsi* (Apidae) workers. Occasional visits by other Hymenoptera and Lepidoptera were observed, but these insects did not perform pollination (Sun *et al.*, 2005; K. Liao, pers. obs.). The rate of pollinator visitation to *Pedicularis* was higher in mixed plots than in pure plots in both populations ($F_{1,764} = 194.52$, $P < 0.001$; Fig. 3). Marked increases in pollinator visitation rates were associated with increased density of *Pedicularis* individuals, and pollinator visitation rates of dense plots were significantly higher than those in sparse plots in both populations ($F_{1,764} = 42.78$, $P < 0.001$). However, the ANOVA test showed that pollinator visitation rates in mixed plots were higher than those in pure plots, even after correcting for flower density ($F_{1,764} = 217.64$, $P < 0.001$). The number of consecutive floral visits to individual *Pedicularis* displays was higher in pure plots than in mixed plots in both populations ($\chi^2 = 162.38$, $P < 0.001$; Fig. 4), and this difference was not affected by population ($\chi^2 = 1.76$, $P = 0.18$). Successive flower visits per individual of *Pedicularis* in pure plots were higher than those in mixed plots in both sparse

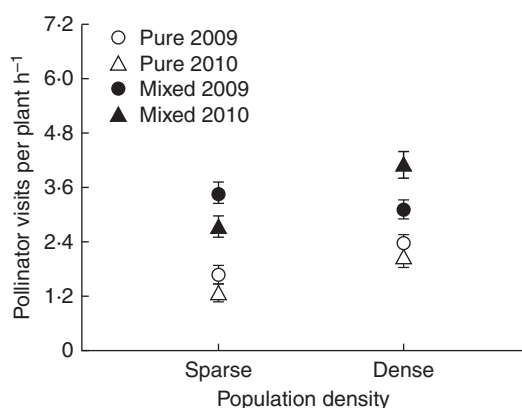


FIG. 3. The number of pollinator visits to *P. monbeigiiana* individuals in pure and mixed plots, as indicated, for sparse and dense populations (see text for details). Each value is the mean (\pm s.e.) number of pollinator visitations per *P. monbeigiiana* individual per hour.

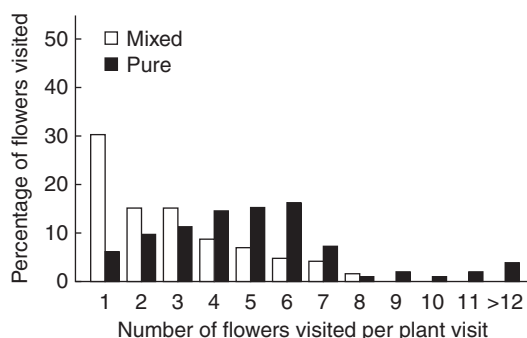


FIG. 4. Distribution of successive flower visits within plants of *P. monbeigiiana* in pure and mixed plots. The number of plant visits was 237 in pure plots and 445 in mixed plots.

and dense plots [mean \pm standard error (s.e.) = 5.13 ± 0.21 in pure plot, 2.84 ± 0.13 in mixed plot]. Interspecific floral visits, either from *Pedicularis* to *Vicia* or in the reverse direction, were rare in both populations or comprised $< 1\%$ of all visitations.

Pollen deposition on stigma

In both pure and mixed plots, pollen deposition per stigma (mean \pm s.e. = 19.03 ± 1.16 , $n = 120$ in the pure plot, 22.38 ± 1.51 , $n = 120$ in the mixed plot) frequently exceeds the number of ovules per flower (mean \pm s.e. = 12.17 ± 0.26 , $n = 240$). There were no significant effects of the presence of *Vicia* ($F_{1,236} = 2.45$, $P = 0.07$) or population ($F_{1,236} = 2.23$, $P = 0.08$) on pollen deposition on the stigma of *Pedicularis*, nor was the presence of *Vicia* \times population effect significant ($F_{1,236} = 1.05$, $P = 0.37$).

Female reproductive success

Using a MANOVA, we found that plot type (pure or mixed plot), pollen supplementation and plot density had significant effects on female reproductive success (i.e. fruit set and seed set; Table 1). Population was not associated with female reproductive success (Table 1). In pure plots, plants with supplemental pollination had significantly higher fruit set than plants in the open-pollinated control treatment, experiencing 1.7 and 0.92 times higher fruit set in sparse and dense plots (Fig. 5A). However, pollen supplementation only increased fruit set by 0.38 and 0.31 times in mixed plots in sparse and dense plots (Fig. 5A). Similarly, plants that received supplemental pollen experienced 2.1 and 1.1 times higher seed set compared with open-pollinated control plants in pure plots in sparse and dense plots, respectively, but pollen supplementation only increased seed set by 36% and 35% in mixed plots in sparse and dense plots, respectively (Fig. 5B). Therefore, the increase in female reproductive success via supplemental hand-pollination was more in high-density pure plots.

DISCUSSION

Previous studies have reported that rewarding species can facilitate pollination of adjacent non-rewarding plants, attracting a greater abundance of pollinators to mixed floral stands (Laverty, 1992; Johnson *et al.*, 2003; Juillet *et al.*, 2007). In our study, the number of pollinator visits to *Pedicularis* in mixed plots was markedly higher than in pure plots, indicating that *Vicia* with its rich nectar may acts as a magnet species for *Pedicularis* that provides only pollen as a reward to the foraging bumble-bee. Similar patterns of bumble-bee foraging behaviour were reported by Ghazoul (2006) who found that bumble-bees visited *Cirsium arvense* and *Hypericum perforatum* primarily for nectar, but collected pollen from the nectar-deficient *Raphanus raphanistrum*. Thus, our results support the hypothesis of facilitation of pollination due to enhanced floral resource diversity (Ghazoul, 2006).

However, if shared pollinators prefer the co-flowering species to a less attractive focal species, this may offset the advantage accruing from an increased number of pollinator

TABLE 1. Effect of plot type (pure or mixed), population, treatment (supplemental hand pollination or control), plot density (low or high) on levels of fruit set and seed set in *Pedicularis monbeigiana*

Factor	MANOVA (d.f. = 2, 303)		Fruit set (d.f. = 1, 304)		Seed set (d.f. = 1, 304)	
	Wilks' λ	F	SS	F	SS	F
Plot type	0.91	16.15***	1.53	41.57***	1.37	22.46***
Population	0.99	0.07	0.05	2.94	0.05	1.92
Treatment	0.98	1.05**	10.33	107.12***	8.34	113.27***
Plot density	0.97	1.98**	0.29	6.38**	0.77	14.35**
P × Pop	0.69	88.23***	0.03	1.36	0.03	0.61
P × T	0.92	13.26***	0.68	21.32**	1.09	18.13***
P × D	0.95	5.03**	0.45	15.31**	0.89	10.26**

In the factor column, P × Pop denotes population by plot type interaction, P × T denotes treatment by plot type interaction, P × D denotes plot density by plot type interaction. Plot and individual were included as two random factors in models. Asterisks indicate MANOVA or univariate ANOVAs that are statistically significant: ** $P \leq 0.01$, *** $P \leq 0.001$.

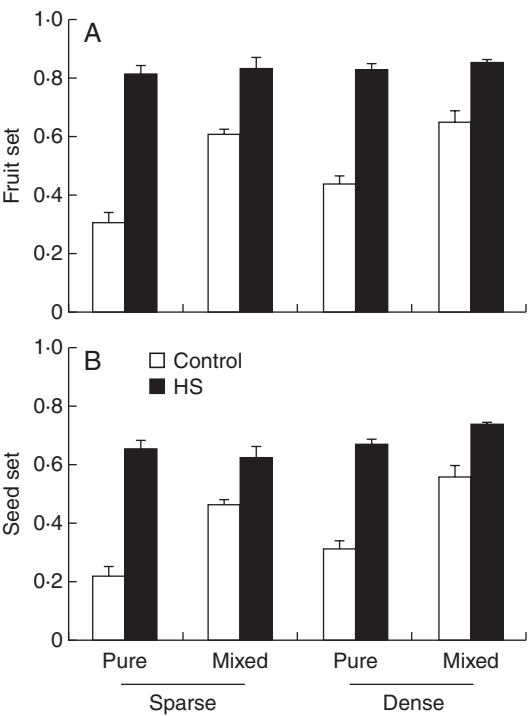


FIG. 5. Female reproductive success of plants that were supplementally pollinated by hand (HS) or were naturally pollinated (control) in different plots of *P. monbeigiana*: (A) mean fruit set; (\pm s.e.) (B) mean seed set per fruit (\pm s.e.).

visits and it may even reduce the pollination service to the focal species (Brown *et al.*, 2002; Muñoz and Cavieres, 2008; Kandori *et al.*, 2009). Density and relative abundance of co-flowering species may determine this foraging preference, thereby influencing the direction and mechanisms of interaction (Rathcke, 1983; Muñoz and Cavieres, 2008; Morales and Traveset, 2009; Flanagan *et al.*, 2010). Our results show that *Pedicularis* can receive more bumble-bee visits when growing with *Vicia* in either sparse or dense plots. A similar positive effect was also reported by Molina-Montenegro *et al.* (2008) who demonstrated an increase in pollinator visitation rates at three different densities

of the focal species. Such benefits which are not dependant on the flower density of the focal species may be explained by the fact that the dense plots from natural populations in both our study and that of Molina-Montenegro *et al.* (2008) were still not dense enough to influence the interaction.

In flowering plant communities, pollinator movement between flowers of different species may cause heterospecific pollen deposition and conspecific pollen loss and, thus, lower the reproductive success of the focal plant (reviewed in Morales and Traveset, 2008). However, the indiscriminate pollinator shifts between the two study species were rarely observed during all the observation periods, indicating that flower constancy occurs in the shared pollinator, *Bombus richardsi*. The ethological isolation based on flower-constant foraging behaviour will prevent heterospecific pollen transfer with its negative effects on plant reproductive fitness (Grant, 1994; Gegear and Laverty, 2005). Two possible reasons may explain why bumble-bees always visit conspecific flowers in a single foraging bout to a mixed floral stand. (1) The two species are markedly different in their floral morphology and colour, which increases the costs of switching between species (Laverty, 1994; Chittka *et al.*, 1999). By contrast, in almost all cases, where competition has been observed, co-flowering species have similar floral morphology or colour to the focal species (Caruso, 2000; Muñoz and Cavieres, 2008; Matsumoto *et al.*, 2010). Our results provide support for the hypothesis that facilitative effects can occur in interaction among morphologically diverse floral species (Ghazoul, 2006; Hegland *et al.*, 2009; Sargent *et al.*, 2011). (2) Both our study species are pollination-specialists; this is particularly true for *Pedicularis* which in China is exclusively pollinated by *Bombus* (Macior *et al.*, 2001; Wang and Li, 2005). Previous studies have indicated that pollination-generalist co-flowering species are particularly likely to become competitors (Memmott and Waser, 2002; Lopezaraiza-Mikel *et al.*, 2007; Muñoz and Cavieres, 2008). In addition, our results suggest that shared specialized pollinators serving multiple species are more likely to result in facilitative interaction.

Hand-supplemented pollination at the individual level significantly increased fruit set of *Pedicularis* in pure plots, but it had a minimal effect in mixed plots in both populations.

This indicates that the presence of *Vicia* alleviates pollinator limitation in *Pedicularis*. However, seed set supplemented by hand pollination was similar in mixed and pure plots, all being below 75 % (Fig. 5B). This relatively low value indicates that resource limitation was present in both types of plots. This result is in agreement with available information for other species of *Pedicularis*, which have been shown to be resource limited for seed set (Yang *et al.*, 2005; K. Liao, unpubl. res.). In addition, we found that *Pedicularis* individuals always have higher fruit set when growing associated with *Vicia*, whether in sparse or in dense plots. Taken together, the consequence of such a facilitative effect on reproductive success is mostly due to an increase in pollinator visitation rates rather than resource availability or density (Moragues and Traveset, 2005; Molina-Montenegro *et al.*, 2008). Furthermore, fruit set in individuals growing in sparse plots is much greater than in those in dense plots; this result demonstrates that low-density populations are more likely to derive greater benefit from shared pollinators than high-density populations (Moeller, 2004; Ghazoul, 2006; Muñoz and Cavieres, 2008; Jakobsson *et al.*, 2009).

Compared with the control, supplemental outcrossing significantly increased seed set in pure plots, but had only a slight effect on mixed plots, indicating that seed set was pollen-limited only in pure plots. More recently, several studies have suggested that pollination limitation due to supplemental outcrossing pollination masked geitonogamous pollination in nature (Aizen and Harder, 2007; Vaughton *et al.*, 2010). In our study, in both pure and mixed plots, pollen deposition frequently exceeded the number of ovules, and the successive flower visits per plant in mixed plots were significantly less than those in pure plots, suggesting that seed set of *Pedicularis* in the absence of *Vicia* may be limited by pollen quality via geitonogamous pollen rather than pollen quantity. In a previous study, artificial geitonogamous pollination experiments demonstrated that seed set of *Pedicularis* was considerably reduced (Sun *et al.*, 2005). There are two non-exclusive mechanisms which may lower geitonogamy in *Pedicularis* in mixed plots rather than in pure plots: (1) a smaller floral display size per plant in mixed plots may reduce the probability of geitonogamous pollen transfer (Galloway *et al.*, 2002; Lau, *et al.*, 2008); and (2) a higher proportion of floral colour change in *Pedicularis* might contribute to the reduced level of geitonogamy (Weiss, 1991; Ida and Kudo, 2010). Less-rewarding purple-phase flowers of *Pedicularis* make insects visit more successive flowers when foraging mixed inflorescences than pure pre-change inflorescences and, hence, floral colour change in *Pedicularis* functions as a mechanism for enhancing inter-individual pollen transfer and reducing intra-individual pollen transfer (Sun *et al.*, 2005).

In summary, the presence of the co-flowering species *V. dichroantha* can facilitate pollination and increase reproductive success of the focal species *P. monbeigiana* via both quantity (mitigating pollinator limitation) and quality (reducing geitonogamy) effects. Moreover, the extent of positive effects was negatively correlated with the floral density. In addition, our results show that *P. monbeigiana* is capable of a flexible reaction to the presence of co-flowering *V. dichroantha* by way of floral display size and floral colour-change variations and thereby balance between enhancing

attraction and relieving the negative effects of geitonogamy on seed production in the plants. This study also suggests that successive pollinator movements among flowers within a plant, as well as pollinator visitation rates and interspecific flower switching, may be important determinants of the direction and mechanisms of interaction among multi-flower species. Our understanding of the role of pollinator foraging behaviour in influencing the outcome of interactions among flowering species has been enhanced by the present study.

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