

## Pollinator shift and reproductive performance of the Qinghai–Tibetan Plateau endemic and endangered *Swertia przewalskii* (Gentianaceae)

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**Abstract** Reproductive failure results in many plant species becoming endangered. However, little is known of how and to what extent pollinator shifts affect reproductive performance of endangered species as a result of the artificial introduction of alien insects. In this study we examined breeding systems, visitor species, visiting frequency and seed set coefficients of *Swertia przewalskii* in two years that had different dominant pollinator species (native vs. alien). Flowers of this species were protandrous and herkogamous and insects were needed for the production of seeds. The stigmatic receptivity of this species was shorter than for other gentians. No significant difference in seed set coefficient was found for hand-pollinated plants between the two years, indicating that pollinator shift only had a minor effect on this plant's breeding system. The commonest pollinators in 2002 were native bumblebees, alien honeybees and occasional solitary bees, however, only alien honeybees were observed in 2004. The flower visitation rate in both years was relatively high, although the total visit frequency decreased significantly in 2004. The control flowers without any treatment produced significantly fewer seed sets in 2004 than in 2002. In the past decade the seed production of this species may have partly decreased due to pollination by alien honeybees, however, we suggest that they might have acted as alternative pollinators ensuring seed production of *S. przewalskii* when native pollinators were unavailable. The main reason that this plant is endangered is probably the result of habitat destruction, but changes in land use, namely intensified

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agricultural practice and unfavorable animal husbandry have also contributed to its decline. We recommend that in-situ conservation, including the establishment of a protected area, is the best way to preserve this species effectively.

**Keywords** *Swertia przewalskii* · Endangered species · Alien pollinators · Pollination · Reproduction · Qinghai–Tibetan Plateau

## Introduction

There are diverse reasons why a plant species might become endangered. The most important one is probably poor reproduction during the species' life cycle, which can reduce the recovery time of existing populations. Therefore, understanding the reproductive processes of endangered species will contribute greatly to its successful conservation (e.g. Wiens et al. 1989; Navarro and Guitián 2002). Many factors, including decreased pollen quality (Byers 1995), poor stigma receptivity (He et al. 2000) and the absence of pollinators (e.g. Kwak and Jennersten, 1991; Burd 1994; Evans et al. 2004) can result in decreased sexual reproduction and seed production. Such decreases can result in plant species becoming endangered or extinct if the species is already threatened. This is of particular concern where populations are scattered, small and isolated (see Wagner and Mitterhofer 1998). For rare and outcrossing annuals and perennials, fluctuations in the number of pollinators (species and frequencies) between years create variability in the number of seedlings that establish within a population (Bierzychudek 1981). In addition, the worldwide introduction of honeybees may disturb native plant–pollinator interactions, particularly for those outcrossing species that have adapted to specialized native insect pollinators. Such introduction of alien pollinators can reduce the reproductive success of plant species and may be one of the important factors leading to their endangerment (Paton 1997; Gross and Mackay 1998; Kato et al. 1999; Kato and Kawakita 2004). However, Dick (2002) demonstrated that introduced African honeybees have become important pollinators of the canopy tree, *Dinizia excelsa* (Fabaceae) and may have aided the recovery of this endangered species. These introduced bees have also altered the genetic structure of remnant populations by mediating frequent long-distance gene flow. Pollinators are generally less reliable in alpine habitats (Stenström and Molau 1992), although it remains unknown how the introduction of alien pollinators affects the pollination systems of local species and the role they may play in the reproductive performance of those that are endangered.

Most Gentianaceae species are restricted to highland habitats (Ho and Liu 2001). Previous research has revealed that most gentians are self-compatible, but pollinator visits are vital for their reproduction due to protandry and herkogamy (e.g. Spira and Pollak 1986; Webb and Littleton 1987; Petanidou et al. 1998, 2001; Bynum and Smith 2001; Duan et al. 2005). Moreover, some gentians have evolved diverse strategies in adaptive responses that appear to enhance their reproductive success in arid alpine habitats, including autogamy in annual gentians (Spira and Pollak 1986), longer duration of pollen shedding and stigma receptivity (e.g. Webb and Littleton 1987; Petanidou et al. 2001) and floral closure to protect pollen shedding during thunderstorms and when temperatures decrease (Bynum and Smith 2001; He et al. 2006). In spite of these adaptations, a few perennial gentians are now endangered and are close to extinction. Intensified agricultural practices

are one of the important factors that have decreased their reproductive output (Raijmann et al. 1994; Petanidou et al. 1995; Luijten et al. 2000; Lennartsson and Oostermeijer 2001; Lienert et al. 2002).

*Swertia przewalskii* Pissjauk. (Gentianaceae), a rare alpine perennial, is only distributed in the Qilian and Menyuan counties of the north-eastern Qinghai–Tibetan Plateau in the Qinghai province. Despite being restricted to a small area along the southwest valley of the Qilian Mountains, extending for about 100 km, this species had high abundance only 20 years ago (Yang et al. 1991). However, the number of individuals has decreased greatly in recent years and this species has now become extremely endangered. In the natural distribution range of *S. przewalskii*, alien honeybees have been artificially introduced by beekeepers because a large number of nearby grasslands have been used for planting cole (*Brassica napus*) to produce colza oils which have high commercial value. The abundance of these honeybees fluctuates between years depending on the number of beekeepers and how many hives they can carry. These honeybees, as well as native bumblebees, visit alpine plants in this region (see, for instance, He and Liu 2004; Duan et al. 2005). In this study, we investigated the reproductive ecology of *S. przewalskii* and discuss possible causes for its endangered status. Specifically, we aimed to address whether there was a difference in the reproductive performance of this species between two years when native bumblebees and alien honeybees were the dominant pollinators, respectively.

## Materials and methods

### Study site

Twenty years ago, *S. przewalskii* is continuously distributed in the marsh wetlands along the Babao and Datong rivers and forms four large populations in Menyuan county, Qishizui town, the Haibei Alpine Meadow Ecosystem Research Station and Qilian County. At present, according to our recent investigation, most individuals in the sparse populations have disappeared, while the abundance of individuals in the four large sites has decreased to several hundred plants from more than ten thousand. We therefore chose only one large site around the Haibei Alpine Meadow Ecosystem Research Station for our experiments, which contained enough individuals for our controlled experiments and pollinator observations. This station is located on the north-east Qinghai–Tibetan Plateau (Lat. 37°29′–37°45′ N, Long. 101°12′–101°23′ E) at 3200 m above sea level. The average annual air temperature here is –1.7°C with extremes of 27.6°C (maximum) and –37.1°C (minimum), and average annual precipitation ranging from 426 to 860 mm. All of our experiments were carried out from late July to early August, and observations of pollinators were made in mid August in 2002 and 2004. The wetlands where *S. przewalskii* grows are dominated by *Kobresia tibetica* (Poaceae). In 2003, we recorded all similar visiting species as in 2002, however, after two weeks all fenced plants used for experiments and observations were unexpectedly damaged by yaks.

### Breeding system

Firstly, we fixed eight flowers in FAA, and then dissected ovules and counted the pollen in the laboratory according to the method described by Dafni (1992). The

pollen:ovule coefficient was used to crudely estimate the breeding system of *S. przewalskii*.

Ten flowers whose stigmas had just opened were labeled each morning, daily from July 27th to August 2nd in 2002. Each flower was emasculated and bagged and then artificially pollinated with pollen from different individuals on August 2nd. The experiment was designed to test the duration coefficient of stigma receptivity through the production of seed sets. A month later, but before the fruits dehisced, we collected all the fruits from each treatment. To obtain the seed set coefficient we counted the matured seeds, aborted seeds and unfertilized ovules. The seed set coefficient was calculated using the following formula, seed set = no. of matured seeds/(no. of matured seeds + no. of aborted seeds + no. of unfertilized ovules).

Before flowers opened in 2002 and 2004, we randomly selected 60 flowers from different individuals, 30 of which were emasculated and bagged to test apomictic seed production and the others were bagged without emasculation to examine whether *S. przewalskii* can be spontaneously self-pollinated without insect involvement. We then labeled 60 flowers on different plants, 30 of which were emasculated and left to freely pollinate to investigate the degree to which *S. przewalskii* depended on vectors for seed production, while the others were left to pollinate under natural conditions as controls. We further emasculated 80 flowers and bagged them for the following two experiments: 40 of the flowers were pollinated artificially with fresh pollen from the same plant, while the rest were pollinated by hand with pollen from different plants.

#### Flower visitors

During the full flowering period we recorded how many species visited *S. przewalskii* and their behavior during the two years. We further selected 12 individual plants, on which all the open flowers were used as focal flowers, to record the visiting frequencies of all the pollinators from 10 to 12 in August in 2002 (21 h in total) and four days from 10 to 13 in August in 2004 (26 h in total) at different time intervals (9:00–12:00, 13:00–15:00 and 15:00–17:00). We then calculated the visiting frequencies of each visitor and the total frequency of active visitors for each flower (times/h).

#### Statistical analysis

We used One-way ANOVA and Post hoc-LSD tests (SPSS Inc., 1997) to compare: (1) seed set among control, emasculated but freely pollinated, selfed within an individual plant and outcrossed flowers in 2002 and 2004, and seed set among flowers that had been artificially outcrossed on different stigma receptivity dates in 2002; (2) seed set of the same treatment between 2002 and 2004; (3) visiting frequencies of the same pollinator species from different time intervals in the two years; and (4) visiting frequencies of all visitors between the two years. All data were tested for normality using 1 Sample K-S in Nonparametric tests before comparison. Nonparametric tests (Kruskal–Wallis Tests for K-Independent Samples) were used to analyze data which were not normally distributed.

## Results

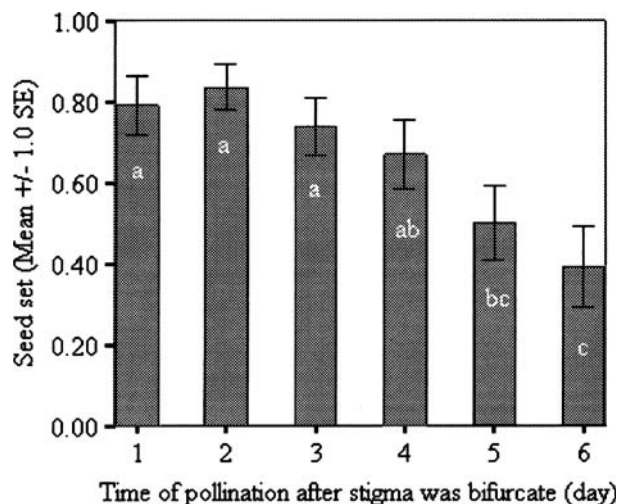
### Breeding system

The ovule abundance per flower ranged from 38 to 106 (average,  $\pm 1.0$  SE;  $74.5 \pm 6.9$ ,  $N = 8$ ), while the number of pollen grains ranged from 12,150 to 31,950 and averaged  $18,862.5 \pm 2438.3$  per flower ( $N = 8$ ). The pollen:ovule ratio varied from 156.7 to 420.4 (average  $266.4 \pm 36.3$ ), indicating that the breeding system of *S. przewalskii* is facultative xenogamy (Dafni 1992). *S. przewalskii* flowered from late July to late August, with the blue anthers surrounding the pistil shedding pollen first. After plants had finished shedding pollen, the anthers moved to outside with the filaments, and the stigma then opened. Therefore, *S. przewalskii* shows characteristics of dichogamy and herkogamy (Lloyd and Webb 1986; Webb and Lloyd 1986). When temperatures decreased and during thunderstorms, we observed no floral closure or movement, as in other gentians (He et al., 2006).

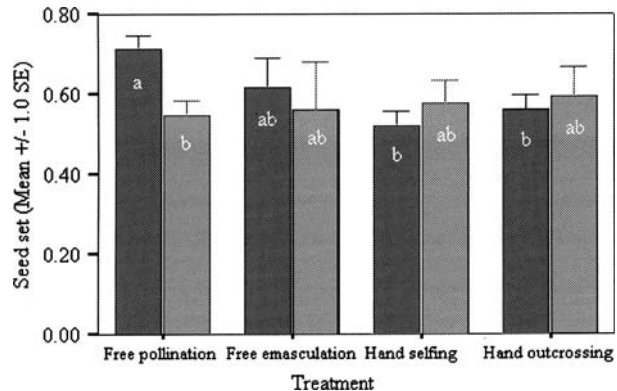
Flowers labeled to test the duration of stigma receptivity produced seeds successfully, but the seed set produced strongly depended on the age of the flowers. The seed set coefficient was higher on the second day, although not significantly, than on all other days and it was still 0.4 on the seventh day (Fig. 1). Therefore, the stigma can remain receptive for six days when a flower has not been pollinated. However, not all ovules had been fully pollinated with sufficient fresh pollen even at the highest receptivity stage (Fig. 1), indicating that once-pollination is insufficient to ensure the successful pollination of all ovules.

There was no evidence of apomixis or spontaneous self-pollination in *S. przewalskii* as neither the bagged flowers with or without emasculation produced seeds. The seed set coefficient for naturally pollinated emasculated flowers was high, and there was no significant difference between naturally pollinated emasculated and control flowers between the two years (Fig. 2). Seed set coefficients of naturally pollinated emasculated flowers did not differ significantly between the two years ( $F = 0.200$ ,  $P = 0.659$ ) (Fig. 2), but there was a significant difference between 2002

**Fig. 1** Seed set coefficients from flowers pollinated at different times after the stigmas had become receptive. Bars with the same letter indicate that the difference was not significant at the 0.05 probability level



**Fig. 2** Seed set coefficients from different treatments in 2002 and 2004, denoted by black bars and gray bars, respectively. Bars with the same letters indicate that the difference was not significant at the 0.05 probability level



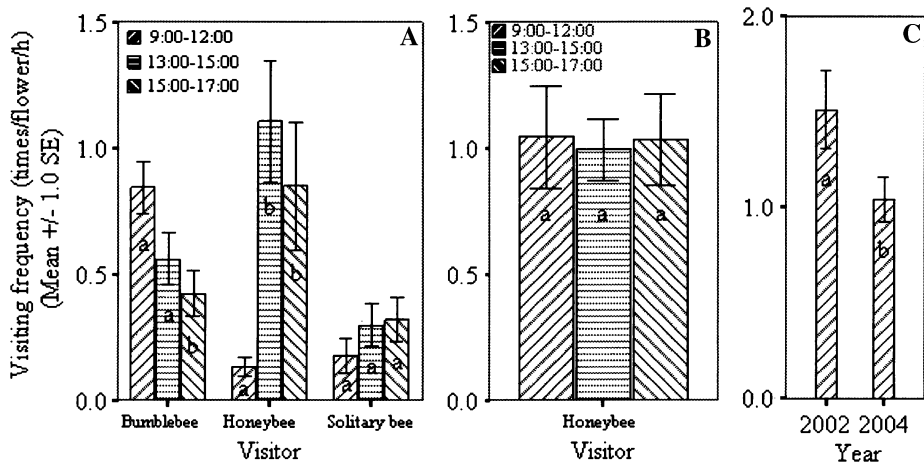
and 2004 in the seed set coefficients of naturally pollinated flowers ( $F = 13.145$ ,  $P = 0.001$ ) (Fig. 2).

All flowers selfed with pollen from the same individual and outcrossed flowers successfully yielded seeds, and no significant difference in the seed set coefficient was found between them (Fig. 2). In addition, the seed set coefficient in the two years did not differ significantly for plants that were hand selfed ( $F = 0.738$ ,  $P = 0.394$ ) and hand outcrossed ( $F = 0.187$ ,  $P = 0.667$ ) (Fig. 2). However, the seed set coefficients from both selfed and outcrossed flowers were lower than that of naturally pollinated flowers in 2002, but not in 2004 (Fig. 2). These comparisons indicate that the control flowers might have received a second pollination, resulting in larger seed set coefficients in 2002. In 2004, however, the control flowers suffered more pollen limitation due to lower pollination from bees and the lack of a second pollination event.

### Flower visitors

According to the behavior of flower visitors, bumblebees (*Bombus keshimirensis* Friese), honeybees (*Apis mellifera* L.) and solitary bees were considered to be the frequent pollinators of *S. przewalskii* in 2002, while other small insects, such as flies, are probably not legitimate pollinators as they only visited flowers for short periods. The frequent flower pollinators landed with the corolla petals either side of their bodies, probing for nectar at the base of the corolla using their proboscises, with the hind part of the body placed at the center of the corolla. Usually they circled the center of the corolla to collect nectar from each petal. During this movement, their hind legs and part of their body continuously touched the anthers or stigmas of the male or female stage flowers. Large quantities of pollen were attached to the hind parts of bumblebees, honeybees and solitary bees.

There were three taxa of the frequent pollinators in 2002 and the visiting frequencies of bumblebees and honeybees were higher than those of solitary bees ( $F = 6.514$ ,  $df = 2$ ,  $P = 0.002$ ), although visitation rates of bumblebees and honeybees varied little ( $F = <0.001$ ,  $P = 0.988$ ). In addition, bumblebees visited *S. przewalskii* more frequently from 9:00 to 12:00 than during other time intervals ( $F = 4.686$ ,  $df = 2$ ,  $P = 0.016$ ), while honeybees visited flowers mainly from 13:00 to 17:00 ( $F = 6.162$ ,  $df = 2$ ,  $P = 0.005$ ). No significant difference was found in the visiting frequency of solitary bees between the different time intervals ( $F = 0.912$ ,  $df = 2$ ,  $P = 0.412$ ) (Fig. 3A).



**Fig. 3** Visiting frequencies of flower visitors in different time intervals in 2002 (**A**) and 2004 (**B**), and the total visiting frequency averaged across all visitors for each of the two years (**C**). For each comparison, means with the same letter indicate that the difference was not significant at the 0.05 probability level

In 2004, however, no solitary bees were observed to visit flowers, and bumblebees visited only once during 26 hours' of observations, therefore, honeybees were the only major pollinators. Visiting frequencies of honeybees in the three time intervals did not differ significantly ( $F = 0.027$ ,  $df = 2$ ,  $P = 0.973$ ) (Fig. 3B). Overall, across all pollinators, there was a significant difference between the total visiting frequency between 2002 and 2004 ( $F = 4.586$ ,  $P = 0.04$ ) (Fig. 3C).

## Discussion

### Breeding systems

Little is known about the floral processes and breeding systems in *Swertia*, which is a large genus containing approximately 150 species from the family Gentianaceae (Ho et al. 1994). Most gentians are protandrous and herkogamous with different floral morphologies during the male and female stages (Lloyd and Webb 1986; Webb and Lloyd 1986; Webb and Littleton 1987; Petanidou et al. 1998, 2001; Bynum and Smith 2001; Duan et al. 2005). Such a combination of dichogamy and herkogamy also exists in *S. przewalskii* and as a consequence of these floral syndromes, spontaneous self-pollination is prevented. Bagged flowers without emasculation produced no seeds, similarly suggesting the dependence on pollinators for seed production. However, hand self-pollinated flowers yielded the same seed set coefficient as the outcrossed flowers (Fig. 2), suggesting that this species is self-compatible and the continuous foraging of pollinators might lead to geitonogamous self-pollination (Duan and Liu 2003). This breeding system remained consistent when the dominant pollinator changed between 2002 and 2004, as there was no difference in the controlled breeding experiments between these two years (Fig. 2).

The infrequent pollinator visits in arid alpine environments are especially disadvantageous to the reproduction of gentians (Spira and Pollak 1986). In



circumstances where pollinators are rare, a “sit and wait” strategy of increased floral longevity may be the only means for flowers to successfully reproduce (Ashman and Schoen 1994). Prolonging pollen presentation and duration of stigma receptivity could increase pollination chance and enhance reproductive success. Such a strategy has been reported for most gentians (Webb and Littleton 1987; Petanidou et al. 1998, 2001; Bingham and Orthner 1998; Bynum and Smith 2001; He et al. 2006). However, pollen shedding of *S. przewalskii* lasted on average only 5.2 h in a study by Duan and Liu (2003), which is much shorter than the 3–5 days observed in other gentians with floral closure, such as the North American gentian *Gentiana algida* (Bynum and Smith 2001), the European gentian *G. pneumonanthe* (Petanidou et al. 2001) and *G. straminea* (Duan et al. 2005). The present investigation further suggested that the duration of female receptivity in *S. przewalskii* (Fig. 1) is shorter than that of other gentians (Webb and Littleton 1987; He et al. 2006). Although the seed set coefficient remained at 0.4 on the 6th day of the female stage in *S. przewalskii*, this coefficient is lower than those perennial gentians with floral closure on the same female duration day. For example, Webb and Littleton (1987) reported that the seed set coefficient of two New Zealand alpine *Gentiana* species decreased to zero on the 8th and 10th days, but retained a seed set coefficient of 0.6 on the 6th day of the female phase. All these results suggest that the total floral longevity of *S. przewalskii* is shorter than for other gentians; and this might partly account for the lower seed set coefficients reported for this species when the visiting frequency of native pollinators decreased and then stopped.

#### Pollinator shift and seed set production

The reproductive success in flowering plants may depend on a number of factors, including pollen limitation, resource limitation, predation and the physical environment (Lee 1988). In alpine species that are dependent on insects for pollination, pollen limitation may be most important, since pollinators are generally less reliable than in other environments (Stenström and Molau 1992). The disruption of the interaction between pollinators and plants may account for the endangered status of some species, especially for those growing in unreliable pollinator environments (e.g. Kunin 1992; Byers 1995; Argen 1996; Huang and Guo 2002). Introduced honeybees may have serious effects on plant–pollinator interactions, for example they may cause drastic decreases in native bee populations, changes in patterns of gene flow among plants, or increased reproductive fitness of invasive exotic weeds (Gross and Mackay 1998). Furthermore, introduced honeybees are considered inadequate substitutes for native pollinators (Buchmann and Nabhan 1996) and potential competitors of native bees. They can also reduce the reproductive success of local bumblebees by consuming nectar, which has the potential to cause cascading effects on native communities (Thomson 2004). The native pollinators may become extinct or endangered due to insufficient floral resources when introduced honeybees become established (Kato et al. 1999; Kato and Kawakita 2004). Therefore, the introduced honeybees are thought to have serious effects on local plant–pollinator interactions. However, in some cases the introduction of honeybees may have rescued some endangered plant species by acting as substitute pollinators following declines in native pollinator numbers (Dick 2002).



In the present study, pollinator species changed greatly between the two years. There were three pollinators visiting *S. przewalskii* in 2002, compared with only one (honeybees) in 2004. Bumblebees are important pollinators of alpine ecosystems (Bingham and Orthner 1998). Although the total visiting frequency in both years (Fig. 3) was higher than those reported for other alpine plants (e.g., 0.0029 times/flower/min in the Andes and 0.0017 times/flower/min in North America, Arroyo et al. 1985; Bingham and Orthner 1998), there was a marked decrease in 2004 when honeybees dominated. Such a decrease in visiting rates may account for the reduced seed set coefficients reported in 2004 (Fig. 3). In addition, pollen limitation of this species may be correlated with the second forage of insects, with this assumption being corroborated by hand-pollination experiments. Even though hand-pollinated plants were supplied with abundant pollen, this did not ensure pollination of all ovules even during the highest period of stigma receptivity (at the second day of the female phase). Artificially outcrossed flowers did not have larger seed set coefficients, which were actually lower than those of control flowers that were naturally pollinated by native bumblebees in 2002 (Fig. 2). This pattern has also been observed in another endangered gentian, *Gentianella uliginosa* (Petanidou et al. 1998). Some species in the tribe Delphinieae (Bosch et al. 2001) were reported to have similar pollination limitations on reproductive success, especially *Delphinium nelsonii*, in which seed set coefficients from hand cross-pollination decreased by 30% relative to natural pollination in the field (Bierzzychudek 1981). In these species, flowers in the field usually received more than one visit, which ensured all ovules were pollinated (Bierzzychudek 1981; Bosch et al. 2001). In 2004, however, seed set coefficients of hand-pollinated flowers were slightly higher than those of flowers naturally pollinated by honeybees. This finding indicates that alien honeybees reinforce the pollen limitation of this species. A possible explanation for this is that honeybees will not make a second visit to the same flower because it has reduced amounts of nectar. In addition, the possibility of competition between native bumblebees and alien honeybees could not be dismissed, although we found that both species pollinated *S. przewalskii* in 2002. These results seem to suggest that alien honeybees have serious affect on the reproductive performance of *S. przewalskii* because their pollination effects are lower than those of bumblebees. However, there are two reasons why the inter-annual fluctuations in native bumblebee abundance in these alpine habitats may not only be caused by the introduction of alien honeybees to this study site. Firstly, oscillation in the numbers of native pollinators has been previously reported in alpine areas (Körner 1999), which may be due to the unpredictable climatic changes of these habitats. Secondly, we monitored the inter-annual changes in bumblebee abundance in the central Qinghai–Tibetan Plateau (lat. 34°21' N, long. 100°29' E, alt. 4000 m) where no honeybees have been introduced and found the same fluctuations in bumblebee populations during the past 4 years (authors' unpublished data). In 2004, the dominant honeybees still produced relatively high seed set coefficients (Fig. 2) and we conclude that the introduced honeybees have played only a minor role in endangering *S. przewalskii* in the past decade. Furthermore, we suggest that these alien honeybees might have acted as alternative pollinators when native pollinators' numbers were reduced.

## Conservation and endangered status

The most important causes of the endangered status of *S. przewalskii* are probably wetland habitat destruction and intensified agricultural practices. The animal husbandry in this area may also contribute to its endangered status. In the past decade, more than 20% of the grasslands have been grubbed to cultivate cole, barley and wheat (according to the local farmers). These changes in land use have not only directly destroyed specimens of *S. przewalskii*, but have also shrunk the wetlands on both sides of the Babao and Datong rivers in the valley bottoms. Some small lakes and streams nearby the rivers have also dried up and disappeared in recent years. Most individuals of *S. przewalskii* disappeared when the wetlands were destroyed. The remaining wetland grasslands were further damaged by the unfavorable animal husbandry. In spite of the reduced grasslands, the numbers of yaks and sheep have doubled in this area since the grassland was partitioned and contracted to private herders approximately 20 years ago (data according to the local government estimates). The wetland grasslands in the valley bottom have been used as winter herding pasture. The livestock generally returns to this area for the winter from the summer pastures at higher altitudes at the end of September when *S. przewalskii* has finished its reproduction. Unfortunately, in recent years the livestock have returned to the winter pastures earlier because of reduced grass in the summer pastures. Furthermore, we found that some livestock did not leave the winter pasture during the summer season between July and September. Some livestock in this area are now also fed with additional artificial fodder made from cole, barley and wheat stems and the wetlands have become exercise sites for those livestock retained during the summer season. The activities of these livestock not only affect the reproduction of *S. przewalskii*, but also directly destroy growing individuals. During this study some individuals used for breeding experiments were destroyed by yaks and sheep. In addition, we found that some local communities collected *S. przewalskii* as medicines, since most species of this genus in the Qinghai–Tibetan Plateau are regarded as effective cures (Yang et al. 1991).

The human activities described above not only destroy existing populations of *S. przewalskii*, but are also responsible for those destroyed in the past. This species is now fragmented into only four separated sites, which might reduce reproductive success, lower the total number of seeds produced and possibly compromise long-term population persistence (Valdivia et al. in press). The creation of a mosaic by the agricultural activities also prevents genetic exchange occurring between populations. Although we observed sufficient pollinators, decreasing plant numbers will undoubtedly decrease their attractiveness to pollinators (Sih and Baltus 1987; Olesen and Jain 1994), while changes in pollinator diversity will further reinforce the endangered status of this species.

We suggest the best way to effectively protect this endangered species is to establish a conservation area along the Dabao and Qilian rivers in the Qilian valley. In this protected area, further agricultural exploitation and the collection of wild plants should be prohibited, with herding practices being restored as they were 20 years ago. When a continuous wetland habitat forms, the seeds from the four sites can be artificially cultivated in the uninhabited area in order to rapidly restore a continuous distribution. However, ex-situ conservation is more difficult than in-situ conservation. Under this scenario, a suitable site should be carefully selected, and seeds should be collected from the four existing sites to establish an artificial and

mixed population. It is important for this site to be near to the species' natural distribution because the artificial population would have to rely on the specific pollinators (bumblebees) for more effective pollination.

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