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# Reproduction of *Hedysarum scoparium* (Fabaceae) in patched habitat is pollen limited, but not just pollinator limited

ChengChen PAN<sup>1\*</sup>, LinDe LIU<sup>2</sup>, HaLin ZHAO<sup>1</sup>, JiLiang LIU<sup>1</sup>, YueLi HOU<sup>2</sup>, Li ZHANG<sup>2</sup>

<sup>1</sup> Cold and Arid Regions Environment and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China;

<sup>2</sup> School of Life Sciences, Ludong University, Yantai 264039, China

**Abstract:** Pollen limitation of plant reproduction occurs across Angiosperms, particularly those in patched habitats. We investigated the relationship between pollen limitation and patch variables (patch size, visitation frequency) in the desert plant *Hedysarum scoparium* (Fabaceae), which is an important xerophyte in the arid and semi-arid regions of Northwest China and can grow well as a pioneer plant in shifting sand dunes. We observed insect visitation to *H. scoparium* over two flowering seasons and estimated pollen limitation using fruit set and seed production. Our results indicate that fruit set and seed production increased significantly with pollen supplementation compared with open pollination. *Hedysarum scoparium* was pollinated by over 8 species of bees, with 88.4% of visits made by introduced honeybees (*Apis mellifera*). Bee visitation varied significantly among the patches of habitats, but not associated with patch size of habitat. In general, pollen limitation occurred more strongly during fruit set than during seed production. The patches that received higher rates of pollinator visits were less pollen limited for fruit set. Pollen limitation for seed production, however, was not associated with pollinator visitation frequency. We conclude that pollen limitation in *H. scoparium* was caused by more than one reason, not just pollinator visits.

**Keywords:** *Hedysarum scoparium*; pollen limitation; pollinator limitation; honeybees; fruit set; seed production

A lower fruit set and/or seed production caused by a scarce pollen receipt (Burd, 1994) is ubiquitous across Angiosperms (Larson and Barrett, 2000; Ashman *et al.*, 2004; Knight *et al.*, 2005; Knight *et al.*, 2006), particularly those in patched habitats (Aizen *et al.*, 2002). The term “pollen limitation” has been used to describe this phenomenon. Pollen limitation disrupts plant–animal interactions, especially plant–pollinator interactions, limits plant recruitment, and so can influence the long-term persistence and viability of plant population (Groom, 1998; Hobbs and Yates, 2003; Morgan *et al.*, 2005), and increase the extinction risk of small, low-density patches (Aizen and Feinsinger, 2003; Harris and Johnson, 2004; Ghazoul, 2005; Aguilar *et al.*, 2006).

Habitat patchiness is one of the main results of current global change and has been considered one of the

most important causes of extinction at the population and species levels (Hanski, 1998; Lindenmayer and Fischer, 2006). When pollen limitation is observed in such habitats, besides the shortage of mates for cross-pollination (Cunningham, 2000; Duncan *et al.*, 2004), it is often interpreted as evidence for insufficient visitation by pollinators (pollinator limitation of reproduction; Cunningham, 2000; Aizen and Feinsinger, 2003; Harris and Johnson, 2004; Ghazoul, 2005; Aguilar *et al.*, 2006; Schleuning *et al.*, 2011). For example, Aizen and Feinsinger (1994a) found that many species in forest patchy distribution showed pollinator limitation. Schleuning *et al.* (2011) found that in *Heliconia metallica*, the patchy distribution increased pollen limitation of reproduction due to the

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\* Corresponding author: ChengChen PAN (E-mail: panchengchen@163.com)

low visitation rates of pollinators. Two general evidences have been proposed to support the broad assumption that plants in patched habitats have fewer visitors. First, patchiness threatens pollinator communities (Jennersten, 1988; Rathcke and Jules, 1993) due to the scarcity of suitable habitat and other resources to support resident pollinator populations (McIntyre and Hostetler, 2001; Steven *et al.*, 2003). Second, pollinator abundance can decrease due to the lower attractiveness to the small patch size of habitat or to the lower density of flowering plants (Steffan-Dewenter and Tscharntke, 1999; Goverde *et al.*, 2002). However, the cause of pollen limitation for plants in patched habitats needs not always to be pollinator visits. *Echinacea angustifolia* in fragmented prairie, for example, was pollen-limited but not pollinator-limited (Wagenius and Lyon, 2010). And some authors have suggested that in equilibrium, reproduction in plants should be limited by more than one factor (Haig and Westoby, 1988; Dogterom *et al.*, 2000).

Patchy distribution of plant communities has been recognized as one of the most conspicuous ecological features in arid and semiarid regions throughout the world (Kotliar and Wiens, 1990; Aguiar and Sala, 1999). However, its effects on ecological processes in general, plant reproduction in particular, have received little attention in the Hexi Corridor region, where land degradation, in the form of desertification typically, is a very serious ecological problem (Qi *et al.*, 2003). As global environmental change progresses, desertification advances at an accelerated pace (Qi *et al.*, 2003), which may foster reduction of suitable habitats and increase patchiness. Thus, there is a need for more empirical studies to detect the effects of habitat patchiness on critical ecological processes such as the reproductive performance of plant species. These studies will improve prediction of plant development and provide a sound basis for establishing conservation guidelines in arid and semiarid regions prone to desertification, in the Hexi Corridor region for instance.

We investigated these issues in *Hedysarum scoparium* (Fabaceae), a shrub which is an important xerophyte in the arid and semiarid desert areas of Northwest China and can grow well as a pioneer plant in shifting sand dunes. It is well known for its capability

to live in desertified habitats (Liu and Zhao, 2001). The study was carried out in the middle of the Hexi Corridor region of Gansu province, adjacent to the southern edge of the Badain Jaran Desert in China. Small, isolated patches supporting small *H. scoparium* populations are frequent in this region.

In this study, pollinator assemblage composition, visitation frequency and pollen limitation were explored to determine whether pollen limitation is pollinator mediated. A pollen supplementation experiment was performed to ascertain the role of pollen limitation in fruit set and seed production (Knight *et al.*, 2005). Specifically, we determined (i) the pollinator assemblages and visitation frequencies among different patches; (ii) the occurrence of pollen limitation across six focal patches, quantified with the net reproductive rate ( $R_0$ ); and (iii) the relationship between the frequency of pollinator visitation and the intensity of pollen limitation.

## 1 Materials and methods

### 1.1 Study area

The study area, the middle part of the Hexi Corridor region in Gansu province of Northwest China, is located between 39°19'–39°19'N and 100°02'–100°21'E at the southern edge of the Badain Jaran Desert, with an altitude ranging from 1,368 to 1,380 m. This region is connected with dense shifting dunes as well as Gobi, and is characterized by a temperate continental climate, dry and hot in the summer and cold in the winter. The annual mean precipitation is 117 mm, with 65% occurring in the summer, while the annual mean evaporation is over 2,390 mm. Annual mean temperature is 7.6°C, while the absolute maximum can reach 39°C and minimum –27°C. The mean temperature in the growing season (late May to early October) is 20.4°C. Plants here are principally woody species such as *Haloxylon ammodendron*, *Calligonum mongolicum*, *C. gobicum*, *C. potanini*, *C. chinense*, *Caragana korshinskii*, *Hedysarum scoparium*, *Nitraria sphaerocarpa*, and *Reaumuria soongorica*. Except *H. ammodendron* which often grows up to small trees, the others belong to shrub species.

### 1.2 Species

*Hedysarum scoparium* (Fabaceae), is a perennial,

large shrub usually growing in arid and semi-arid desert regions. It often grows to 2–4 m high. The species is characterized by low density and patchy distribution in the study area, and its patch size ranges from 2 to 5,000 m<sup>2</sup> with single units of 1–50 shrubs. *Hedysarum scoparium* is self-compatible, but not autogamous (Pan *et al.*, 2010). It produces racemose inflorescences and nectar-secreted, hermaphroditic flowers with degraded wings that open during daylight hours. Flowering occurs in late May and lasts into late September with twice flowering peaks in approximately mid-June and mid-August. During its second flowering peak, *H. scoparium* is virtually the only dominant species in full bloom. The distribution range of this species in China covers the Hexi Corridor region, the Badain Jaran Desert, the Tengger Desert and the Gurbantunggut Desert (Pan *et al.*, 2010). For this study, six patches were deliberately selected. Patches were 200 m apart at least.

### 1.3 Experimental design of pollen limitation

To estimate the degree of pollen limitation, we conducted a pollen-supplementation experiment in each of the six focal patches in September 2010. In each patch, we labelled 12 plants at the same flowering stage. All plants for the experiment were similar in size. In 6 randomly designated plants, we labelled 8 inflorescences, adding outcross pollen in 4 inflorescences (Pollen added (PA) treatment) and leaving the other 4 as control (C treatment). Four inflorescences were also labelled from the 6 remaining plants as a procedural control (CC treatment). PA and C flowers used in this experiment were chosen from the same part of the stalks with flowers in order to avoid any noisy outcome caused by the inherent effect of flower position on reproduction and pollen limitation. CC flowers were used to detect the effect of pollen supplementation on the re-allocation of resources from C flowers (Wesselingh, 2007; Gómez *et al.*, 2010). This protocol makes our results conservative and our conclusions robust. PA flowers were administered pollen from individuals 20 m away at least. We pollinated all new receptive flowers from 11:00–15:00 every day. In total, 4,615 flowers from 72 plants were used in this experiment.

After maturation, we counted the number of ex-

perimental flowers that had produced fruits. These fruits were taken to the laboratory for determination of the seed number per fruit.

To measure pollen limitation, the following components of plant reproductive output were used: (i) fruit set, proportion of flowers setting fruit; and (ii) seed production, number of seeds per fruit. We determined pollen limitation index (PL index, a measure of the magnitude of pollen limitation) for each of the both reproductive components. The PL index was calculated as:  $PL = 1 - (R_C/R_{PA})$ . Where  $R_C$  is the fruit set or seed production of C treatment and  $R_{PA}$  the fruit set or seed production of PA treatment (thus, we had two PL indices,  $PL_C$  for C plants and  $PL_{CC}$  for CC plants; Gómez *et al.*, 2010). Each of the plant individuals in the experiment was used to calculate PL index according to Larson and Barrett (2000), and thereby PL index is always expressed at plant level. The PL index ranges from 0 (indicating no pollen limitation) to 1 (indicating the highest pollen limitation).

### 1.4 Flower visitors and visitation frequency

Pollinator observations were carried out in September 2009 and 2010. Observations were conducted to identify flower visitors and to determine their visitation frequencies in the field on sunny days. Six plants were monitored in the 6 focal patches and each patch was observed for two days within the study period. We observed the area every 30 min from 08:00 to 18:00, with a total of 60 hours of field observations. During observations, the species and the number of visits made by each flower visitor were recorded. Most individual flower visitors were identified in the field, but some specimens were captured and sent to specialists at Ludong University, Yantai, China, for identification. We only treated the insects carrying pollen grains of *H. scoparium* on their bodies and touching flower sex organs as potential pollinators (Pérez-Barrales, 2007).

Visitor visitation frequency ( $V_f$ ), visiting number per flower per hour, was then calculated as:  $V_f = V/(F \cdot T)$ , where  $V$  is the total number of visits to flowers,  $F$  the total number of flowers in the patch, and  $T$  the observation time in hours (Cosacov *et al.*, 2008). Also calculated is the relative frequency of visits by each insect species (percentage of visits), which provides a relative abundance index for each species in

each patch. Differences among patches in  $V_f$  were tested with one-way ANOVA.

### 1.5 Data analysis

Separate analyses were performed for comparing inter-treatment differences in reproductive output. To compare the fruit set and seed production between PA and C treatments, PA and CC treatments, C and CC treatments, we performed repeated-measures ANOVAS, using treatment as the within-subject factor and patches as the between-subject factor. Data from individual flowers belonging to the same treatment and plant were averaged (Gómez *et al.*, 2010).

As there was no evidence of resource re-allocation among flowers (see Results), all the subsequent analyses were performed on the C and PA flowers of treated plants.

In order to determine how well the magnitude of pollen limitation reported for fruit set was correlated with the magnitude of pollen limitation for seed production per plant, we correlated both indices (Knight *et al.*, 2005). In order to determine the effect of patch size on  $V_f$ ,  $V_f$  on mean  $PL_C$  index for fruit set and seed production, simple linear regression analyses were performed among the studied patches (Cosacov *et al.*, 2008).

Most statistical analyses were carried out using the statistical software package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

## 2 Results

### 2.1 Pollen limitation

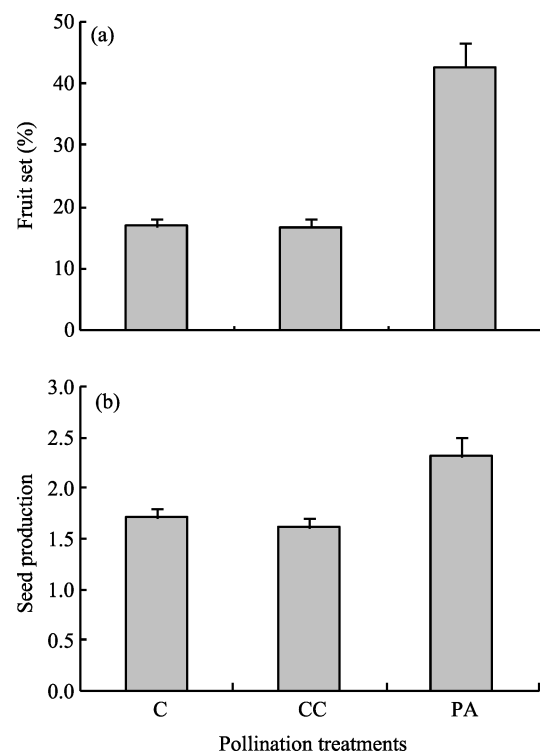
Based on comparisons of the performance of supplemented flowers with that of C and CC flowers, the experimental pollen supplementation was significantly increased in the net fruit set and seed production of the plants (Fig. 1). Fruit set did not differ significantly between C flowers ( $17.0 \pm 1.1\%$ ) and CC flowers ( $16.8 \pm 1.4\%$ ). However, it differed significantly between PA flowers ( $42.8 \pm 4.1\%$ ) and C and CC flowers (Fig. 1a; Table 1). Pollen supplementation significantly increased seed production per flower in PA flowers ( $2.3 \pm 0.2$  seeds per fruit; Fig. 1b; Table 1) compared to C flowers ( $1.7 \pm 0.1$  seeds) and CC flowers ( $1.6 \pm 0.1$  seeds).

The  $PL_{CC}$  indices for fruit set and seed production

were  $0.613 \pm 0.073$  and  $0.276 \pm 0.023$ , respectively, whereas the  $PL_C$  indices for fruit set and seed production were  $0.624 \pm 0.062$  and  $0.248 \pm 0.056$ , respectively (i.e. pollen supplementation increased the net fruit set of the plants by 61% and 62%, and seed production by 28% and 25%, depending on procedural control and control treatment considered). And, no significant differences were found in pollen limitation among/ between patches in fruit set and seed production, since all patches were pollen limited according to  $PL_{CC}$  and  $PL_C$  index estimates (Table 2). There was no evidence that pollen limitation for fruit set was correlated with pollen limitation for seed production (Pearson's  $r=0.1114$ ,  $P=0.5117$ ,  $n=36$  plants; Fig. 2).

### 2.2 Flower visitor assemblage

At least 12 different species of insect taxa visiting the flowers of *H. scoparium* were recorded in the six focal patches. In general, this assemblage was taxonomically diverse, composed of insects belonging to over 7 families and 2 orders (Table 3). At least 8 species of bees including an unidentified bee of *Bombus* were observed visiting *H. scoparium*. Almost all the bee



**Fig. 1** The mean (a) fruit set and (b) seed production under different pollination treatments. Seed production is the mean number of seeds per fruit. Vertical bars denote standard errors. Pollination treatments: C, control; CC, procedural control; PA, pollen added (see text for details).

**Table 1** Effect of pollination treatments on *Hedysarum scoparium* reproductive output. PA, pollen added treatment; C, control treatment; CC, procedural control treatment; Seed production, mean number of seeds per fruit per patch

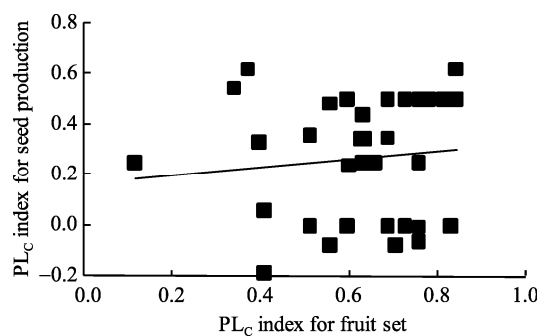
		Fruit set				Seed production		
		<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>	<i>MS</i>	<i>F</i>	<i>P</i>
PA vs. C	Treatment (T)	1	1.19	126.54	0.0001	6.06	28.12	0.0001
	Patch (P)	5	0.01	0.54	0.749	0.21	0.98	0.440
	T×P	5	0.01	1.00	0.421	0.33	1.53	0.195
PA vs. CC	Treatment (T)	1	1.21	103.16	0.0001	7.06	24.44	0.0001
	Patch (P)	5	<0.01	0.24	0.944	0.27	0.93	0.468
	T×P	5	0.01	1.25	0.297	0.04	0.14	0.983
C vs. CC	Treatment (T)	1	<0.01	0.01	0.914	0.04	0.14	0.709
	Patch (P)	5	0.01	1.24	0.300	0.31	1.14	0.348
	T×P	5	<0.01	0.24	0.945	0.24	0.88	0.502
PL <sub>C</sub> index				0.623±0.061		0.248±0.056		
PL <sub>CC</sub> index				0.598±0.036		0.276±0.023		

Note: PL index refers to pollen limitation index calculated comparing  $R_0$  of plants belonging to pollen added treatment with plants belonging to procedural control treatment (PL<sub>CC</sub>) and control plants (PL<sub>C</sub>)

**Table 2** Patch size, visitation frequency for each patch of *Hedysarum scoparium* ( $V_f$ ) and estimates of pollen limitation for fruit set and seed production. Patch size refers to the number of flowering plants in the patch.  $V_f$  is visits/(flower·h)

Patch	Patch size	PL <sub>C</sub> index			PL <sub>CC</sub> index	
		$V_f$	Fruit set	Seed production	Fruit set	Seed production
1	12	3.64±0.24 <sup>a</sup>	0.525±0.076	0.194±0.124	0.619±0.075	0.313±0.093
2	17	1.70±0.15 <sup>b</sup>	0.708±0.058	0.159±0.105	0.667±0.034	0.368±0.112
3	35	3.12±0.28 <sup>a</sup>	0.618±0.065	0.314±0.098	0.535±0.143	0.289±0.137
4	27	1.59±0.12 <sup>b</sup>	0.623±0.047	0.321±0.059	0.613±0.062	0.304±0.177
5	21	2.46±0.26 <sup>ab</sup>	0.664±0.027	0.359±0.084	0.716±0.142	0.257±0.056
6	19	2.82±0.27 <sup>a</sup>	0.600±0.107	0.240±0.144	0.529±0.129	0.291±0.100

Note: Different superscript letters indicate significant differences at  $\alpha < 0.05$ .

**Fig. 2** Relationship between two measures of pollen limitation index (PL<sub>C</sub> index), fruit set per plant and number of seeds per fruit per plant ( $n=36$  plants)

species exhibited similar behavior that would lead to pollination, e.g. taking nectar, contacting anthers, carrying pollen on their bodies (abdomen, head, thorax and/or legs), and contacting styles. Marked differences

in relative abundance occurred for flower visitors (Table 3). The introduced honeybees (*Apis mellifera*) were the most abundant visitor to *H. scoparium*, comprising 76.5%–90.7% of the total visits in each patch, 88.4% on average. A large number of honeybee individuals were observed foraging at the same time on different flowers of *H. scoparium*. Another abundant pollinator occurring in most sites was *Amegilla* spp. (based on behavior and pollen loads), contributing to about 5.4% of the total visits. *Amegilla* spp. visitors including at least three species (*Amegilla montivaga*, *A. nigricornis*, and *A. salviae*) were not identified in the field, since all these species were of similar looks and displayed similar foraging behavior. “*Amegilla* spp.” thus refers to these three species. The rarer bees (*Anthidium septemspinosum*, *Halictus zonulus*, *Lasi-*

**Table 3** Insects visiting *Hedysarum scoparium* flowers in the six studied patches.  $V_f$  is visits/(flower·h). P means the percentage of visits (relative to the total number of visits) attributable to a given taxa for each patch

Species	1		2		3		4		5		6	
	$V_f$	P	$V_f$	P	$V_f$	P	$V_f$	P	$V_f$	P	$V_f$	P
<b>Hymenoptera</b>												
<i>Apis mellifera</i>	3.27	89.8	1.30	76.5	2.83	90.7	1.43	89.9	2.17	88.2	2.55	90.4
<i>Amegilla</i> spp.	0.15	4.1	0.23	13.5	0.17	5.4	0.05	3.1	0.09	3.7	0.14	5
<i>Anthidium septemspinosum</i>	0.01	0.3	0.01	0.6								
<i>Halictus zonulus</i>	0.08	2.2	0.05	2.9	0.06	1.9	0.02	1.3				
<i>Lasioglossum scitulum</i>							0.04	2.5				
<i>Megachile spissula</i>	0.04	1.1	0.06	3.5					0.10	4.1	0.04	1.4
<i>Xylocopa nasalis</i>	0.01	0.3			0.01	0.3					0.05	1.8
<i>Bombus</i>	0.03	0.8			0.01	0.3	0.03	1.9	0.03	1.2		
<b>Diptera</b>												
<i>Syrphus vitripennis</i>	0.04	1.1	0.03	1.8	0.03	1	0.02	1.3	0.05	2	0.03	1.1
<b>Lepidoptera</b>												
<i>Autographa mandarina</i>			0.01	0.6					0.01	0.4		
<i>Coenonympha amaryllis</i>	0.01	0.3										
<i>Pieris rapae</i>			0.01	0.6	0.01	0.3			0.01	0.4	0.01	0.4

*oglossum zonulum*, *Megachile spissula*, *Xylocopa nasalis* and an unidentified species of *Bombus*), hoverfly *Syrphus vitripennis*), butterflies (*Coenonympha amaryllis*, *Pieris rapae*) and moth (*Autographa mandarina*), due to their much lower visiting frequencies, might play a minor role in pollination of *H. scoparium* flowers, if any at all.

$V_f$  differed significantly among patches (One-way ANOVA,  $F_{5,239}=8.29$ ,  $P<0.05$ ; Table 2). The highest value of  $V_f$  was  $3.64\pm0.5$  insects/(flower·h) and the lowest  $1.59\pm0.3$  insects/(flower·h). There was no evidence that bee visitation increased with patch size ( $R^2=0.021$ ,  $P=0.78$ ,  $n=6$  patches; Fig. 3). A total of 32 honeybee hives were found within or in the immediate vicinity (<1 km) of the studied patches.

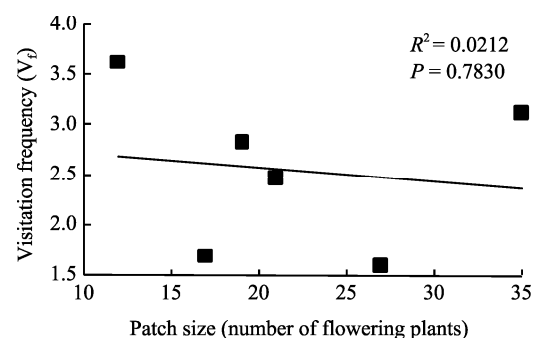
### 2.3 Effect of visitor visitation frequency on pollen limitation

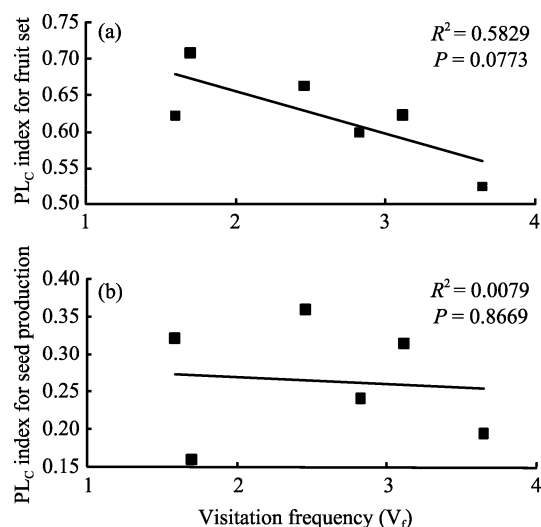
Examination of the relationship between visitor visitation frequency and  $PL_C$  index revealed a trend of decreasing  $PL_C$  index for fruit set with increasing visitor visitation frequency, which was not significant ( $R^2=0.5829$ ,  $P=0.0773$ ,  $n=6$  patches; Fig. 4a). Pollen limitation for seed production, however, was not related to visitor visitation frequency ( $R^2=0.0079$ ,  $P=0.8669$ ,  $n=6$  patches; Fig. 4b).

## 4 Discussion and conclusion

Our experiment demonstrates that *H. scoparium* is pollen-limited as suggested by the significant differences between the pollen supplementation treatment and any of the two control treatments across patches (Table 1).

To study pollen limitation, the traditional pollen supplementation experiment was performed. However, pollen supplementation experiments may indicate higher magnitude of pollen limitation when only a fraction of plant flowers receive the experimental

**Fig. 3** Visitation frequency in relation to patch size. Patch size is the number of flowering plants in the 6 patches.



**Fig. 4** Relationships between two measures of pollen limitation index ( $PL_C$  index for fruit set per patch and  $PL_C$  index for seed production per patch) and visitation frequency. Seed production is the mean number of seeds per fruit per patch.

treatment relative to whole-plant treatment, because many plant species can reallocate resources among flowers (Knight *et al.*, 2005). Unfortunately, we could not apply the whole individual plant to control or experimental treatments, since an individual plant can produce thousands of flowers. To avoid misleading results, we used two controls following the method from Wesselingh (2007) and Gómez (2010), one control on the manipulated plants and another on the unmanipulated ones. If resource allocation existed in *H. scoparium*, reproduction of the unmanipulated plants would be higher than that of the manipulated ones. Notably, our results showed that reproductive output did not differ between flowers from the manipulated plants and those from the unmanipulated ones, and the reproductive output of flowers from the manipulated plants was a bit higher than that from the unmanipulated ones. In fact, under resource allocation, controls on non-manipulated plants would be expected to have higher rather than lower reproduction than controls on manipulated plants. This suggested that pollen added to some flowers did not induce resources reallocation among flowers.

The magnitude of pollen limitation in *H. scoparium* varied between both reproductive components, being more intense in fruit set than in seed production. To determine how well the magnitude of pollen limitation for fruit set (the most commonly measured response

variable; Burd, 1994; Knight *et al.*, 2005; Knight *et al.*, 2006) was correlated with the magnitude of pollen limitation for seed production (the best response variable; Knight *et al.*, 2005), we correlated both indices for the 36 experimented plants (6 plants per patch), and no correlation was found. However, Knight *et al.* (2005) correlated the pollen limitation index for fruit set and seed production for 63 data records, and found a strong correlation between both reproductive components, concluding that pollen limitation for fruit set was a good indicator of pollen limitation for seed production. So our result proposes a caution on this conclusion. A similar caution has been proposed by Gómez *et al.* (2010), who found strong pollen limitation during seed production but a weak one during fruit set in a mega-generalist species, *Erysimum mediohispanicum*, and concluded that a good correlation between fruit set and seed production might occur in plant species bearing a small and fixed number of ovules per flower. *Hedysarum scoparium* flowers bear 3–5 ovules, and when fruits are produced, one to several ovules may remain unfertilized. In this case, fruit set alone can not predict pollen limitation accurately.

Pollen limitation in patched habitats may be related to the pollination environment (Wilcock and Neiland, 2002; Knight *et al.*, 2005). Some studies showed that pollinator visitation frequency was a primary factor contributing to pollen limitation in many plant species (Duan *et al.*, 2007; Cosacov *et al.*, 2008; González-Varo *et al.*, 2009). To support this idea, we found a negative relationship across patches between pollinator visitation frequency and *H. scoparium*  $PL_C$  index for fruit set. This relationship indicates that increased pollinator visitation frequency may decrease the intensity of pollen limitation for fruit set among the patches. Our results suggest that flower pollinator abundance benefits *H. scoparium* reproduction. Pollinator visitation frequency can decrease due to the less attractiveness to small populations than large ones (Steffan-Dewenter and Tschardt, 1999; Goverde *et al.*, 2002). Contrary to previous studies, pollinator visitation frequency did not increase with patch size. This could be resulted from the large foraging range of honeybees (*A. mellifera*) and the decision of the bee keepers to settle hives seasonally to take advantage of the available flower resource (Steffan-Dewenter and

Tscharntke, 2000). Exotic pollinators introduced are often considered to be threats to native pollinators abundance and diversity, and thought to disrupt specialized relationships between native pollinator and their plants (Roubik, 1980). And the presence of non-native pollinators is expected to increase the magnitude of pollen limitation in native plants (Knight *et al.*, 2005). However, introduced pollinators can also replace lost or declining native pollinators to some extent (Traveset and Richardson, 2006), and decrease the magnitude of pollen limitation in plants. In this study region, the native bee species (*Megachile bicolor* and *M. takoensis*) that were frequently observed in August were rarely observed in September. Pollinator visitation frequency decreased to  $0.45 \pm 0.11$  insect/(flower·h) before honeybees were introduced in September, while fruit set was  $(8.1 \pm 0.5)\%$  in September (Pan *et al.*, unpublished data). The introduced honeybees increased visitation frequency significantly. *Apis mellifera* is a generalist forager and visits flowers with different pollination syndromes (Roubik, 1980; Aizen and Feinsinger, 1994b). As shown in some studies, *A. mellifera* had lower pollination effectiveness than the native pollinator (Hansen *et al.*, 2002; Fumero- Cabán and Meléndez-Ackerman, 2007). *Apis mellifera* agglutinates the pollen grains with nectar and places them into corbiculae on the hind tibia (Simpson *et al.*, 1977) that would reduce the chance of pollen being scooped into the stigmas. However, in this region, introduced *A. mellifera* was as efficient as other native bee-pollinators at depositing pollen (*A. mellifera*,  $5.82 \pm 1.01$  grains per visit on average; *Ame-*

*gilla* spp.,  $5.5 \pm 2.32$ ) (Pan *et al.*, unpublished data). And, it visited flowers much more frequently than other pollinators. Once visitation frequencies were taken into account, *A. mellifera* was proved to be the most important pollinator at depositing pollen in that it 'rescued' fruit set as effective pollinators and reduced the magnitude of pollen limitation for fruit set in the desert species *H. scoparium*, irrespective of the future fate of the seeds developing inside. However, high pollinator visitation frequency did not reduce the intensity of pollen limitation for seed production. McIntosh (2002) and Kéry and Matthies (2004) reported that the reduction in the number of seeds per fruit or plant in small populations can be a consequence of lower habitat quality (water, light, climatic variation and soil conditions) or inbreeding depression, which may lead to low pollen-tube survival or high zygote death (Harder and Aizen, 2010). We assumed that these factors have contributed to the reduced reproductive output in this species, although we have no physical data to support this. As a whole, these findings suggest that pollen limitation in *H. scoparium* should be caused by more than one factor, not just pollinator visits. The results of this study provide an insight into *H. scoparium* conservation and/or possible man-aided reproduction technique.

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## References

- Aguir M R, Sala O E. 1999. Patch structure, dynamics and implications for the functioning of arid ecosystems. *Tree*, 14(7): 273–277.
- Aguilar R, Ashworth L, Galetto L, *et al.* 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, 9(8): 968–980.
- Aizen M A, Feinsinger P. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, 75(2): 330–351.
- Aizen M A, Feinsinger P. 1994b. Habitat fragmentation, native insect pollinators, and feral honey bees in argentine Chaco Serrano. *Ecological Applications*, 4(2): 378–392.
- Aizen M A, Ashworth L, Galetto L. 2002. Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *Journal of Vegetation Science*, 13(6): 885–892.
- Aizen M A, Feinsinger P. 2003. Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. In: Bradshaw G A, Marquet P A. *Disruptions and Variability: the Dynamics of Climate, Human Disturbance and Ecosystems in the Americas*. Berlin: Springer-Verlag, 111–129.
- Ashman T L, Knight T M, Steets J A, *et al.* 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85(9): 2408–2421.



- Burd M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review*, 60(1): 83–139.
- Cosacov A, Nattero J, Cocucci A A. 2008. Variation of pollinator assemblages and pollen limitation in a locally specialized system: the oil-producing *Nierembergia linariifolia* (Solanaceae). *Annals of Botany*, 102(5): 723–734.
- Cunningham S A. 2000. Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London B*, 267(1448): 1149–1152.
- Dogterom M H, Winston M L, Mukai A. 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*; Ericaceae). *American Journal of Botany*, 87(11): 1584–1591.
- Duan Y W, Zhang T F, Liu J Q. 2007. Interannual fluctuations in floral longevity, pollinator visitation and pollination limitation of an alpine plant (*Gentiana straminea* Maxim., Gentianaceae) at two altitudes in the Qinghai-Tibetan Plateau. *Plant Systematics and Evolution*, 267(1–4): 255–265.
- Duncan D H, Nicotra A B, Wood J T, *et al.* 2004. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. *Journal of Ecology*, 92: 977–985.
- Fumero-Cabán J J, Meléndez-Ackerman E J. 2007. Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *American Journal of Botany*, 94(3): 419–424.
- Ghazoul J. 2005. Pollen and seed dispersal among dispersed plants. *Biological Reviews*, 80(3): 413–443.
- Gómez J M, Abdelaziz M, Lorite J, *et al.* 2010. Changes in pollinator fauna cause spatial variation in pollen limitation. *Journal of Ecology*, 98(5): 1243–1252.
- González-Varo J P, Albaladejo R G, Aparicio A. 2009. Mating patterns and spatial distribution of conspecific neighbours in the Mediterranean shrub *Myrtus communis* (Myrtaceae). *Plant Ecology*, 203(2): 207–215.
- Goverde M, Schweizer K, Baur B, *et al.* 2002. Small-scale habitat fragmentation effects on pollinator behaviour: experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biology Conservation*, 104(3): 293–299.
- Groom M J. 1998. Allee effects limit population viability of an annual plant. *The American Naturalist*, 151(6): 487–496.
- Haig D, Westoby M. 1988. On limits to seed production. *The American Naturalist*, 131(5): 757–759.
- Hansen D M, Olesen J M, Jones C G. 2002. Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds. *Journal of Biogeography*, 29(5–6): 721–734.
- Hanski I. 1998. Metapopulations dynamics. *Nature*, 396: 41–49.
- Harder L D, Aizen M A. 2010. Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society of London: Series B*, 365(1539): 529–543.
- Harris L F, Johnson S D. 2004. The consequences of habitat fragmentation for plant-pollinator mutualisms. *International Journal of Tropical Insect Science*, 24(1): 29–43.
- Hobbs R J, Yates C J. 2003. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany*, 51(5): 471–488.
- Jennersten O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, 2(4): 359–366.
- Kéry M, Matthies D. 2004. Reduced fecundity in small populations of the rare plant *Gentianopsis ciliate* (Gentianaceae). *Plant Biology*, 6(6): 683–688.
- Knight T M, Steets J A, Vamosi J C, *et al.* 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology and Systematics*, 36: 467–497.
- Knight T M, Steets J A, Ashman T L. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany*, 93(2): 271–277.
- Kotliar N B, Wiens J A. 1990. Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos*, 59(2): 253–260.
- Larson B M H, Barrett S C H. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of Linnean Society*, 69(4): 503–520.
- Lindenmayer D, Fischer J. 2006. *Habitat Fragmentation and Landscape Change: An Ecological and Conservation Synthesis*. Washington D.C.: Island Press.
- Liu Z M, Zhao W Z. 2001. Shifting-sand control in central Tibet. *Ambio*, 30(6): 376–380.
- McIntosh M E. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology*, 162(2): 273–288.
- McIntyre N, Hostetler M E. 2001. Effects of urban land use on pollinator (Hymenoptera: Apodidea) communities in a desert metropolis. *Journal of Applied and Theoretical Biology*, 2(3): 209–218.
- Morgan M T, Wilson W G, Knight T M. 2005. Plant population dynamics, pollinator foraging, and the selection of self-fertilization. *The American Naturalist*, 166(2): 169–183.
- Pan C C, Liu L D, Hou Y L, *et al.* 2010. Flowering characteristics and breeding system of *Hedysarum Scoparium* in the middle reaches of Heihe river. *Journal of Desert Research*, 30: 1099–1103.
- Pérez-Barrales R, Arroyo J, Armbruster W S. 2007. Differences in pollinator faunas may generate geographic differences in floral morphology and integration in *Narcissus papyraceus* (Amaryllidaceae).

- ceae). *Oikos*, 116: 1904–1918.
- Qi S, Wang T, Feng J M. 2003. Classification of land degradation in the Heihe River Basin, Northwestern China. *Ecology and Environment*, 12(4): 427–430.
- Rathcke B J, Jules E S. 1993. Habitat fragmentation and plant–pollinator interactions. *Current Science*, 65: 273–277.
- Roubik D W. 1980. Foraging behavior of competing Africanized honeybees and stingless bees. *Ecology*, 61(4): 836–845.
- Schleuning M, Templin M, Huamán V, *et al.* 2011. Effects of inbreeding, outbreeding, and supplemental pollen on the reproduction of a hummingbird-pollinated clonal Amazonian herb. *Biotropica*, 43(2): 183–191.
- Simpson B B, Neff J L, Moldenke A R. 1977. *Prosopis* flowers as a resource. In: Simpson B B. *Mesquite: its Biology in Two Desert Scrub Ecosystems*. Pennsylvania: Dowden, Hutchinson and Ross, Stroudsburg, 84–107.
- Steffan-Dewenter I, Tschamtk T. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia*, 121(3): 432–440.
- Steffan-Dewenter I, Tschamtk T. 2000. Resource overlap and possible competition between honey bees and wild bees in central Europe. *Oecologia*, 122(2): 288–296.
- Steven J C, Rooney T P, Boyle O D, *et al.* 2003. Density-dependent pollinator visitation and selfincompatibility in upper Great Lakes populations of *Trillium grandiflorum*. *Journal of the Torrey Botanical Society*, 130(1): 23–29.
- Traveset A, Richardson D M. 2006. Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution*, 21(4): 208–216.
- Wagenius S, Lyon S P. 2010. Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology*, 91(3): 733–742.
- Wesselingh R A. 2007. Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist*, 174(1): 26–37.
- Wilcock C, Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Sciences*, 7(6): 270–277.



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