

**FRAGMENTATION AND POLLINATION CRISIS IN THE SELF-INCOMPATIBLE *IRIS BISMARCKIANA* (IRIDACEAE), WITH IMPLICATIONS FOR CONSERVATION**

BOSMAT SEGAL,<sup>a</sup> YUVAL SAPIR,<sup>b,†</sup> and YOHAY CARMEL<sup>a,\*</sup>

<sup>a</sup>*Department of Civil and Environmental Engineering,  
Technion—Israel Institute of Technology, Haifa 32000, Israel*

<sup>b</sup>*Department of Evolution, Systematics and Ecology,  
The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

ABSTRACT

Recent evidence suggests that fragmentation effects on pollinators may drive plant species to extinction even if their immediate habitats are not affected. We hypothesize that species at high risk of suffering this “pollination crisis” are characterized by the following set of traits: self-incompatibility with synchronous flowering; reliance on a small number of pollinator species whose pollinators are specialists; and the existence of only a few, sparse populations. *Iris bismarckiana* fits this description perfectly. We studied two populations separated by recent urban development in order to (a) determine if the species is suffering a “pollination crisis”, (b) assess which stage of the process limits reproductive success, and (c) evaluate artificial pollination as a viable conservation means for this species. We performed pollination experiments in two populations of *I. bismarckiana*. The overall natural fruit-set of open-pollinated inflorescences was around 13%, while supplementary cross-pollination by pollen from the same population substantially increased the fruit-set to 44%. An even greater fruit-set of 78% resulted from cross-pollination with pollen from the second population. Artificial cross-pollination also increased the number of seeds per capsule. Our results suggest that fragmentation may link the effects of inbreeding depression and pollinator activity to generate a “pollination crisis”, expressed as significant reduction in sexual reproduction. We propose that supplementary cross-pollination, as well as transplanting of seeds, seedlings, and adult plants, may increase seed production and improve the survival chances of *I. bismarckiana*.

*Keywords:* conservation, inbreeding depression, fruit-set, *Iris* section *Oncocyclus*, pollen limitation, seed production, self-incompatibility, supplementary artificial pollination

\*Author to whom correspondence should be addressed. E-mail: yohay@technion.ac.il

†Present address: Department of Genetics, University of Georgia, Athens, Georgia 30602, USA.

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## 1. INTRODUCTION

Accelerating rates of land cover changes result in unprecedented habitat fragmentation worldwide. In the last decade, concerns were raised that fragmentation effects on pollinators may drive rare plants to extinction, even if their immediate habitats are not affected (Murcia and Feinsinger, 1996; Kearns et al., 1998). Fragmentation can potentially disrupt natural plant–pollinator mutualism by decreasing pollinator visitations and/or decreasing the proportion of outcross pollen deposition (Buchmann and Nabhan, 1996; Kevan and Phillips, 2001; Biesmeijer et al., 2006). Recently, evidence of negative effects of fragmentation on reproductive success has been demonstrated. For example, Cunningham (2000) found fewer pollen grains per stigma in fragmented habitats compared to undisturbed continuous habitats of two shrub species. He also found that supplementation of pollen increased fruit-set in both species examined. Similarly, Donaldson et al. (2002) found that fragment size and its distance from large populations influenced fruit-set and the abundance of certain pollinators.

This phenomenon, known also as a “pollination crisis” (Allen-Wardell et al., 1998), may affect certain species more than others and could possibly be accelerated by the effect on plant–pollinator interactions. Bronstein (1995) suggested two factors that determine sensitivity of pollination systems to fragmentation: synchronous flowering vs. asynchronous flowering, and generalized vs. specialized pollinators. We hypothesize that a plant species at higher risk of suffering a “pollination crisis” may be characterized by the following properties:

- It is rare, with isolated populations;
- It reproduces via synchronous flowering (Bronstein, 1995);
- It has relatively few pollinating species (Donaldson et al., 2002);
- It has a highly specialized pollination system (Bronstein, 1995; Biesmeijer et al., 2006);
- It has a low rate of selfing due to self-incompatibility (Aigner, 2004).

To date, no assessment of what constitutes a “pollination crisis” has been done on a species that possesses the full range of these properties. Here we provide evidence of the effect of a “pollination crisis” in a species that exhibits a combination of all these traits, and thus can be used as a test case for our hypothesis.

The *Iris bismarckiana* Regel is a rare and endemic species (Avishai and Zohary 1980; Sapir et al., 2002). Following a recent decline, it is now limited to a few isolated populations (Sapir and Walczak, 2002). Its habitat has become largely fragmented in the last century, mainly due to urbanization. It belongs to the section *Oncocyclus*, where all the species are self-incompatible (Avishai and Zohary, 1980; Sapir et al., 2005), pollinated obligatorily by males of a solitary bee (*Eucera* spp., Sapir et al., 2005). It has synchronized flowering with its pollinators (Y. Sapir, pers. observ.). Satisfying all the above criteria, it may be highly susceptible to a disruption of its pollination system, and is likely to suffer reduced sexual reproduction.

In order to assess the effect of fragmentation and the degree of the “pollination crisis” in *Iris bismarckiana*, we defined four stages within the sexual reproduction process, as follows:

- (1) Pollen may be limited due to low pollen production or to short pollen longevity (Ashman et al., 2004).
- (2) Pollination may be limited by low abundance or low activity of pollinators (Buchmann and Nabhan, 1996).
- (3) Plant mating systems may be fully or partially self-incompatible (Charlesworth et al., 2005), which, in combination with pollinator behavior, may limit the fruit-set and seed production.
- (4) Resource availability may also limit the fruit-set and seed production (Harper, 1977).

Using this classification, we may then ask which of the sexual reproduction stages is most affected by disruption of the pollination system. We studied these four situations and their contribution to fruit-set and seed production in *I. bismarckiana* in two populations in northern Israel. Our specific goals were the following:

- (1) To evaluate if, and to what extent, *I. bismarckiana* is suffering a “pollination crisis” and consequently a reduction in its sexual reproduction;
- (2) To identify which of the four stages of the reproduction process are limiting *I. bismarckiana*’s sexual reproductive success;
- (3) To assess the effectiveness of supplementary artificial pollination in enhancing reproduction in *I. bismarckiana* populations as a possible conservation practice for preventing population decline.

## 2. MATERIALS AND METHODS

### 2.1. STUDY ORGANISM: IRIS BISMARCKIANA

Irises of the section *Oncocylus* (Siems.) Baker (*Irises*: Iridaceae) are rhizomatous geophytes characterized by a large flower growing individually on each flowering stem (Avishai and Zohary, 1980; Sapir et al., 2002). Their habitat is semiarid and montane areas throughout the Middle East (Mathew, 1989). *Irises bismarckiana* is unique in its bicolor flowers (Fig. 1a) and its relatively sparse clones. It grows leaf fans 30–50 cm tall during the winter and may produce a single flower per ramet in the spring. The flower and leaves wilt at the beginning of May, and the plant is dormant until the next season.

The flowers of *I. bismarckiana* consist of three pollination units, called “pollination tunnels” (Chaudhary, 1972; Fig. 1b). Self-incompatibility in *Oncocylus* irises was suggested by Avishai and Zohary (1980). Sapir et al. (2005) assessed selfing rates in three species (*I. atrofusca*, *I. atropurpurea*, and *I. hermona*), and found low to zero selfing. The selfing rate of *I. bismarckiana* was not assessed, and we hypothesized that it would be similarly low.

Like other *Oncocylus* irises, *I. bismarckiana* is pollinated obligatorily by solitary male *Eucera* spp. bees (Sapir et al., 2005). The bees shelter within the flower overnight and are rewarded by the heat absorbed in the dark-colored parts after sunrise (Sapir et al., 2006). This is an asymmetrical specialization: *Oncocylus* irises are dependent on

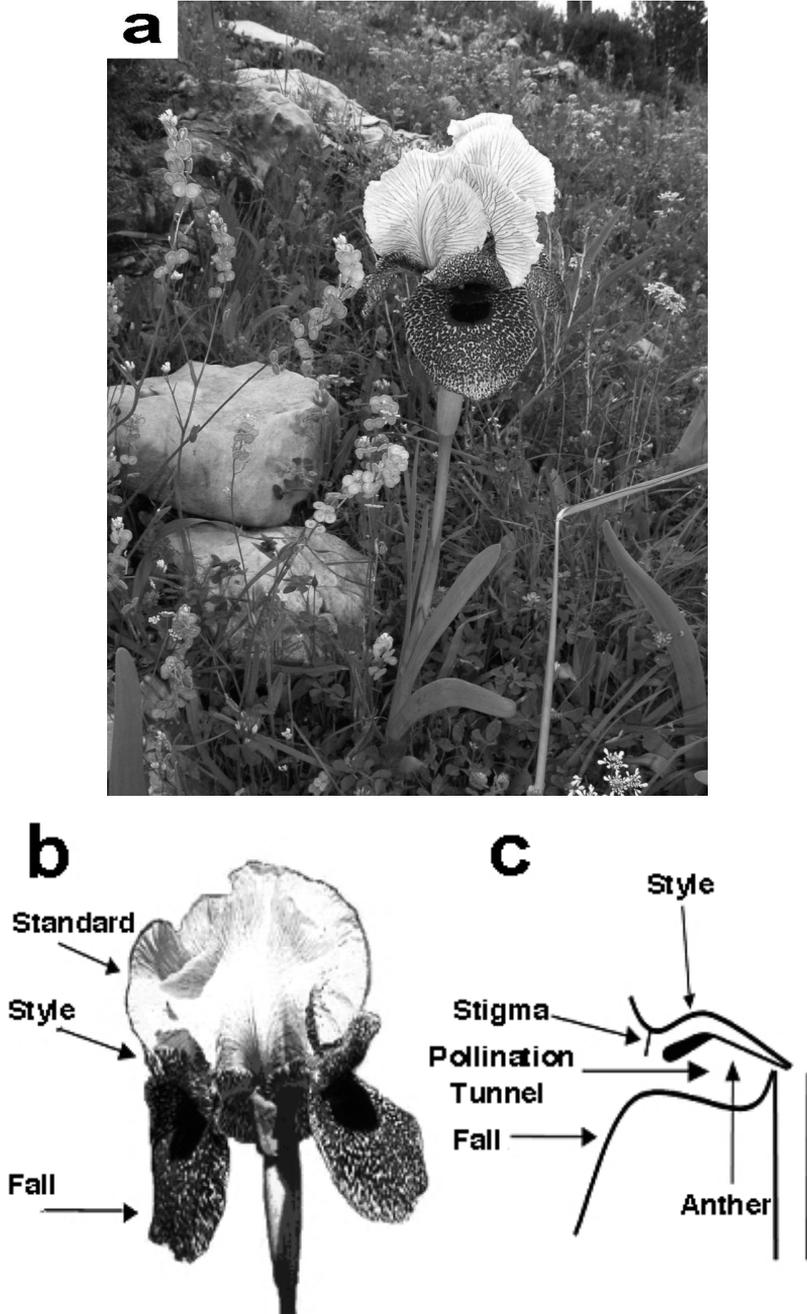


Fig. 1. The flower of *I. bismarckiana* with the sexual reproductive organs designated. (a) Whole flower. (b) Close-up. (c) A schematic diagram of one pollination unit.

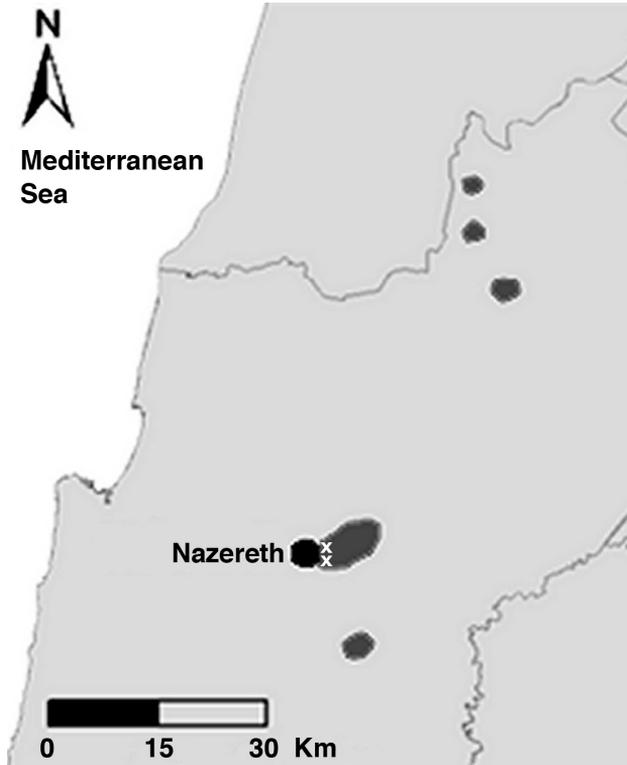


Fig. 2. Distribution of *I. bismarckiana* (gray patches) in Israel. The two study sites, marked by an 'x', were near the city of Nazareth.

sheltering bees so that they can be pollinated, but the bees are not specific to this iris and can find shelter in other plant species as well (Sapir et al., 2005). Avishai and Zohary (1980) predicted that large bees (such as *Xylocopa* and *Bombus*) may play a role in the pollination of *Oncocyclus* irises. Evidence from Lebanon suggests that some *Oncocyclus* irises are on rare occasions visited by male *Xylocopa* bees (L. Saad, pers. comm.); however, these bees have never been observed in *I. bismarckiana*. We assume that most pollen transfer is performed by sheltering male *Eucera* bees.

Large-scale urbanization of many natural or traditionally farmed lands in Israel has resulted in a decline of *I. bismarckiana* populations, which are now limited to a few isolated populations (Fig. 2). The species is at the top of the Israeli list of endangered plants. In 1963, one of the few remaining *I. bismarckiana* habitats, located on an eastern-facing slope of the Nazarene hills in the Lower Galilee, was designated as a nature reserve called the Nazarene Iris Reserve. Qualitative assessments of the Iris Reserve population suggest a decreasing number of flowers and a negative population growth (D. Kaplan, pers. comm.). This reduction is unlikely to be a result of direct human or animal interference, although minor effects of insects on the flowers were noticed.

## 2.2. STUDY SITES

The experiments concerned two populations of *I. bismarckiana* located about 1500 m apart, on the eastern slopes of the Nazarene hills, 550 m asl (marked "x" in Fig. 2). Both sites consist of terra-rossa soil cover on top of hard limestone bedrock. The area receives an annual average precipitation of 650 mm as winter rains between November and March. The vegetation consists of typical Mediterranean grassland and dwarf shrubs.

The Iris Reserve site encompasses 13.7 ha south of the village of Ein Mahel. The region was cultivated until 1963 by the inhabitants of the village, using traditional methods that presumably did not damage the irises. Following its designation as a nature reserve in 1963, cultivation ceased, while controlled sheep and cow grazing was permitted in order to limit vegetation growth and reduce fire risk. The second site is located 1500 m northwest of the Iris Reserve, on the eastern and southern slopes of Mt. Yona. The Mt. Yona population is larger and denser. All of the Iris Reserve area was used in this study, and only a small portion of Mt. Yona was used (0.8 ha).

## 2.3. POPULATION DENSITY

We used several long transects to estimate plant density (ramet density) in the Iris Reserve. Density of flowering plants was estimated in both sites. During the peak of the flowering season, the entire population of flowering plants in the Iris Reserve was counted and marked, as well as in the study area within Mt. Yona. Very few flowers appeared at the end of the season, and these were not recorded.

## 2.4. POLLINATION EXPERIMENTS

We performed the experiment at both sites in the 2003 flowering season (7 March 2003 to 30 March 2003). The flowering season in Mt. Yona started and ended about two weeks earlier than in the Iris Reserve, presumably due to the more southerly aspect of its slopes. Flowers were assigned randomly to one of four treatment groups: (a) natural pollination: flowers were left open; (b) local supplementary cross-pollination: flowers were artificially pollinated with pollen taken from a flower at the same site, growing at least 10 m apart (in order to ensure that the two flowers belonged to different clones); (c) remote supplementary cross-pollination: flowers were artificially pollinated with pollen taken from the other site, 1500 m apart; (d) self-pollination: flowers were pollinated by their own pollen and covered by a fine mesh to prevent natural cross-pollination.

Flowers in local and remote cross-pollination treatments were left open to natural pollination. Hence the artificial pollination is supplementary. Self-pollination of self-incompatible plants may be a negative intervention; therefore, complying with the Nature and Parks Authority regulations, we did not perform self-pollination treatments in the Iris Reserve, only at Mt. Yona.

All artificial pollination treatments were carried out by emasculating the stamens and brushing them against the three stigmas of another flower for cross-pollination, or of the same flower for self-pollination. Anthers of *I. bismarckiana* contain large amounts of pollen grains, and pollen of one anther was usually sufficient for pollinating all three stigmas. Emasculated flowers were used as both pollen donors and pollen recipients.

Table 1

Fruit set in *Iris bismarckiana* at two study sites in the Nazarene Hills, Israel. First visit recorded visible ovary development, second visit recorded seed production as measures of fruit set. Self-pollination was performed only at the Mt. Yona site

	Iris Reserve			Self-pollination	Mt. Yona		
	Cross-pollination		Natural pollination		Cross-pollination		Natural pollination
	Remote source	Local source			Remote source	Local source	
Sample size	16	16	34	12	24	23	52
Fruit-set	16	11	5	7	17	8	12
1st survey	(100%)	(69%)	(15%)	(58%)	(71%)	(35%)	(23%)
Fruit-set	15	10	5	2	16	7	6
2nd survey	(94%)	(63%)	(15%)	(17%)	(67%)	(30%)	(12%)
Abortions	6	1	1	5	1	1	6
Seeds per fruit ( $\pm$ s.d.)	38.33 ( $\pm 12.32$ )	31.25 ( $\pm 9.66$ )	17.00 ( $\pm 10.12$ )	16.50 ( $\pm 3.54$ )	24.79 (12.52)	20.50 ( $\pm 15.15$ )	16.83 ( $\pm 10.80$ )

Flowers were assigned each day to each of the three treatments in a ratio of 2:1:1 for natural pollination, for local, and for remote supplementary cross-pollination, respectively. This ensured that the different treatments took place under similar meteorological conditions in spite of the difference in flowering time among individuals in the populations. Each treated flower was marked by a plastic tag with an individual identification number. Sample sizes for each treatment in each of the study sites are given in Table 1.

Flowers at both sites were surveyed twice for fertilization success. Four weeks after establishing the experiment, each tagged flower was examined for ovary development (first survey). Two weeks later, the tagged flowers were surveyed again for ripe fruits (second survey). Visible development of the ovary in the first survey, and seed production in the second survey, were counted as setting fruit. Flowers with a fruit-set in the first survey but no seeds in the second survey were presumably aborted. The rate of seed abortions could not be quantified since some seed abortions could have occurred prior to visible ovary development. The fruits of the tagged flowers were collected, and total number of seeds in each fruit was recorded.

### 2.5. POLLINATOR SURVEY

In order to estimate pollinator activity, we surveyed flowers for night-sheltering male bees at both sites. Visitation rate in open flowers was surveyed on 28 March 2003. A total of 88 and 55 flowers were surveyed for night-sheltering insects in the Iris Reserve and Mt. Yona, respectively. The flowers in all three pollination tunnels of each flower were monitored less than one hour before sunset. Flowers were chosen for monitoring at random along transects at both sites.

## 2.6. STATISTICAL ANALYSIS

Fruit-set (the proportion of flowers that set fruits) and mean number of seeds per flower were compared for differences between treatments. Fisher's exact test (Sokal and Rohlf, 1995) was used for comparing fruit-set rates in the different treatments, as well as pollinator numbers between sites. In cases where the numbers were too large to use Fisher's exact test (total counts of more than 100 flowers), the G statistic that approximates the Fisher statistic was used (Sokal and Rohlf, 1995). The number of seeds per fruit in the different treatments was compared using the Wilcoxon–Mann–Whitney test (Sokal and Rohlf, 1995). Two-tailed probability results  $< 0.05$  were considered statistically significant.

## 3. RESULTS

### 3.1. POPULATION DENSITY

Plant (ramet) density in the Iris Reserve was quite high, with an average of 13,200 plants/ha. In contrast, flower density in the same area was only 8.5 flowers/ha. On average, only one of 1500 plants made flowers. In Mt. Yona, flower density was much higher than in the Iris Reserve, with an average of 191 flowers/ha. This higher density compared to the Iris Reserve seemed to be a result of both higher ramet density and a higher proportion of individuals flowering.

### 3.2. FRUIT-SET

We were able to find ~70% of the tagged stems in both study areas. We believe that most of the flowers were still there, somewhere, but were not found because of the high grass. Only flowers that were found in the fruit-set survey were considered in the analysis. Thus, the actual sample size is smaller than the number of treated flowers. Fruit-set rates in natural conditions were 15% in the Iris Reserve and 12% in Mt. Yona (Table 1). Higher fruit-set rates of 63% in the Iris Reserve ( $p = 0.001$ ) and 30% in Mt. Yona (marginally significant,  $p = 0.056$ ) were recorded for the local supplementary artificial pollination. Remote supplementary pollination resulted in even higher fruit-set rates, 94% in the Iris Reserve and 67% in Mt. Yona, ( $p < 0.001$  in both cases).

The differences in fruit-set rates between the two sites in all treatments were not significant (Mann–Whitney test,  $p > 0.05$  in all cases); therefore, we pooled the fruit-set results from both sites for further analyses. Fruit-set rates in the natural pollination, the local, and the remote supplementary cross-pollination were 13%, 44%, and 78%, respectively (all comparisons significant at the  $p < 0.001$  level). In the self-pollination experiment, a high initial fruit-set rate of 58% was recorded, but all the self-pollinated fruits except two were aborted (Table 1).

### 3.3. SEED PRODUCTION

A total of 53 capsules were examined for number of seeds. An increase in the number of seeds per fruit from natural pollination to local supplementary pollination to remote source supplementary pollination was evident in both sites. The mean number of seeds

per fruit in the natural pollination experiments was 16.8 in the Iris Reserve and 17.0 at Mt. Yona. In the Iris Reserve, the mean number of seeds per fruit increased sharply to 31.3 in the local supplementary pollination ( $p = 0.033$ ), and to 38.3 in the remote supplementary pollination ( $p = 0.006$ ). At Mt. Yona, the mean number of seeds per fruit in the local source and remote source artificial pollination experiments were 20.5 and 24.8—larger but not significantly different than their control. Mean number of seeds per fruit at the Iris Reserve was higher than at Mt. Yona in both local and remote cross-pollination treatments (significant only for remote pollination,  $p = 0.018$ ).

### 3.4. POLLINATOR ACTIVITY

Most of the insects found were *Eucera* spp. male bees, but we also incorporated in the analyses a few *Amphicoma* spp. beetles (Coleoptera: Glaphyridae), assuming that they may play some role in pollen transfer, in spite of being herbivores on the flowers. A total of 43 insects were found in 32 flowers in the two sites; 22 flowers (out of 88) in the Iris Reserve hosted 29 insects, and 10 flowers (out of 56) hosted 14 insects in Mt. Yona. Differences between the two sites were not significant (Fisher exact test,  $p = 0.27$ ). Calculated over both sites, the average number of night-sheltering insects per flower was  $0.29 \pm 0.62$  SE bees per flower ( $1.34 \pm 0.6$  for flowers that hosted at least one insect).

## 4. DISCUSSION

*Iris bismarckiana* belongs to the section *Oncocyclus*, in which several species were found to be self-incompatible (Avishai and Zohary, 1980; Sapir et al., 2005). Here we found that fruits were developed in 7 of 12 self-pollinated flowers, yet only two of these fruits produced seeds. This confirms that *I. bismarckiana* is rather self-incompatible. The selfing rate in *I. bismarckiana* is not zero, as found in other species (Sapir et al., 2005), but is still lower than the average 0.41 of herbaceous perennial species (Barrett et al., 1996). Selfing rates in other species of irises from other sections range from 21.4% to 74.1% (Planisek, 1983; Motten, 1986; Kron et al., 1993; Zink and Wheelwright, 1997; Wilson, 2001; Wheelwright et al., 2006). The differences in selfing rates among *Iris* species have been discussed in detail elsewhere (Sapir et al., 2005). The low selfing rate found here emphasizes the dependence of *I. bismarckiana* on insect pollination for its reproductive success.

Natural fruit-set was low compared to supplementary artificial pollination treatments in both populations. Similarly, supplementary artificial pollination increased seed production. In both parameters (fruit-set and seed production), pollination using remote source pollen outperformed local source pollen. Together, these results show that *I. bismarckiana* sexual reproduction is fairly limited. This is in line with several characteristics that make the species particularly susceptible to a “pollination crisis” (sensu Allen-Wardell et al., 1998) following habitat fragmentation: it is self-incompatible and rare (only a few sparse populations), with synchronous flowering, and a small number of pollinator species that visit only a small fraction of the flowers. These relationships are asymmetric: the *Iris* flowers are visited by a narrow spectrum of insects, but these

insects visit other flowers as well (Sapir et al., 2005). This suggests that the pollination crisis of *I. bismarckiana* may not affect the pollinators directly.

These results also indicate which of the four crucial stages of reproduction may be the “bottleneck” that limits sexual reproduction in *I. bismarckiana*. The increased fruit-set and seed production in the supplementary artificial pollination treatments indicate that pollen quantity and genetic distance are limiting factors. It also implies that resource availability may not be the limiting factor in seed production. While many seeds were aborted in the natural pollination group, very few fruits were aborted in the artificial pollination treatments. This suggests that the cause of seed abortions is genetic rather than environmental. It should be noted, however, that artificially added nutrients could potentially compensate for genetic proximity (Shi et al., 2005).

Our results indicate that both pollinator activity and pollen quality (genetic distance) limit the potentially high rate of sexual reproduction in *I. bismarckiana*. Pollinators were present in about 20% of the flowers. Yet, the substantial increase in the fruit-set in supplementary artificial pollination indicates that pollinator population or activity may not have been sufficient to overcome the genetic constraints. Two of our results suggest that genetic constraints limit seed production: (1) the high rate of seed abortions in naturally pollinated plants and (2) the large increase in fruit-set and seed production, as well as the low seed abortion rate when remote pollen was used, compared to using local pollen. Presumably, genetic distance between clones within each population is small, while genetic distance between the two populations is larger. The high degree of relatedness of individuals within a site may explain the lower fruit-set and seed production, as well as the higher seed abortion rate in natural and local artificial pollinations compared to remote pollination.

The seemingly low pollinator efficiency in naturally pollinated flowers is also probably tied to this genetic problem. Low clonal diversity may result in low availability of compatible mates in each population, and indeed, several studies found that spatial arrangement of clones affects seed production (Charpentier, 2001; Wolf and Harrison, 2001; Lennartsson, 2002). Much of the pollinator activity in such conditions would be ineffective. Seed production was correlated with genetic distance between clones in a recent study of a clonal, self-incompatible plant (Aigner, 2004), corroborating earlier claims that the joint effects of genetic structure and pollination service would particularly affect clonal species that are self-incompatible (Charpentier, 2001).

Urban development around the study area during recent decades isolated the two populations, and natural cross-pollination between them has probably ceased. This fragmentation presumably acted to link the two “pollination crisis” factors in this species: while remote cross-pollination is no longer possible, local cross-pollination is probably limited by both pollinator activity and genetic similarity.

The current situation, in which only a single *I. bismarckiana* population is protected in a nature reserve, does not ensure long-term survival of the species. Taking into account the low rate of seed production, and the apparent low genetic diversity in these populations, protection of more *I. bismarckiana* populations is a prerequisite in any attempt to save the species.

However, this study also shows that given the combined effects of genetic obstacles and low pollinator activity, passive conservation means would not be sufficient. We suggest a set of actions to reduce the current pollination crisis in *I. bismarckiana*: (a) annual supplementary artificial pollination, using remote pollen, of a portion of each of the remaining populations and (b) transplanting of seeds, seedlings, and adult plants between nearby populations in an attempt to widen the genetic variability in each population and enhance future reproductive success.

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