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The effects of pollen limitation on population dynamics of snow lotus (*Saussurea medusa* and *S. laniceps*, Asteraceae): Threatened Tibetan medicinal plants of the eastern Himalayas

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Abstract Pollen limitation reduces seed production and may reduce plant population growth rate. Plants may be particularly prone to pollen limitation if they require pollinators, occur at high elevation, and have human-mediated reductions in plant density due to harvesting. We found that two rare monocarpic Tibetan plant species, known as the Himalayan Snow Lotus, both require pollinators and that seed production in *Saussurea medusa* (Asteraceae) but not *S. laniceps* is limited by pollen receipt. We created deterministic and stochastic stage-structured matrix models for *S. medusa*, and found that pollen supplementation significantly increases population growth rate. However, even when pollen is not limiting, *S. medusa* is likely at risk for extinction in the next 50 years. Our results for this monocarpic plant differ from other population studies on pollen limitation in polycarpic plant species since the magnitude of pollen limitation for seed production was relatively low, and yet the sensitivity of population growth to changes in seed production was relatively high.

Keywords Breeding system · Elasticity analysis · Matrix population models · Pollen limitation · *Saussurea laniceps* · *Saussurea medusa* · Sustainable harvest · Tibetan medicine

Introduction

Understanding the factors that regulate population growth is critical for preservation of rare plant species (Keith 1998) and plants that are harvested for human use (Ticktin 2004). Biotic interactions with other plant and animal members of the community are often important factors influencing the plant population growth rate (Calvo and Horvitz 1990; Ehrlén 1995; Ehrlén and Eriksson 1995; Garcia and Ehrlén 2002; Maron and Crone 2006; Parker 1997). For example, the majority of plant species rely on animal pollinators for reproduction (Buchman and Nabhan 1996) and thus, the abundance and behavior of pollinators influence the seed production and can potentially affect the population dynamics of plants (Ashman et al. 2004; Knight et al. 2005).

Plants that occur at high elevation may have difficulty attracting pollinators. In high alpine habitats, lower temperatures, high winds, and short growing seasons create a stressful environment for most pollinators. Several studies have shown that flowers are

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more likely to be visited by pollinators at low elevations than at high elevations (Duan et al. 2007; Kearns and Inouye 1994; Utelli and Roy 2000). Low visitation by pollinators may result in lower pollen receipt and lower seed production for plants. Alternatively, many high elevation plant species are self-compatible and/or can autogamously self-pollinate (Berry and Calvo 1989), and are thus less dependent on pollinators.

Pollen supplementation experiments and matrix population models can be used in combination to quantify whether plant reproduction is limited by pollen receipt and whether pollen limitation significantly reduces population growth rate and viability. Pollen supplementation experiments compare flowers given supplemental pollen (i.e., those experimentally given a saturating amount of pollen from conspecific individuals) to control flowers (Burd 1994). If flowers have higher fruit or seed set when given supplemental pollen, then it is inferred that fecundity is limited by pollen (pollen limited). Results from the pollen supplementation experiment can be incorporated into a matrix population model; if population growth rate or viability is lower in the control compared to the supplement treatment, then pollen limitation is expected to decrease the abundance of the plant population. Pollen limitation might not decrease population growth rate if reproductive plants given supplemental pollen have lower survivorship or if they are more likely to retrogress to a non-reproductive stage class (Ehrlén and Eriksson 1995). Further, pollen limitation might have trivial effects on population growth rate if the growth rate is not sensitive to changes in fecundity (Ashman et al. 2004; Knight et al. 2005).

There have been over 1,000 pollen supplementation experiments conducted in natural plant populations (Knight et al. 2005), but only a handful of studies that have evaluated the population effects of pollen limitation (Bierzychudek 1982; Ehrlén and Eriksson 1995; Franco and Silvertown 2004; Garcia and Ehrlén 2002; Knight 2004; Parker 1997; Price et al. 2008; Ramula et al. 2007). Many of these studies have found relatively small effects of pollen limitation on plant population dynamics (reviewed by Ashman et al. 2004). However, this result may be partially because most of the plants that have been studied to date are polycarpic, and variations in annual seed production should have less effect on population dynamics of plants that have multiple opportunities to reproduce compared to those that are monocarpic (Franco and Silvertown 2004; Ramula et al. 2008; Silvertown et al. 1993).

In plant species that are harvested by humans, pollen limitation might reduce the number and proportion of individuals that can be sustainably harvested from the population. Harvesting reduces demographic vital rates, and such effects can be incorporated into a matrix population model to assess how different intensities of harvesting affect population growth rate and extinction risk (Raimondo and Donaldson 2003). Several studies have quantified the effects of harvesting on population dynamics (reviewed by Ticktin 2004); however, to date, none have considered the joint effects of pollen limitation and harvesting on plant populations.

In this study, we investigate the breeding system, level of pollen limitation, and population dynamics of two rare monocarpic plant species, *Saussurea laniceps* Hand.-Mazz. and *Saussurea medusa* Maxim, which occur at high elevations in the eastern Himalayas and are harvested for traditional Tibetan and Chinese medicines. Prior to this study, the breeding system and pollination ecology of these species were not known. Specifically, we examine (1) whether insect visitors are necessary for seed set, (2) the identity and visitation frequency of their floral visitors, (3) the magnitude of pollen limitation, (4) the consequences of pollen limitation on population growth rate and on the probability of extinction in 50 years in the absence of harvesting and (5) the consequences of pollen limitation on population growth rate and extinction risk in the presence of human harvesting of different intensities and as a function of the frequency of years that are suitable for germination and seedling establishment. We compare our results with other studies considering the consequences of pollen limitation on population growth.

Materials and methods

Study system

Saussurea laniceps and *S. medusa* (Asteraceae) (Fig. 1) are generally found at elevations above 4,000 m in the eastern Himalayas. Inhabiting distinctly different niches, populations of *Saussurea laniceps* favor rocky cliff habitats and occur in Sichuan, and Yunnan provinces and Tibet, while *S. medusa* is found

Fig. 1 Inflorescences of **a** *Saussurea laniceps*, in which each flowering head is surrounded by pubescent bracts and creates holes for bumblebees to enter, and **b** *S. medusa*, where flowering heads are exposed at the top of the plant and easily accessible

on the more abundant loose rock soils known as scree in Gansu, Qinghai, Sichuan, and Yunnan provinces and Tibet. These two sister species (Raab-Straube 2003) occur in populations separated by several hundreds of meters and in spatially distinct areas. Both species are slow growing (7–10+ years), non-clonal, monocarpic, and perennial herbs. Reproductive plants of *S. laniceps* produce a single enlarged inflorescence with 6–36 capitula (hereafter referred to as flowering heads) each consisting of 33–86 florets. Each flowering head is individually surrounded by pubescent bracts on the inflorescence, distinctly separating flowering heads from each other. Holes in the pubescence allow entrance by high alpine bumblebees. In contrast, the single large inflorescence of *S. medusa* consists of 2–50 flowering heads densely packed together on the top of an enlarged individual plant, with each flowering head having between 2 and 15 individual florets. These flowers are fully exposed and easily accessible to pollinators. Flowers of both species bloom for 3–4 weeks during the monsoon season (between late-July and late-August). However, pollen is only released when it is not raining and is most abundant when the plants receive direct sunlight (W. Law, pers. obs.), which is not common during the flowering period of these species due to monsoons.

Saussurea laniceps and *S. medusa*, known in this area as “Snow Lotus”, are harvested during flowering but before seed set. Both species are used in Tibetan and Chinese medicine to treat high blood pressure, headaches, and a category of problems known as “women’s diseases”, which can range from ailments associated with pregnancy to dysmenorrhea (Yang and Chuchengjiangcuo 1989). Since time of harvest is during the only flowering period of a plant, collected individuals never have the opportunity to reproduce. Collection pressure is increasing on medicinal plants in this area, and *S. laniceps* is a species that is being threatened (Law and Salick 2007). *Saussurea laniceps* is the Snow Lotus preferred by collectors because it is larger and people believe it to be more potent, thus bringing a higher price; it is also the rarer species. *Saussurea medusa* has a wider distribution and is often only collected when *S. laniceps* cannot be found. However, as *S. laniceps* populations decline, collectors and the markets are switching to *S. medusa*, bringing increased harvesting pressure.

Study sites

The Menri or Medicine Mountains (transliterated to *Meili* in Chinese) are situated on the border of northwest Yunnan and southeast Tibet with Mt. Khawa Karpo (28°26′20″N latitude, 98°41′05″E longitude) the highest peak and one of eight sacred Tibet mountains. The Menri are within one of the most biologically diverse temperate ecosystems on earth (Mittermeier et al. 1998) and, as its name indicates, is a traditional collecting area for Chinese and Tibetan medicinal plants. For each species, we identified two study populations. For *S. laniceps*, one population is found at an elevation of 4,600 m in northern Menri which we will refer to hereafter as “laniceps I.” The other population is found in the central Menri at 4,400 m, referred hereafter as "laniceps II." For *S. medusa*, the first population was found at 4,200 m, east of Menri (medusa I). The other population was at an elevation of 4,300 m in the mountains of northern Menri (medusa II).

Breeding system experiments

In order to determine whether flowers of *S. laniceps* and *S. medusa* were capable of setting seed in the absence of pollinators (i.e., are capable of autonomous self-fertilization), we experimentally excluded pollinators from flowers by covering the entire inflorescence with fine mesh. In 2004 within medusa II and laniceps II, respectively, we sampled 20 individuals of *S. medusa* and 23 flowering heads of *S. laniceps* on four individual plants (flowering heads were used as opposed to individuals for this species because of the low number of flowering individuals). For each species, we compared the seed production per flowering head (*S. laniceps*) or per individual (*S. medusa*) in the bagged treatment with those given outcross pollen (those in the pollen supplementation experiment, see below) using the non-parametric Kruskal–Wallis Test since data was not normally distributed. If individuals produce significantly more seeds per head in the outcross treatment compared to the bagged treatment, then this indicates that the species is not fully autogamous and that pollinator visitation facilitates seed production. All statistical analyses were conducted using SYSTAT (2000).

Pollen supplementation experiments

To quantify the magnitude of pollen limitation, multiple flowering heads on an inflorescence of *S. laniceps* were randomly assigned to control (no pollen supplementation) or pollen supplement treatments. In 2004, most experimental individuals were lost to harvesting in laniceps I, so experimentation was not attempted in this population in 2005. Thus, pollen supplementation experiments are only available for the laniceps II population for *S. laniceps* in both 2004 and 2005. Sample sizes were 29 flowering heads per treatment from six (control) and seven (supplemented) individuals in 2004 and 20 flowering heads from four individuals for each of the control and supplemented treatments in 2005. For *S. medusa* 20 flowering individuals in each population were randomly assigned to one of two treatments: supplement and control. Multiple flower heads for each individual were manipulated, and the average number of seeds per flower head for each individual was quantified. Pollen supplementation of *S. medusa* was also repeated in 2 years (2004 and 2005).

For supplementation, we first collected pollen from 10 individuals (within the population but not individuals being used in the experiment) using a fine tip paintbrush and tweezers. Then, for flowering heads in the supplement treatment, we applied the freshly collected pollen to receptive stigmas for each flowering head. Florets of both species mature from the center of the plant outwards, and treatments were applied when over 50% of the florets on a plant were in bloom. Due to the inaccessibility of the area, distance between sites, and amount of time required to get to populations, pollen was not reapplied a second time. After 1 month, mature seeds were collected and the numbers of seeds produced per flowering head were counted. For *S. laniceps*, we determined if pollination treatment (supplemented, control), year (2004, 2005), or their interactions influenced seed production per flowering head using an ANOVA (ANOVA was also performed to test for mean seed production per flowering head for each individual plant to test for independence though results did not differ). Similarly, we used an ANOVA for *S. medusa* to determine if pollination treatment, site (medusa I, medusa II), year, or their interactions, influenced the average number of seeds per flowering head for individual plants. All data were approximately normal with equal variance and all analyses were conducted using SYSTAT (2000).

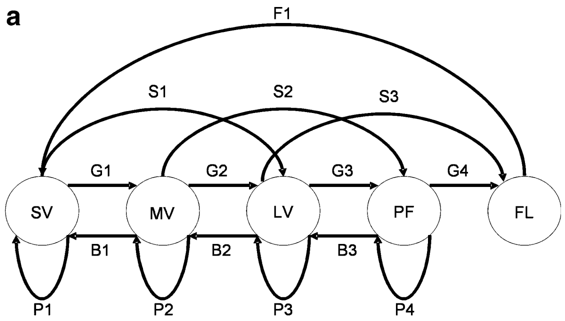
Pollinator observations

Saussurea laniceps flowers were monitored for visitors between 2 and 5 pm on the 24 and 25 of August 2004. Observations were conducted simultaneously by two observers, who switched locations on the second day to control for observer differences. Since flowering heads are surrounded by pubescent (densely hairy) bracts (Fig. 1a, we recorded time spent per visitor in each flowering head (recorded only if visitors were observed to enter pubescent bracts deep enough to contact reproductive parts) and total number of flowering head visited. A total of 4 plants were observed for 3 h each day. *Saussurea medusa* visitor activities were monitored between 3 and 5 pm on 4, 6, and 8 August 2004 and 15–16 August 2005 for medusa II. Flowering individuals in medusa I were monitored from 3 to 5 pm on 26–27 July and 18 August of 2004, and 8–9 August 2005. Each day a different individual plant was observed (total number of flowering plants $n = 10$). Since, all flowers are exposed on the top of the flowering plant for *S. medusa* (Fig. 1b), we recorded the number of visits and the time spent by each visitor as long as visitors were observed to contact reproductive parts. After observations, six pollinators were captured, killed, and pinned for identification by Dr. Paul Williams of the Natural History Museum, London.

Matrix population models

We found that seed production of *S. medusa* but not *S. laniceps* was significantly pollen limited (see results), and therefore here we create a matrix population model for *S. medusa* to determine the consequences of pollen limitation on population growth rate (λ) and extinction risk. Matrix models for *S. laniceps* will be published in Law, Salick, and Knight (in prep.). We collected demographic data for 5 years (2002–2006) in both the medusa I and medusa II. In demographic studies, individuals are typically marked with an adjacent tag or flag (Bierzychudek 1982), but due to repeated pilfering of visible markers, instead we used detailed site mapping employing geological features, compass bearings and distances to identify individuals in our demographic study. We combined the demographic data across both populations for our matrix model, since the vital rates did not differ significantly across populations (unpublished results), and since combining the data allowed for adequate sample size of rare stage classes. In 2002, we measured the number of leaves, length of largest leaf, and overall plant size (height and width) on each individual plant. These size measurements were highly correlated with each other, and therefore we only measured number of leaves in the following years. Individuals were classified into 5 stage classes: small vegetative (SV, 1–3 leaves), medium vegetative (MV, 4–7 leaves), large vegetative (LV, 8–20 leaves), pre-flowering (PF, more than 20 leaves), and flowering (FL). Vegetative individuals of *S. medusa* were mapped and followed in medusa I ($N = 333$) and in medusa II ($N = 264$) (see Appendix for stage-specific sample sizes). Censuses were carried out annually to measure survivorship and growth from 2002 to 2006 to obtain transition probabilities used in the matrix model.

Possible stage transitions from 1 year to the next are shown in Fig. 2. For plants in many stage classes, individuals can remain in the same class (parameters P1, P2, P3, P4), retrogress back to the previous stage class (B1, B2, B3), advance to the next largest stage class (G1, G2, G3, G4), or advance two stage classes (i.e., skip a size class due to very high growth from 1 year to the next, S1, S2, S3). This is a monocarpic species, and all flowering plants die; parameter F1 is the number of small vegetative plants produced per flowering individual in a single year. In the absence of harvesting, F1 is calculated as the average number of flowering heads per plant (r) multiplied by the average number of seeds per flowering head (s) multiplied by germination rate (g): $\mathrm{F1} = r \times s \times g$. Parameters r and s were measured in 2004 and 2005. In our matrix population models, we pool data across sites and years: $r = 25.31$ and $s = 1.657$ and 2.217 for individuals in the control and pollen supplement treatments, respectively. Seeds are not modeled as a



b

$$A = \begin{pmatrix} \mathrm{P1} & \mathrm{B1} & 0 & 0 & \mathrm{F1} \\ \mathrm{G1} & \mathrm{P2} & \mathrm{B2} & 0 & 0 \\ \mathrm{S1} & \mathrm{G2} & \mathrm{P3} & \mathrm{B3} & 0 \\ 0 & \mathrm{S2} & \mathrm{G3} & \mathrm{P4} & 0 \\ 0 & 0 & \mathrm{S3} & \mathrm{G4} & 0 \end{pmatrix}$$

Fig. 2 **a** Life cycle of *S. medusa*. Five stage classes are represented (*SV* small vegetative, *MV* medium vegetative, *LV* large vegetative, *PF* pre-flowering, *F* flowering individual). *Arrows* represent probabilities of transitioning from one stage class to the next and fecundity in a one year time step.**b** Stage-specific transition probabilities and fecundity are summarized in the matrix separate stage class because there is no dormancy; reproductive plants produce seeds, and seeds that germinate become small vegetative plants in a single year.

In order to determine the germination rate (g) of *S. medusa*, we collected seeds and recorded their germination rates at an outdoor field site in Zhongdian County (4,000 m, rocky habitat) and in the greenhouse of the Shangri-la Alpine Botanical Garden (3,000 m, shaded outdoor environment). These areas provided high elevation environmental conditions similar to our natural populations and allowed for us to more easily and more frequently monitor seed germination. Seeds were collected, wrapped in moist towels, placed in plastic bags, and kept in cool conditions until they could be placed in seeds trays and covered with 2 mm of soil. In 2003, all 800 seeds of *S. medusa* failed to germinate in both field and greenhouse conditions. Germination did not occur even when seeds were scarified with sand paper or when they were treated with gibberellic acid. We suspected that seeds were not viable, since they were flat instead of plump. To test for viability, 240 seeds of *S. laniceps* and 325 seeds of *S. medusa* were subjected to tetrazolium tests (Moore 1973). Tetrazolium tests indicated that on average, only 16% of seeds produced were viable, and also indicated that all plump, fleshy seeds are viable. All of these lines of evidence suggest that the germination rate of *S. medusa* seeds from 2003 to 2004 was ~0%. Further, since this species does not form a persistent seed bank (seeds decompose after a year), this result indicates reproductive failure for *S. medusa* in 2003–2004. We conducted a second germination experiment in 2004, this time within medusa II using six seed baskets each with 20 viable seeds in 2004. We again found no germination in 2005 (Law 2007). We conducted identical germination evaluation for *S. laniceps*, and found that in 2004 there was no germination, but in 2005, 100% of the seeds germinated. Because *S. laniceps* and *S. medusa* are sister species with similar seed characteristics, we assume that *S. medusa* can also have years of high germination. This is confirmed by our field observations of sporadic dense stands of germinating *S. medusa* (W. Law, personal observations) in 2003. Based on these seed germination experiments and observations, our best estimate for *S. medusa* is that germination is 0% in two out of every 3 years and 100% in one out of every 3 years. However, since we cannot accurately quantify the frequency of years that allow germination for *S. medusa* at this time, we examine a range of frequencies in our stochastic matrix population model (see below).

To parameterize a deterministic matrix population model, we calculated the average stage-specific vital rates across both populations and all years to create a single demographic matrix, **A**, for the control and supplement treatments (see Fig. 2 and Appendix for matrix structure and data for each year). Control and supplement matrices only differ in a single matrix element, F1. For this deterministic matrix model, we assume that germination rate, g, is 0.33 and that harvesting is absent ($h = 0$). The matrix population model is $\mathbf{n}_{t+1}=\mathbf{A}*\mathbf{n}_t$, where the vector $\mathbf{n}_t$ gives the number of individuals in each stage at time t (Caswell 2001). The asymptotic population growth rate, λ, is the dominant eigenvalue of **A**. Calculation of λ determines whether a population can persist ($\lambda \geq 1$) or not ($\lambda < 1$).

We used elasticity analysis of the control pollination matrix to determine the proportional sensitivity of λ to changes in the matrix element a_{ij}:

$$e_{ij} = \frac{a_{ij}}{\lambda}\frac{\partial\lambda}{\partial a_{ij}} = \frac{\partial(\log\lambda)}{\partial(\log a_{ij})}$$

(de Kroon et al. 1986; Caswell 2001).

In order to obtain 95% confidence intervals for λ and to test whether λ differed between control and supplement pollination treatments, we used bootstrap resampling and randomization tests (Caswell 2001; McPeek and Kalisz 1993). A bootstrap data set was created by sampling individuals with replacement from the original demographic data set. The original demographic data set for the supplement model contained 717 individuals, while the control model contained 737 individuals. The process of creating a bootstrap data set was repeated 1,000 times for each pollination treatment, to create 1,000 bootstrap data sets and corresponding values of λ by which we obtained 95% confidence intervals around the mean value. To test whether λ was significantly higher with pollen supplementation than for the control, we performed randomization tests ($N = 1{,}000$ runs). All matrix projections and randomization tests were conducted using MATLAB (2003).

In this system, environments are not constant, and thus we analyze population growth and extinction risk in variable environments separately for control and supplement pollination treatments. In addition to pollen limitation, we consider the roles of harvesting (proportion of flowering plants harvested ranging from 0 to 1) and frequency of years suitable for germination. We calculate stochastic growth rate using a computer simulation. Each of our four matrices for *S. medusa* (see Appendix) represents a possible state for the environment. We assume that each state has an equal probability of occurring in the future and that environmental conditions in 1 year are independent of conditions in the previous years (i.e., environments do not cycle in a predictable manner). We used $\mathbf{n}_{t+1}=\mathbf{A}^*\mathbf{n}_t$ to project population size from t to $t+1$. Our initial population vector was set to the stable stage distribution of **A** with an initial population size of 1. We project population size over many successive time intervals, using a matrix drawn at random each time interval. For each matrix, element F1 is the product of $r \times s \times g \times (1-h)$, where h is the proportion of flowering plants harvested. r, s and h are the same across all four matrices ($r = 25.31$ and $s = 1.657$ and 2.217 for individuals in the control and pollen supplement treatments, respectively, h is a value between 0 and 1). In each year, g is either 0 or 1, and each of these has a probability of being chosen in each year; we examine a range of probabilities for choosing 0 or 1 ranging from choosing 1 all of the time (i.e., every year is suitable for seed germination) to choosing 1 with probability 0.143 (i.e., ratio of suitable to unsuitable years for germination is 1:6). We simulated population growth increments 50,000 times to estimate of λ_S and 95% confidence intervals in λ_S. We modified MATLAB code provided in Morris and Doak (2002) for these analyses.

We quantified the probability that the population falls below the quasi-extinction threshold in 50 years using a simulation approach similar to that used to estimate of λ_S. Our initial population vector was set to field estimates of population size and structure for *S. medusa* in 2006: [100; 300; 200; 40; 20]. At each time step a matrix and germination probability was randomly chosen as above. We replicated this simulation 5,000 times and calculated what proportion of those 5,000 populations dropped below 25 individuals (quasi-extinction threshold). We modified MATLAB code provided in Morris and Doak (2002) for these analyses.

Comparison of studies on population effects of pollen limitation

The population growth rate of *S. medusa* might be more affected by pollen limitation than other species if its population dynamics are more sensitive to changes in fecundity (i.e., if it has a high elasticity value for the F1 matrix element that is expected for a monocarpic plant species). To test this hypothesis, we compared the effect of pollen limitation on seed production per flowering plant and the effect size of pollen limitation on deterministic population growth rate for the monocarpic plant, *S. medusa* with similar data from the other published studies that have been conducted on polycarpic plants (*Arisaema triphyllum* (Bierzychudek 1982); *Cytisus scoparius* (Parker 1997); *Lathyrus vernus* (Ehrlén and Eriksson 1995); *Primula veris* (Garcia and Ehrlén 2002); *Trillium grandiflorum* (Knight 2004)). For each study, we calculated an effect size (Gurevitch et al. 1992) for difference in seed production per flowering plant in supplement and control treatments using the log response ratio ($\ln(\text{Seeds}_{\text{supplement}}) - \ln(\text{Seeds}_{\text{control}})$). Similarly, we calculated an effect size for λ in supplement treatments minus controls ($\ln(\lambda_{\text{supplement}}) - \ln(\lambda_{\text{control}})$). Finally, we showed the elasticity of λ to changes in fecundity for each plant species.

Results

Saussurea laniceps

Breeding system & pollen supplementation experiments

Bagged flowering heads of *S. laniceps* produced no viable seeds, whereas flowering heads given outcross pollen (supplement treatment) produced 13.5 ± 1.7 seeds/flower head (mean ± standard error shown for all results; $\chi^2 = 8.415$, df $= 1$, $P = 0.004$). However, seed production was not significantly different when compared by pollination treatment (supplement vs. control), year, or their interaction (Table 1, Fig. 3b).

Pollinator observations

Saussurea laniceps was observed to be visited by the two generalist bumblebee species *Bombus*

Table 1 ANOVA results to test for differences in the number of *Saussurea laniceps* seeds produced per flowering head across two pollination treatments (supplement, control) and over 2 years (2004, 2005)

Source	Sum-of-squares	df	Mean square	*F* ratio	*P*
Treatment	10.897	1	10.897	0.316	0.581
Year	10.887	1	10.887	0.316	0.582
Treatment * year	23.592	1	23.592	0.684	0.420
Error	586.502	17	34.500		

rufofasticatus Smith and *B. festivus* Smith. When populations of *S. laniceps* are in bloom, these bumblebees are commonly observed visiting this species and are rarely observed visiting other flowering species (i.e., spatio-temporal specialists). These bumblebees are most active on sunny days from the early afternoon through 5 pm. On average, bumblebees visit flowers 5.67 ± 1.03 times per hour. Once a bumblebee found a flowering individual, on average it visited 4.16 ± 0.39 flowers per flowering head and spent an average of 9.41 ± 0.87 s per flowering head.

Saussurea medusa

Breeding system & pollen supplementation experiments

Bagged individuals produced significantly fewer seeds (0.3 ± 0.2 seeds/flowering head) than supplemented individuals (2.2 ± 1.0 seeds/flowering head, $\chi^2 = 39.453$, df = 1, $P < 0.001$). Pollen supplemented individuals produced significantly more seeds than control individuals, indicating pollen limitation (Fig. 3b; Table 2). Seed production was also significantly different between years; plants produced more seeds in 2004 than in 2005, especially in medusa II (year by site interaction, Table 2).

Pollinator observations

Only *Bombus rufofasticatus* Smith was observed to visit *S. medusa*. Similar to *S. laniceps*, these bumblebees were commonly observed visiting *S. medusa* as spatio-temporal specialists. The amount of time pollinators spent visiting a flowering individual was

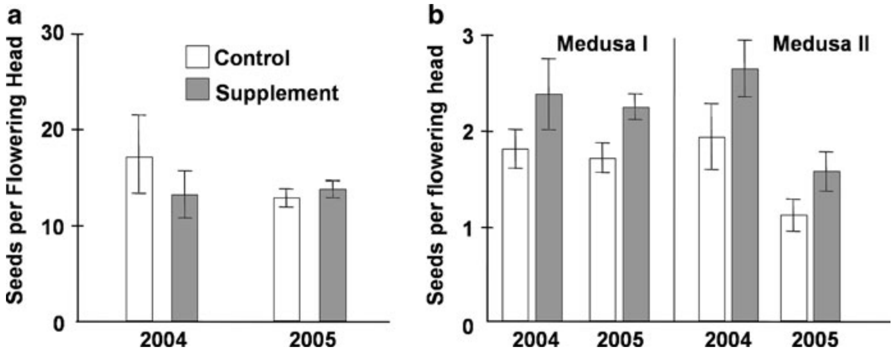


Fig. 3 Pollen supplementation experiment results (mean and one standard error) for **a** *Saussurea laniceps*. Pollen supplementation did not significantly increase seed production in either year of study. **b** *S. medusa* produces significantly more seeds per flowering head in supplemented compared to the control treatment

Table 2 ANOVA results to test for differences in the number of *Saussurea medusa* average seeds produced per flower head for individual plants across two pollination treatments (supplement, control), two populations (medusa I and medusa II), and 2 years (2004, 2005)

Source	Sum-of-squares	df	Mean square	*F* ratio	*P*
Treatment	10.59	1	10.59	11.884	0.001
Year	8.898	1	8.898	9.985	0.002
Population	1.307	1	1.307	1.467	0.228
Treatment * year	0.102	1	0.102	0.114	0.736
Treatment * population	0.04	1	0.04	0.045	0.832
Year * population	6.564	1	6.564	7.366	0.008
Treatment * year*population	0.246	1	0.246	0.276	0.600
Error	114.954	129	0.891		

31.5 ± 8.03 s per flowering head, and pollinators visited an individual 9.75 ± 1.95 times per hour.

Population dynamics

Experiments of *S. medusa* populations revealed that pollen supplemented individuals produced more seeds than control individuals (respectively, 2.2 ± 1.0 and 1.7 ± 1.0 seeds/flowering head, $F = 8.669$, df = 1, $P = 0.004$). The deterministic population growth rate, λ, was higher in the pollen supplemented (1.16 ± 0.07) compared to the control groups (1.10 ± 0.07, $P = 0.062$). Elasticity analysis revealed that matrix element F1 (transition from flowering plant to small vegetative plant) had the largest elasticity value (0.16, Table 3). Therefore, changes in this element are expected to result in large changes in λ.

Similarly, the stochastic population growth rate (λ_S) of *S. medusa* was significantly higher for pollen supplemented plants relative to controls ($\lambda_S = 1.09 \pm 0.01$ for supplemented plants and $\lambda_S = 1.05 \pm 0.01$ for control plants; $P < 0.05$ Fig. 4a).

As harvesting intensity increases, λ_S decreases (Fig. 4a). As the level of harvesting increases, the difference in λ between pollen supplementation and control treatments lessens (Fig. 4a). At high harvesting levels, most of the plants in the population experience complete loss of reproductive success and pollen limitation becomes unimportant. In the absence of pollen limitation, more than 50% of the adult plants can be harvested before the population

Table 3 Elasticity values of matrix elements (see Fig. 2 for meaning of matrix element codes) for *Saussurea medusa* plants in the control pollination treatment

Matrix element	Elasticity
P1	0.05
P2	0.10
P3	0.11
P4	0.02
G1	0.12
G2	0.10
G3	0.06
G4	0.07
S1	0.05
S2	0.02
S3	0.08
B1	0.01
B2	0.02
B3	0.01
F1	0.16

F1, the number of small vegetative plants produced per reproductive individual, has the highest elasticity values of all matrix elements, and thus changes to this element will have a large effect on λ

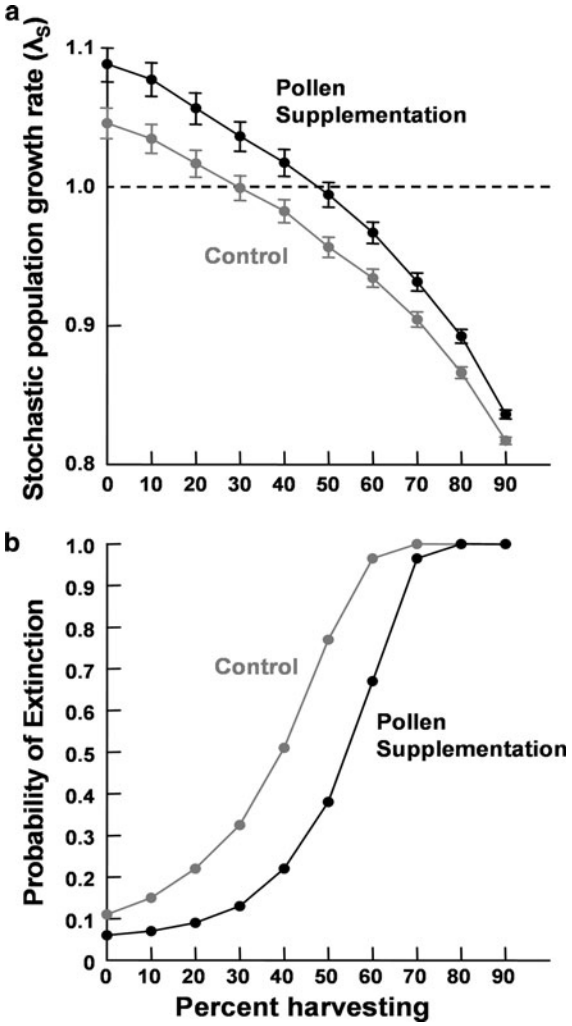


Fig. 4 **a** Stochastic population growth rates (and 95% confidence intervals) and **b** extinction probability (probability of extinction in 50 years) of *S. medusa* under pollen supplementation and control treatments at different intensities of human harvesting, ranging from 0 to 100% of the reproductive plants in the population. The *dotted line* shows $\lambda = 1$; below this threshold the population is projected to decline toward extinction, whereas above this threshold the population is projected to grow

growth rate is significantly below $\lambda_S = 1$, whereas in the presence of pollen limitation, when harvesting levels exceed 30% the threshold of $\lambda_S = 1$ is reached.

Saussurea medusa is still at risk of extinction even at harvesting levels that appear sustainable based on the stochastic population growth rate ($\lambda_S > 1$ in Fig. 4a). Populations are considered viable if they are projected to have <5% probability of extinction in the next 50 years. *Saussurea medusa* has a 6% probability of extinction without harvesting and with pollen supplementation (Fig. 4b). Thus, no amount of harvesting will be viable for this Snow Lotus species. Extinction probability reaches >90% once more than 60% of plants in the population are harvested.

We simultaneously examine the effects of pollen limitation, harvesting, and ratio of years suitable and unsuitable for germination. These factors all influence stochastic population growth rate and extinction probability (Fig. 5), however, under the ranges of these factors examined here, harvesting and ratio of years suitable and unsuitable for germination have the greatest effects on the population.

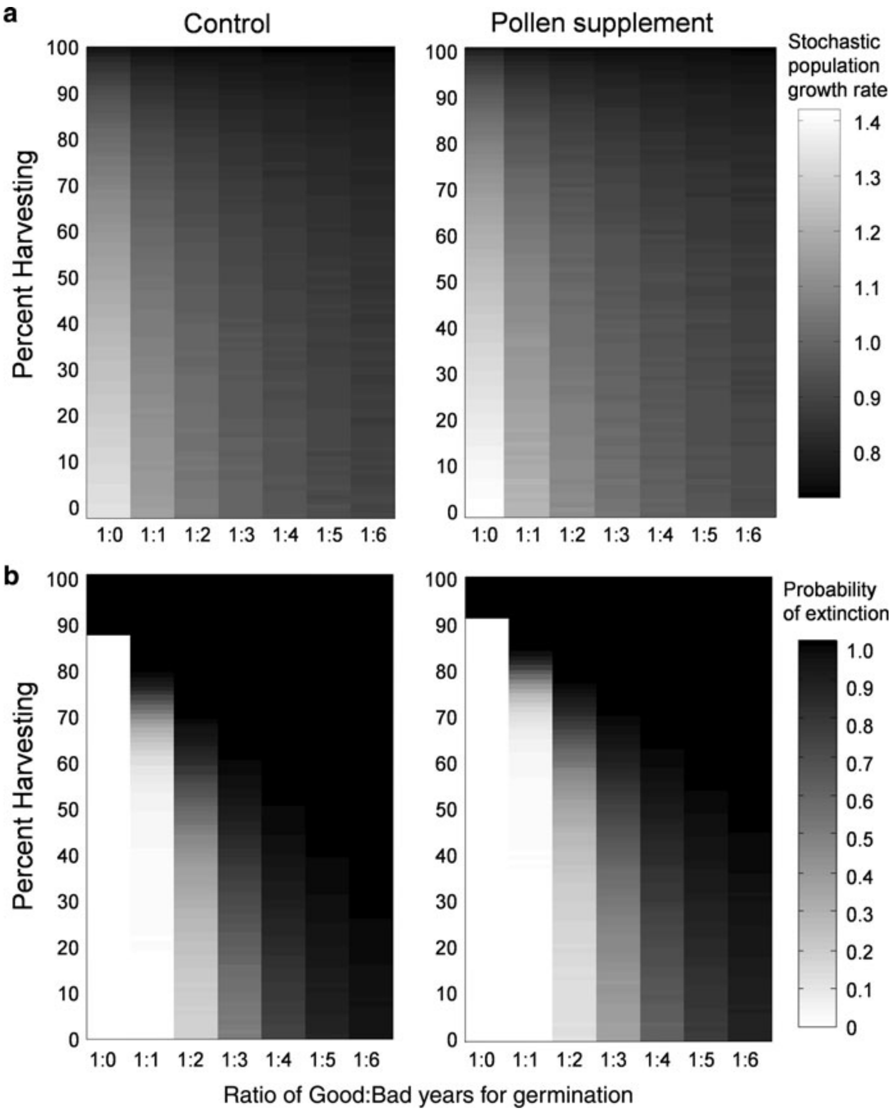


Fig. 5 The *scale* shows a range of **a** stochastic population growth rate and **b** probability of quasi-extinction in 50 years as a function of harvesting intensity and ratio of years that are suitable and unsuitable for germination. *Left panels* show control pollination treatments and right panels show supplement pollination treatments

Comparative pollen limitation

Seed production of *S. medusa* is limited by pollen, however, the effect size (i.e., magnitude of pollen limitation) is one of the lowest observed amongst the species in which population studies have been conducted (Fig. 6a). However, *S. medusa* has one of the highest values for elasticity of fecundity (i.e., λ is very sensitive to changes in seed production and germination, Fig. 6b). Thus, when we look at the effect size for pollen limitation on population growth rate, it is similar for *S. medusa* and *Arisaema triphllum* (0.07) and only *Cytisus scoparius* has a higher value (0.27, Fig. 6c).

Discussion

Monocarpic plants have only one opportunity to reproduce and population dynamics tend to be sensitive to perturbations in their seed production (Silvertown et al. 1993; Franco and Silvertown

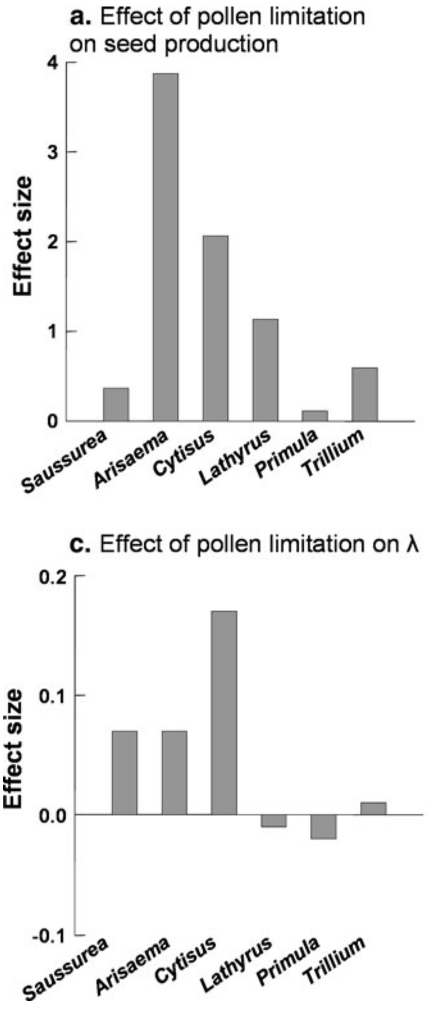


Fig. 6 Comparisons of the effect size of pollen limitation on seed production and population growth rate (λ) and the elasticity of λ to changes in fecundity between *S. medusa* compared to polycarpic species. *Saussurea medusa* has **a** one of the lowest magnitudes of pollen limitation, effect size = (ln ($\text{seeds}_{\text{supplement}}$) − ln ($\text{seeds}_{\text{control}}$)), **b** one of the highest values for elasticity of λ to changes in fecundity, and **c** relatively high effect of pollen limitation on population growth rate, effect size = (ln ($\lambda_{\text{supplement}}$) − ln($\lambda_{\text{control}}$))

2004). *Saussurea laniceps* and *S. medusa* are monocarpic plants that occur at high elevations and low densities. We found that these species are not capable of setting seed in the absence of pollinator visitation and are only pollinated by two species of bumblebees. However, the pollinators we observed appeared to specialize on these plant species when they are in bloom and were frequently observed visiting. Despite this, seed production of *S. medusa* was significantly limited by pollen receipt, and this limitation was observed in both populations and in both years of study. This suggests that *S. medusa* may frequently receive inadequate quantity or quality of pollen from its pollinators. The population growth rate (λ) of *S. medusa* is sensitive to perturbations in the fecundity of plants in the population (this vital rate had the highest elasticity value) and reductions in fecundity due to pollen limitation led to a reduction in λ.

Saussurea laniceps is the medicinal Snow Lotus of preference. It currently faces heavy harvest pressure, and it is found in decreasing abundance (matrix models of harvest pressure in Law 2007 and Law, Salick, and Knight in prep.). As the abundance of *S. laniceps* decreases, market demand shifts to the more common *S. medusa* (W. Law, pers. obs.). In contrast, according to our deterministic and stochastic matrix population models, *S. medusa* populations are expected to grow in the absence of harvesting even when fecundity is pollen limited. However, due to high environmental stochasticity in these populations, *S. medusa* has >5% probability of extinction in the next 50 years in the absence of harvesting and with ambient levels of pollen limitation. This high probability of extinction likely results from the small initial number of individuals in these populations and the high temporal variation in demographic vital rates (e.g., germination). Thus, it is possible that not even small intensities of harvesting are sustainable for this species. Population growth and extinction probabilities estimated for *S. medusa* are influenced by the ratio of years that are suitable versus unsuitable for germination. Future field germination studies are needed to determine a more accurate representation of this ratio for this species, to determine the mechanisms that result in low seed viability and sporadic recruitment, and to examine whether or not germination conditions might become more favorable or less favorable in the face of global climate change.

Our matrix population model may also be optimistic in that we do not explicitly incorporate Allee effects. As harvesters decrease the size and density of the population, plants may have a more difficult time attracting pollinators causing the level of pollen limitation to increase with decreasing plant densities (Hackney and McGraw 2001); but see (Berry and Gorchov 2006). Such Allee effects would make extinction even more likely in *S. medusa*. However, in a preliminary analysis with a small sample size, we did not find differences in the level of pollen limitation between isolated and clumped adult plants of *S. medusa* (Law 2007). It is possible that the *Bombus* pollinators specialize on these *Saussurea* species, and that Allee effects are small or absent.

Other population studies of pollen limitation have found that pollen limitation does not significantly decrease population growth rate, even though it does significantly decrease average seed production (reviewed in Ashman et al. 2004). The lack of population effects of pollen limitation is due in some cases to the low sensitivity of the population growth rate to changes in fecundity. This is the case with *Trillium grandiflorum*: pollen limitation results in a 30% decrease in seed production, but a negligible decrease in population growth rate. In *T. grandiflorum*, fecundity had the lowest elasticity value amongst all the demographic vital rates (Knight 2004). For monocarpic species, such as *S. medusa*, elasticity values for fecundity are high (Silvertown et al. 1993), and pollen limitation is expected to have consequences on the population. Indeed, in our study we find that even though pollen limitation resulted in only moderate decreases in seed output, these decreases resulted in a noticeable decrease in population growth. However, we note that all of the matrix population models that have been constructed to examine the population effects of pollen limitation (including ours) do not include the potential for density dependence to counteract benefits of increased seed production. Price et al. (2008) found that density dependence did counteract the positive effects of pollination in a lifetime fitness study on the monocarpic plant, *Ipomopsis aggregata*, and more studies are necessary to see if this is a general phenomenon.

In other polycarpic plant species, the lack of effect or even negative effect of pollen supplementation within the population is due to trade-offs between reproductive success and the survivorship and growth of reproductive plants in future years. Ehrlén and Eriksson (1995) found that pollen supplementation increased the seed production of *Lathyrus vernus* 3.1-fold, but that supplemented plants were more likely to regress in size and made fewer flowers in the next year relative to control plants. As a result, the population effect of pollen supplementation was negligible. Such tradeoffs between seed output and future size of reproductive individuals do no exist in monocarpic plants because reproductive individuals, by definition, die after reproduction.

The great combined effect of pollen limitation on λ that we observed in *S. medusa* was second only to *Cytisus scoparius*. *Cytisus scoparius* is an invasive plant species that has a high population growth rate (and its λ is therefore quite sensitive to changes in fertility), and is highly pollen limited, likely because its *Bombus* pollinators prefer native species (Parker 1997). Amongst the native plant species that have been studied, *S. medusa* pollen limitation has the greatest effect on λ.

In conclusion, the population dynamics of monocarpic plant species that are not capable of autogamous self pollination may be very sensitive to perturbations in the populations or behaviors of their pollinators. Our study demonstrates the consequences of pollen limitation on population effects for the growth and persistence of monocarpic plant species. The fact that Snow Lotus is a high elevation, heavily harvested, popular Tibetan medicinal plant exacerbates its population debility (Law 2007; Law and Salick 2005).

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Appendix

See Tables 4, 5, and 6.

Table 4 The demographic matrix for *Saussurea medusa* in each study year

	SV	MV	LV	PF	F
2002–2003					
SV	0.2	0.076923	0	0	r x s x g x $(1 - h)$
MV	0.4	0.461538	0.259259	0	0
LV	0.1	0.25	0.425926	0.444444	0
PF	0	0.038462	0.092593	0.222222	0
F	0	0	0.055556	0.222222	0
2003–2004					
SV	0.263158	0.044444	0	0	r x s x g x $(1 - h)$
MV	0.368421	0.422222	0.092308	0	0
LV	0.052632	0.322222	0.461538	0.25	0
PF	0	0	0.153846	0.166667	0
F	0	0	0.107692	0.5	0
2004–2005					
SV	0.285714	0.078341	0.008197	0	r x s x g x $(1 - h)$
MV	0.285714	0.40553	0.114754	0	0
LV	0.10989	0.248848	0.442623	0.285714	0
PF	0	0.041475	0.131148	0.142857	0
F	0	0.032258	0.114754	0.464286	0

Table 4 continued

	SV	MV	LV	PF	F
2005–2006					
SV	0.285714	0.027211	0	0	$r \times s \times g \times (1 - h)$
MV	0.357143	0.571429	0.141791	0	0
LV	0.053571	0.176871	0.492537	0.033333	0
PF	0	0.020408	0.119403	0.333333	0
F	0	0	0.044776	0.233333	0

Stages are represented by *SV* small vegetative, *MV* medium vegetative, *LV* large vegetative, *PF* pre-flowering, and *F* flowering individual. For fecundity *r* represents the average number of flowering heads per plant, *s* the average number of seeds per flowering head, *g* germination rate, and *h* the proportion of flowering plants harvested

Table 5 Number of vegetative individuals of *S. medusa* that were mapped and followed in each of the populations

Saussurea medusa									
Medusa I					Medusa II				
	Year 1–2	Year 2–3	Year 3–4	Year 4–5		Year 1–2	Year 2–3	Year 3–4	Year 4–5
SV	9	19	66	41	SV	1	2	28	15
MV	40	63	91	67	MV	12	25	128	84
LV	41	55	74	76	LV	15	10	49	59
PF	7	4	12	12	PF	2	8	15	17

Table 6 Number of flowering individuals of *S. medusa* that were relocated and had seed data collected from in each population

	Medusa I		Medusa II	
	2004	2005	2004	2005
Control	18	20	16	20
Supplement	14	18	14	17

References

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421

Berry PE, Calvo RN (1989) Wind pollination, self-incompatibility, and altitudinal shifts in pollination systems in the high Andean genus *Espeletia* (Asteraceae). Am J Bot 76:1602–1614

Berry EJ, Gorchov DL (2006) Female fecundity is dependent on substrate rather than male abundance, in the wind-pollinated dioecious understory palm *Chamaedorea radicalis*. Biotropica 39:186–194

Bierzychudek P (1982) The demography of Jack-in-the-Pulpit, a forest perennial that changes sex. Ecol Monogr 52:335–351

Buchman SL, Nabhan GP (1996) The forgotten pollinators. Island Press, Washington DC, USA

Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Bot Rev 60:83–139

Calvo RN, Horvitz CC (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. Am Nat 136:499–516

Caswell H (2001) Matrix population models. Sinauer Associates, Inc., Sunderland, MA

de Kroon H, Plaisier A, Groenendael JMv, Caswell H (1986) Elasticities: the relative contribution of demographic parameters to population growth rate. Ecology 67:1427–1431

Duan YW, Zhang TF, Liu JQ (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 Syst Evol 267:225–226

Ehrlén J (1995) Demography of the perennial herb *Lathyrus vernus* I. Herbivory and individual performance. J Ecol 83:287–295

Ehrlén J, Eriksson O (1995) Pollen limitation and population growth in a herbaceous perennial legume. Ecology 76: 652–656

Franco M, Silvertown J (2004) Comparative demography of plants based on elasticities of vital rates. Ecology 84:531–538

Garcia MB, Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. Am J Bot 89:1295–1302

Gurevitch J, Morrow LL, Wallace A, Walsh JS (1992) A meta-analysis of competition in field experiments. Am Nat 140:539–572

Hackney EE, McGraw JB (2001) Experimental demonstration of an Allee effect in American ginseng. Conserv Biol 15: 129–136

Kearns CA, Inouye DW (1994) Fly pollination of *Linum lewisii* (Linaceae). Am J Bot 81:1091–1095

Keith DA (1998) An evaluation and modification of world conservation union red list criteria for classification of extinction risk in vascular plants. Conserv Biol 12:1076–1090

Knight TM (2004) The effects of herbivory and pollen limitation on a declining population of *Trillium grandiflorum*. Ecol Appl 14:915–928

Knight TM, McCoy MW, Chase JM, McCoy KA, Holt RD (2005) Trophic cascades across ecosystems. Nature 437: 880–883

Law W (2007) Ecological and evolutionary impacts of harvesting the Himalayan snow Lotus (*Saussurea laniceps* Maxim. and S. *medusa* Hand.-Mazz.). Evolution, ecology, and population biology. Washington University, St. Louis, p 154

Law W, Salick J (2005) Human-induced dwarfing of Himalayan snow lotus, *Saussurea laniceps* (Asteraceae). Proc Natl Acad Sci USA 102:10218–10220

Law W, Salick J (2007) Comparing conservation priorities for useful plants: botanists and Tibetan doctors. Biodivers Conserv 16(6):747–1759

Maron JL, Crone E (2006) Herbivory: effects on plant abundance, distribution and population growth. Proc R Soc B 273: 2575–2584

MATLAB (2003) MATLAB. Mathworks, Natick, MA, USA

McPeek MA, Kalisz S (1993) Population sampling and bootstrapping in complex designs: demographic analysis. In: Schiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York, London

Mittermeier RA, Myers N, Thomsen JB, Fonseca GABD, Olivieri S (1998) Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. Conserv Biol 12:516–520

Moore RP (1973) Tetrazolium staining for assessing seed quality. In: Heydecker W (ed) Seed ecology. Butterworths, London, UK, pp 347–366

Morris WF, Doak DF (2002) Quantitative conservation biology: theory and practice of population viability analysis. Sinauer Associate, Inc., Sunderland

Parker IM (1997) Pollinator limitation of *Cystisus scoparius* (Scotch broom), an invasive exotic shrub. Ecology 78: 1457–1470

Price MV, Campell DR, Waser NM, Brody AK (2008) Bridging the generation gap in plants: pollination, parental fecundity, and offspring demography. Ecology 89:1596–1604

Raab-Straube EV (2003) Phylogenetic relationships in *Saussurea (Compositae, Cardueae*) sensu lato, inferred from morphological, ITS and *trn*L-*trn*F sequence data, with a synopsis of *Himalaiella* gen. nov, Lipschitziella and Frolovia. Willdenowia 33:379–402

Raimondo DC, Donaldson JS (2003) Responses of cycads of different life histories to the impact of plant collecting: Simulation models to determine important life history stages and population recovery times. Biol Conserv 111:345–358

Ramula S, Toivonen E, Mutikainen P (2007) Demographic consequences of pollen limitation and inbreeding depression in a gynodioecious herb. Int J Plant Sci 168: 443–453

Ramula S, Knight TM, Burns JH, Buckley YM (2008) General guidelines for invasive plant management based on comparative demography of invasive and native plant populations. J Appl Ecol 45:1124–1133

Silvertown J, Franco M, Pisany I, Mendoza A (1993) Comparative plant demography: relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. J Ecol 81:465–476

SYSTAT (2000) SYSTAT version 10. SPSS Institute, Chicago, IL

Ticktin T (2004) The ecological implications of harvesting non-timber forest products. J Appl Ecol 41:11–21

Utelli AB, Roy BA (2000) Pollinator abundance and behavior on *Aconitum lycoctonum* (Ranunculaceae): an analysis of the quantity and quality components of pollination. Oikos 89:461–470

Yang JS, Chuchengjiangcuo (1989) Diqing Zang Yao. Yunnan Min Zu Chu Ban She, Kunming Shi