Pollination of *Oncocyclus* irises (*Iris:* Iridaceae) by Night-Sheltering Male Bees

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Abstract: **Irises in the section *Oncocyclus* (Siems.) Baker (*Iris:* Iridaceae) grow throughout the Middle East and have large and dark-coloured flowers but no nectar reward available to flower visitors. Consequently, no reward-collecting pollinators have been observed visiting the flowers during daytime. The only visitors are solitary male bees (*Eucera* spp.: Apidae) that enter the flowers at dusk and stay there overnight. Here we describe the mating system of *Oncocyclus* irises, and the role of night-sheltering male bees in their pollination system. Pollen viability in *I. haynei* on Mt. Gilboa was very high (> 90%) throughout all floral life stages. Stigmas were receptive in buds and in open flowers, but not in older ones. Self-pollination yielded no fruits in three species, confirming complete self-incompatibility in *Oncocyclus* irises. On average, 1.9 flowers were visited by each male bee before it settled for the night in the last one. Moreover, *Iris* pollen was present on the dorsal side of 38.8% of males caught sheltering in flower models mounted near an *I. atrofusca* population, indicating that pollen is transferred between flowers by night-sheltering solitary male bees. We have surveyed 13 flowering populations of six *Oncocyclus* species for the presence of night-sheltering male bees as well as for fruit set. We found a positive correlation, indicating that sexual reproduction in *Oncocyclus* irises is dependent on night-sheltering solitary male bees. Based on their complete self-incompatibility, the absence of nectar-collecting visitors during the day, and the transfer of pollen grains by the night-sheltering solitary male bees, we conclude that fertilization of *Oncocyclus* irises is totally dependent on pollination by night-sheltering solitary male bees.**

Key words: **Bee pollination, clonal plants, geitonogamy, mating system, night-sheltering, self-incompatibility, solitary bees.**

Introduction

The relationships between flowering plants and their insect pollinators as ecological and evolutionary systems have been intensively studied (Faegri and van der Pijl, 1979; Proctor et al., 1996). Many groups of insects that have been described visiting flowers proved to be their efficient pollinators (Kevan and Baker, 1983). Among the diverse groups of flower-visiting insects, bees are the best adapted and the most important group of pollinators (Kevan and Baker, 1983; Proctor et al., 1996; O'Toole and Raw, 1999), and solitary bees consitute the vast majority of bee species (Michener, 2000).

Bees are attracted to flowers mainly for the reward offered by the flowers; this reward is mainly nectar and pollen, but other types of rewards exist (Proctor et al., 1996). Bees use flowers not only as a food source but also as a shelter. Several flowers have been described as night shelters for various species of solitary male bees (Horovitz, 1976; Dafni et al., 1981; Danforth and Neff, 1992; Gaglianone, 2000). However, in only one case (the orchid *Serapias vomeracea*) has it been shown that night-sheltering male bees served as pollinators (Dafni et al., 1981).

Successful pollinator-mediated fertilization depends not only on pollinators but also on the plant mating system and the origin of the pollen. Mating systems and the rate of fruit set are also often correlated with plant life forms and other life history traits (Stebbins, 1974; Barrett et al., 1996; Sutherland et al., 1999). For example, perennial plants that have a large floral display (i.e., a high number of flowers) may also face the risk of geitonogamy, resulting in inbreeding depression or decreased fruit set in self-incompatible plants (Stebbins, 1974; Barrett et al., 1996; Sutherland et al., 1999). Thus, insect-pollinated perennial plants are predicted to have self-incompatibility mechanisms to reduce the risk of genetic load (Barrett et al., 1996; Morgan et al., 1997).

Irises in the section *Oncocyclus* (Siems.) Baker (*Iris:* Iridaceae) that grow throughout the Middle East are characterized by their dense clonal growth and conspicuous large and mostly dark flowers that grow individually on each flowering stem (Avishai and Zohary, 1980; Sapir et al., 2002). Avishai and Zohary (1980) suggested that large carpenter bees (*Xylocopa* spp.) and bumblebees (*Bombus* spp.) are the major pollinators of *Oncocyclus* irises, but did not present any supporting data. *Oncocyclus* irises have also been reported to be self-incompatible, producing seeds only by cross-pollination (Avishai, 1977; Avishai and Zohary, 1980), but no quantitative data have ever been presented.

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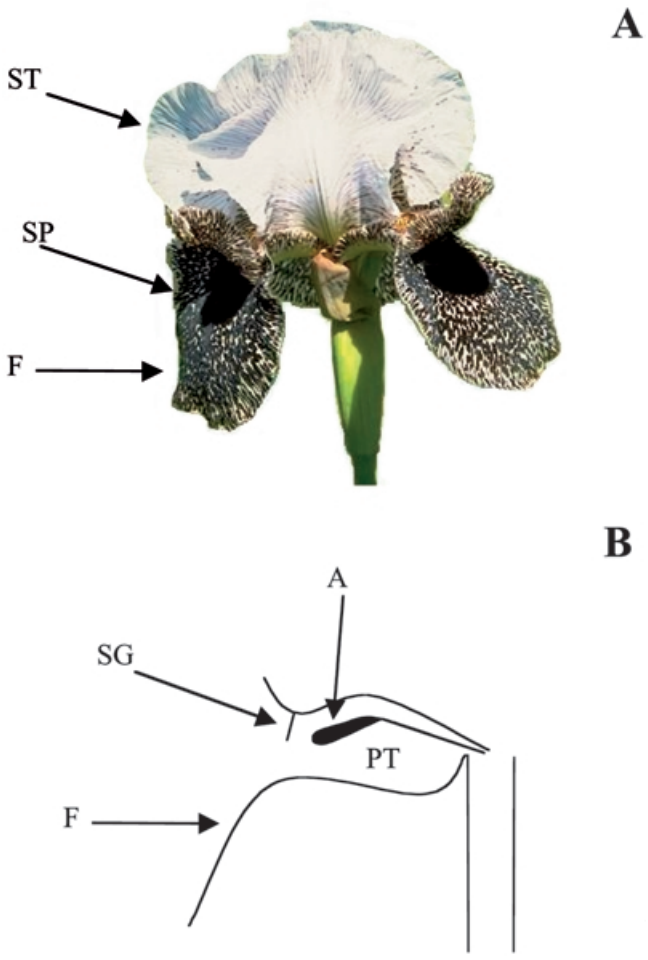

Fig. 1 Flower structure of typical *Oncocyclus Iris*. (**A**) Flower of *Iris bismarckiana*. (**B**) Schematic structure of the iris flower, presenting one of the three flower units ("meranthium"). A, anther; F, fall (outer petal); PT, pollination tunnel; SP, signal patch; SG, stigma, the arrow points to the external face of the stigma; ST, standard (inner petal).

Ten species of the *Oncocyclus* section have been recorded in Israel and adjacent areas (Feinbrun-Dothan, 1986; Fragman et al., 1999; Sapir et al., 2001); all of them are narrow endemic and of high conservation priority (Sapir et al., 2003). These conspicuous flowers are a symbol for nature conservation in Israel and Jordan, and are a focal point for nature lovers during the flowering season. However, in spite of their high conservation priority and horticultural importance, very little is known about their reproductive biology.

The floral structure of *Oncocyclus* irises is similar to other *Iris* and iris-like flowers (terminology follows Avishai and Zohary, 1980, and Sapir et al., 2002). The inner petals (standards) are large and erect while the outer petals (falls), together with the flat style, form three "gullet" units, henceforth referred to as pollination tunnels (Avishai, 1977; Faegri and van der Pijl, 1979; Goldblatt et al., 1989; Goldblatt and Bernhardt, 1999). The roof of the pollination tunnel is formed by the expanded, petaloid style, and its base by the outer petal (Fig. **1**). A single anther adheres to the roof of the pollination tunnel and the stigma is located at the entrance to the tunnel (Fig. **1**). At the end of the flower's life, the wilting style branch curls down and the stigmatic papillae touch hairs on falls that usually carry pollen that has dropped from the open anthers, thus permitting spontaneous self-pollination (Kron et al., 1993). However, seed set may result only in self-compatible species.

Oncocyclus iris flowers have no nectaries and thus offer no nectar reward to flower visitors (Avishai, 1977). The only recorded visitors to *Oncocyclus* flowers are solitary male bees that enter the flowers at dusk and stay there overnight (Ivri and Eisikowitch, 1988; Sapir and Shmida, 2002). In a preliminary survey, we found that most of these bees were *Eucera* spp. males (including the genus *Synhalonia:* Apidae [Michener, 2000]) but *Chalicodoma sicula* (Rossi) (Megachilidae) and a few *Andrena* spp. (Andrenidae) males were also recorded (Sapir and Shmida, 2002). *Eucera* bee species are common and important pollinating species in the Mediterranean region (Nachtigall, 1994; O'Toole and Raw, 1999; Potts et al., 2003). These are long-tongued, ground nesting solitary bees (Shimron, 1984; Kadmon and Shmida, 1992). Males emerge from mid-February to mid-March and disappear 3–4 weeks later; this is more or less coincident with the flowering time of *Oncocyclus* irises. Females emerge about a week later than the males and are active until the end of their nesting period in early June (Shimron and Hefetz, 1985). The activity of male bees commences in the early morning, while virgin females emerge only later in the day (Shimron and Hefetz, 1985). It is important to note that, while *Oncocyclus* irises are narrowly distributed in isolated populations, *Eucera* species are abundant throughout their range (C. O'Toole and A. Shmida, unpublished data).

Here, we describe an exploration of the pollination system of the *Oncocyclus* irises. The goals were to study the mating system and to determine the role of the night-sheltering male bees as pollinators of *Oncocyclus* irises. We hypothesized that solitary male bees are the main pollinators and thus that plant sexual reproduction is highly dependent upon their pollination services.

Materials and Methods

Study sites and species

The study was conducted between 1999–2003, with preliminary observations conducted in the 1997 flowering season (February–March). We studied four populations of different *Iris* species in Israel: (1) *Iris atrofusca* in the Goral Hills (Northern Negev; desert climate), (2) *I. haynei* on Mt. Gilboa (Lower Galilee; semi-arid climate), (3) *I. atropurpurea* at Nes Ziona (Coastal plains; dry Mediterranean climate), and (4) *I. hermona* at Keshet (Golan Heights; Mediterranean climate). The flowers of *I. haynei, I. atrofusca,* and *I. atropurpurea* are uniformly dark-purple to brown, representing the dark-coloured taxa, while flowers of *I. hermona* are bicolor and represent the light-coloured taxa: the inner petals are bluish-white, while the outer ones have dense dark pigmentation (Sapir et al., 2002). These populations/taxa represent the two types of coloration within *Oncocyclus* irises. Additional observations were also done in a few other populations or other species (see Table **2**).

Floral longevity

Floral longevity was studied in 2000 in the *Iris atropurpurea* population in Yakum and the *I. atrofusca* population at Goral, and in 2003 in the *I. hermona* population at Keshet. Flowers were marked with a coloured ribbon at the base of the flower stem on the day of dehiscence of the stamen. These flowers were left open to pollinators and monitored daily. The number of wilting flowers was recorded daily.

Pollen viability and stigma receptivity

To test pollen viability and stigma receptivity we used MTT (thiazolyl blue tetrazolium bromide; M-2128, Sigma-Aldrich Inc.; Rodriguez-Riano and Dafni, 2000). In a preliminary experiment, fresh pollen grains from three flowers of *I. atropurpurea* were stained with MTT, while heated, and subsequently dead, pollen from the same flowers was not stained with MTT.

Pollen viability and stigma receptivity were studied in an *Iris haynei* population on Mt. Gilboa in the spring of 2001. Flowers of three developmental stages were tested: (1) recently opened buds (0 – 1 days; n = 10), (2) open, mature flowers (1 – 5 days; n = 10), and (3) old flowers (> 5 days; n = 5) with an open pollination tunnel but wilting standards. Pollen grains were sampled from all three anthers of each flower and stained with MTT. One hundred pollen grains were counted and the proportion of stained grains was calculated. We used MTT to test the receptivity of all three stigmas of each flower at each floral stage: ten recently opened buds, eight open flowers, and ten old flowers.

Mating system

Artificial pollination experiments were performed in March 2001 in the *Iris haynei* population on Mt. Gilboa and the *I. hermona* population at Keshet. Floral buds were covered with an insect-proof net, and artificially pollinated on the first day of dehiscence of the stamen. Following pollination, the flowers were covered again until fruit set examination. All three stigma branches of each flower were pollinated by attaching an anther of a donor stamen to the stigma. For cross-pollination, donor plants were carefully chosen to ensure that they represent a different clone (genet). Clones can be clearly and easily defined in the field due to the phalanx growth habit of *Oncocyclus* irises. A distance larger than 20 cm without leaf fans was used as an indication of a border between clones. We performed four pollination treatments: (1) cross-pollination of covered flowers, as a control for the artificial pollination procedure, (2) self-pollination of covered flowers, to test for self-compatibility, (3) no pollination of covered flowers as a control for spontaneous self-pollination (autogamy) or apomixis (agamospermy), and (4) no artificial pollination of open flowers to monitor the rate of natural pollination. Spontaneous self-pollination of *I. atropurpurea* flowers was also tested in 2000 at Yakum. Fruit set was checked 6 – 8 weeks after pollination and calculated as the percentage of capsules that developed from the treated flowers.

Daytime observations and night-sheltering male bees

During eight successive flowering seasons (1996 – 2003), a total of about 120 h of pollinator observations were carried on flowers of all study species. Each observation lasted 10 – 15 min, and observations were distributed throughout daytime hours. The observer was located about 2 m from a group of 5 – 7 flowers. Any insect that approached the flowers or entered the pollination tunnel was recorded.

Flowers of 11 populations of 6 *Oncocyclus* species were surveyed between 1997 and 2003 for night-sheltering male bees, as well as for fruit set. Flowers were monitored for night-sheltering male bees in all three pollination tunnels of each examined flower 1 h or less before sunset or before sunrise. Flowers were randomly chosen along transects in the population or, when possible, all open flowers in a certain area were examined. In order to estimate fruit set at the population level, open flowers were randomly chosen and marked with a coloured plastic ribbon at the base of the flowering stem, and monitored for fruit-set 6 to 8 weeks later. The flowers monitored for fruit set were independent of those inspected for night-sheltering male bees.

In *Iris atrofusca* in Goral in 2003, we marked flowers with and without night-sheltering male bees, to correlate directly the night-sheltering of solitary male bees with fruit set at the individual flower level. In certain marked populations of *I. atropurpurea* at Nes Ziona and *I. haynei* population on Mt. Gilboa, we counted the total number of flowers in clones and calculated fruit set as the ratio of fruits to the number of flowers per clone (genet).

Black cones made of plastic foam (Paltziv, Kibutz Ein-Hanatziv, Israel), 4 cm long with a 2-cm entrance diameter, were used as flower models to attract night-sheltering male bees in an *Iris atrofusca* population at Goral. A total of 20 models were mounted for 3 nights near the edge of the population during the peak of the 2003 flowering season. The dorsal side of the thorax of night-sheltering male bees collected from the floral models was examined for the presence of *I. atrofusca* pollen grains. The insects were observed under a dissecting microscope (× 40). The pollen grains were identified by comparison with fresh *I. atrofusca* pollen grains.

The bees collected during this study were identified by C. O'Toole, Hope Entomological Collection, Museum of Natural History, Oxford, UK. The full list of bee species is available upon request from the first author.

Results

Floral longevity

Average floral longevity and its distribution varied between species; *Iris atrofusca* had the longest floral longevity (6.7 ± 1.3 days; n = 85), while *I. hermona* had the shortest longevity (3.6 ± 0.8 days; n = 218), and *I. atropurpurea* had an intermediate longevity (4.8 ± 1.3 days; n = 103). The effect of weather was not examined, but our impression was that cold, cloudy, or rainy days increased floral longevity, while hot days shortened it. In general, the flowering period of a population was 3 to 4 weeks long.

Table 1 Percentage of fruit set in pollination treatments in *Iris haynei* (Mt. Gilboa), *I. hermona* (Keshet), and *I. atropurpurea* (Yakum). All flowers, except the control, were bagged. In Yakum, only the control (open) and spontaneous self-pollination (covered flowers) treatments were done

Treatment	Gilboa *(I. haynei)*	n	Keshet *(I. hermona)*	n	Yakum *(I. atropurpurea)*	n
Artificial cross-pollination	72%	11	80%	10		
Artificial self-pollination	0%	13	0%	5		
Spontaneous self-pollination	0%	11	0%	13	0%	6
Control (natural pollination)	42%	175	30.5%	108	17.4%	132

Table 2 The number and percentage of flowers hosting night-sheltering male bees in several species of *Oncocyclus* irises across several populations and years. N_h = number of flowers checked for night-sheltering male bees; N_f = number of flowers checked for fruit set

Species	Population name	Year	# of host flowers (%)	N_h	Fruit set (%)	N_f
Iris atrofusca	Goral	1997	34 (38.6%)	88	59.8	97
I. atrofusca	Goral	2000	25 (53.2%)	47	34.7	98
I. atrofusca	Goral	2003	43 (39.1%)	110	18.6	97
I. atropurpurea	Nes Ziona	1997	14 (37.8%)	37	19.2	769
I. atropurpurea	Hator	1999	1 (2.6%)	39	29.1	79
I. atropurpurea	Palmahim	1999	6 (6.0%)	100	23.5	98
I. atropurpurea	Nes Ziona	1999	10 (10.0%)	100	24.1	108
I. atropurpurea	Yakum	2000	3 (3.0%)	100	17.4	132
I. atropurpurea	Nes Ziona	2003	6 (4.5%)	133	17.5	114
I. atropurpurea	Rishon Le-Zion	2003	2 (6.3%)	32	14.3	14
I. bismarckiana	Ein-Mahil	2003	4 (6.9%)	58	16.4	55
I. bismarckiana	Nazareth	2003	4 (7.3%)	55	24.7	77
I. haynei	Gilboa	1999	21 (30.9%)	68	53.8	223
I. hermona	Keshet	1999	24 (70.6%)	34	29.9	67
I. hermona	Keshet	2002	7 (25.9%)	27	26.3	38
I. mariae	Secher	2003	1 (2.8%)	36	11.8	17

Pollen viability and stigma receptivity

Pollen viability in *I. haynei* flowers on Mt. Gilboa was high: 94.7 ± 5.4%, 94.5 ± 4.5%, and 97.6 ± 3.8% for young, fully open and old flowers, respectively, with no significant differences in pollen viability among the three stages (Arcsine transformation; ANOVA: $F_2 = 0.33$, $p = 0.72$).

In buds and open flowers, stigmatic papillae of all three stigmas were stained, indicating simultaneous receptivity in all three floral units. In old flowers, none of the stigmas were stained, indicating the end of female function before flower wilting. Only the external face of the stigma was stained, indicating that pollen grains could be deposited on the receptive part of the stigma only upon a bee's entrance into the flower, but not upon its exit.

Mating system

Artificial and spontaneous self-pollination treatments yielded no fruits in *I. haynei* on Mt. Gilboa, in *I. hermona* at Keshet or in *I. atropurpurea* at Yakum. (Table **1**). Artificial cross-pollination yielded a higher percentage of fruit set than did control flowers (Table **1**). However, this difference was significant for *Iris hermona* ($\chi^2 = 6.43$, df = 1, $p = 0.01$), and not significant for *I. haynei* ($\chi^2 = 2.17$, df = 1, $p = 0.14$).

Night-sheltering male bees and fruit set

During eight seasons of observations (1996 – 2003), no reward-collecting visitors were observed approaching *Oncocyclus* iris flowers, except for one case in 1999 at Yakum, where one honeybee (*Apis mellifera* L.) was observed collecting pollen in one flower of *Iris atropurpurea.* The bee entered the pollination tunnel, turned with its legs upward, and grabbed pollen from the anther. However, in all other cases, solitary male bees were the only visitors to the flowers.

Male bees enter the flowers approximately 1 – 1.5 h before sunset and perform a unique probing behaviour prior to settling in a flower for the night. This probing behaviour was difficult to observe due to the unpredictability of the flowers chosen by the male bees and the bee's flight speed. However, ten male solitary bees were observed in five cases: one in *I. haynei* on Mt. Gilboa, three in *I. atrofusca* at Goral, and one in *I. hermona* at Keshet. The male bees landed on the outer petals and immediately entered the pollination tunnel. During entry, the dorsal part of the bee's thorax rubbed against the outer face of the stigma. In almost all cases the male bees did not stay in the first tunnel but left a few seconds later to probe another pollination tunnel of another flower. Male bees visited, on average, 1.9 flowers (± 1.1 SD; range 1 – 4 flowers) before settling down for the night in the last visited flower. This number

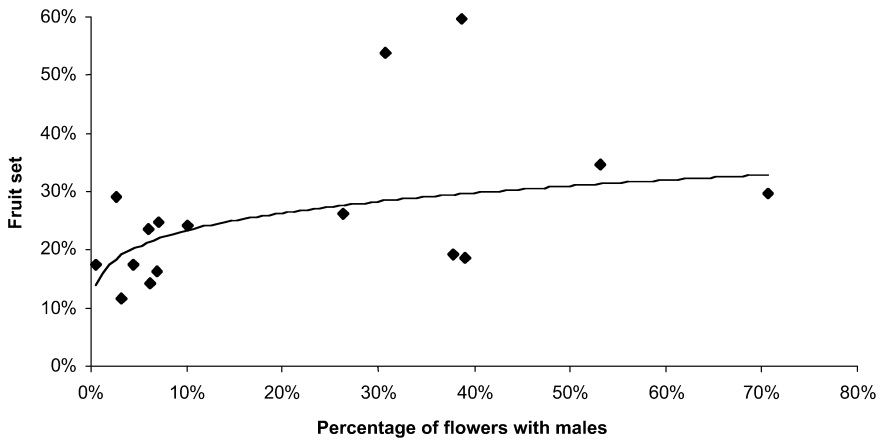

Fig. 2 Fruit set percentage as a function of the percentage of flowers hosting male bees in *Oncocyclus* iris populations. The line represents the best fit non-linear regression model ($y = 0.4008\,x^{0.209}$; $r^2 = 0.259$; $F_{14} = 41.17$; $p < 0.001$). Data points represent the 16 populations presented in Table **2**.

is probably an underestimation because the bee could have already checked several flowers prior to our observation.

The flowering season of *Oncocyclus* irises in Israel is at the end of winter, when cloudy or rainy days are still abundant (Jaffe, 1988). On such days, male bees were observed to enter flowers whenever the sun was hidden behind a cloud.

Male bees sheltering in the pollination tunnel in all the iris populations were often observed to be covered with *Iris* pollen, but the origin of the pollen is not known. Seven out of 18 (38.8%) male bees collected from the black flower models at Goral carried *I. atrofusca* pollen grains on their dorsal side, indicating their potential to affect interfloral pollen transfer.

One third (n = 36) of the *I. atrofusca* flowers that were marked as hosting male bees at Goral produced seeds, while only 9.8% (n = 61) of flowers that did not host bees on the observation nights set fruits; this difference is significant ($\chi^2 = 23.08$, df= 2, $p < 0.0001$). The percentage of fruit set and the percentage of bee-hosting flowers in the populations are presented in Table **2**. The relationships between the percentage of flowers that hosted male bees and fruit set are not linear. Thus, the best-fit non-linear regression model, found by iterations and bootstrapping using SPSS (V. 10.0.5), was $y = 0.4008\,x^{0.209}$ ($r^2 = 0.259$, $F_{14} = 41.17$, $p < 0.001$; Fig. **2**).

Other species occurring in Israel and Jordan *(Iris petrana, I. nigricans, I. lortetii, I. bostrensis)* were scored for night-sheltering male bees. However, these species were not surveyed for fruit set observations. The range of flowers hosting males and the number of male bees in these populations are within the range found here, and these results are utilized elsewhere (Y. Sapir and L. Hadany, in prep.).

The number of fruits per *I. atropurpurea* clone (genet) at Nes Ziona and *I. haynei* on Mt. Gilboa were positively and significantly correlated with clone size, i.e., the number of flowers in a clone (Fig. **3**). Significant linear regression lines were found for both *I. atropurpurea* and *I. haynei* populations ($r^2 = 0.21$; $F_{31} = 8.274$; $p = 0.007$ and $r^2 = 0.50$; $F_{29} = 28.017$; $p < 0.001$, respectively). Two *I. atropurpurea* clones at Nes Ziona had exceptional numbers of flowers. Field observations indicate that these two clones also had longer flowering periods than other clones. One of these two clones was the first (about January 20th) as well as the last (about March 20th) flowering clone in most of the research seasons. The two clones, each with over 100 flowers, were treated as outliers and excluded in an additional analysis, revealing a stronger regression between fruit set and clone size ($r^2 = 0.406$; $F_{29} = 19.805$; $p < 0.001$). However, fruit set per clone (calculated as the percentage of capsules within a clone relative to the number of flowers in the clone) was negatively related to the number of flowers in the clone. It was significant, though non-linear, for *I. haynei* on Mt. Gilboa ($y = 0.982\,x^{-0.3451}$; $r^2 = 0.227$; $F_{28} = 8.22$; $p = 0.008$), but not significant for *I. atropurpurea* at Nes Ziona ($r^2 = 0.005$; $F_{31} = 0.147$; $p = 0.704$).

Discussion

This study suggests that night-sheltering male bees are the main, if not the sole, pollinators of *Oncocyclus* irises, which are homogamous and totally self-incompatible and thus depend on their pollinators for sexual reproduction. The supporting pieces of evidence are: (1) lack of any other significant visitors to flowers of *Oncocyclus* irises, (2) *Iris* pollen grains were found on the thorax of male bees in flower models that served as alternative night shelters, (3) evident self-incompatibility that prevents self-fertilization, and (4) a significant positive correlation between fruit set and the number of night-sheltering bees.

Our results reveal that *Oncocyclus* flowers are homogamous, i.e., the male and female organs function simultaneously. Pollen grains are viable from dehiscence of the stamen throughout the floral life span, which can last 5 or more days, and the stigma is also receptive from bud opening until flower wilt. Moreover, pollen was observed on the hairs at the base of the pollination tunnel due to gravity or to bees' movements while sheltering. The structure of the pollination tunnel enables spontaneous deposition of self pollen on the stigma when the wilting style curls down and the stigmatic papillae touch these hairs (Kron et al., 1993). However, even though self-pollination might occur due to pollinator activity or spontaneously, autogamy is reduced by the location of the stigmatic receptive tissue and totally avoided by the flowers total self-incompatibility.

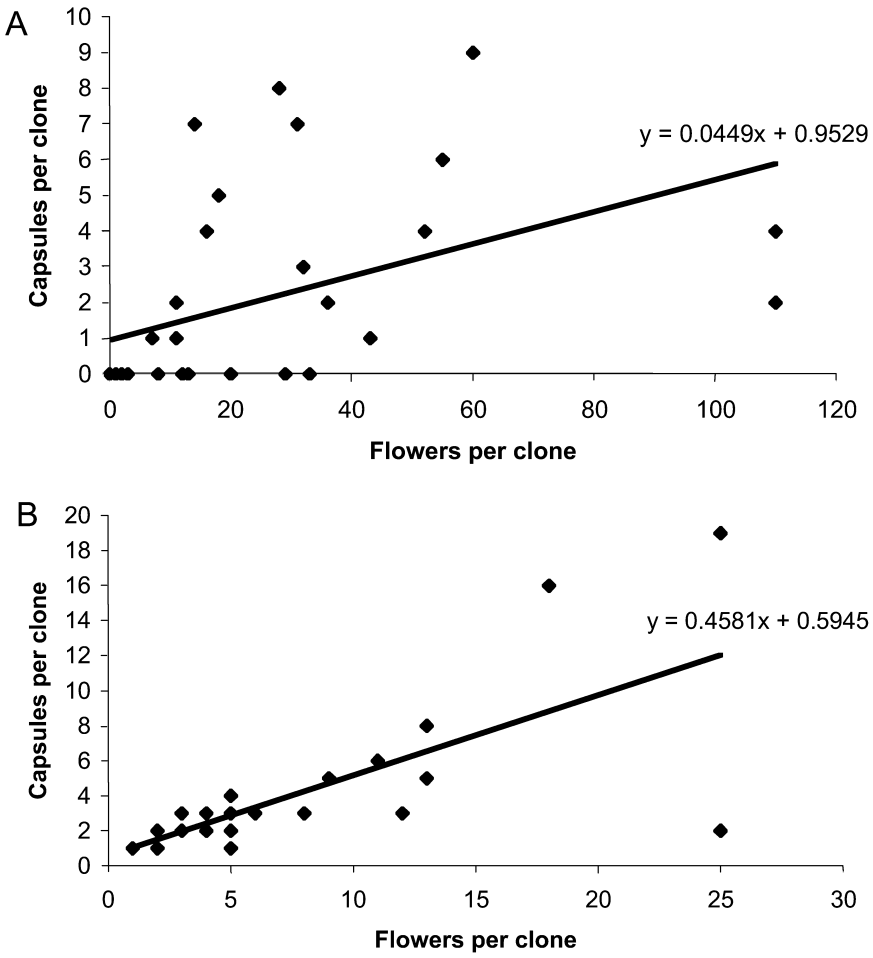

Fig. 3 The percentage of fruit set as a function of the number of flowers in the same clone for *Iris atropurpurea* in Nes Ziona (**A**) and *I. haynei* on Mt. Gilboa (**B**).

Self-incompatibility reduces the risk of inbreeding depression, expected in cases of autogamy (pollen transfer within a flower) and geitonogamy (pollen transfer between flowers but within a clone; Handel, 1985; Charlesworth and Charlesworth, 1987). This is especially so in *Oncocyclus* irises where autogamy can occur between the three pollination units of a single flower and geitonogamy between flowers of a genet (Faegri and van der Pijl, 1979; Kron et al., 1993). Thus, the phalanx clonal growth of *Oncocyclus* irises with high flower density is expected to result in a high rate of self-incompatibility (Morgan et al., 1997; Zhang, 2000). Indeed, we have found complete self-incompatibility in the *Oncocyclus* species examined, an extreme case relative to the mean selfing rate of 0.41, typical of herbaceous perennials (Barrett et al., 1996).

The number of fruits per clone was linearly correlated with the number of flowers per clone (up to 60 flowers), implying that larger clones with more flowers are also more attractive to pollinators (Klinkhamer et al., 1989; Conner and Rush, 1996). However, the combination of a high rate of geitonogamy with complete self-incompatibility may explain the relatively low fruit set in large clones with more than 100 flowers, as well as the negative relationships between percentage of fruit set and number of flowers per clone.

The positive relationships found across species between fruit set and the percentage of flowers that hosted male bees demonstrate the important role of night-sheltering male bees as pollinators of *Oncocyclus* irises, mainly in the absence of any other floral visitors and the observed complete self-incompatibility. The significantly higher fruit set in flowers that hosted male bees overnight than in other flowers, and the twofold higher fruit set revealed in artificially cross-pollinated flowers over openly pollinated flowers (Table **1**) suggest that fruit set might be limited by the number or the activity of night-sheltering solitary male bees. It is, however, important to note that flowers that hosted no male bees on any of the observation nights could still have been pollinated on other nights by night-sheltering male bees, because floral life span is 5 days or more.

In contrast to our results, earlier crossing experiments in other *Iris* species (Planisek, 1983; Kron et al., 1993; Zink and Wheelwright, 1997; Wilson, 2001) resulted in selfing rates ranging from 21.4% (Kron et al., 1993) to 71% (Planisek, 1983) for spontaneous self-pollination and 74.1% for artificial self-pollination (Kron et al., 1993) flowers. The *Oncocyclus* species examined in this study are the only irises demonstrating complete self-incompatibility, implying that they experience a high degree of selection for outcrossing, hence maintaining their apparent high degree of genetic diversity (Arafeh et al., 2002).

The average percentage fruit set of openly pollinated flowers in 16 *Oncocyclus* populations was 26.3%, and rarely exceeded 35% (Table **2**). This is about half of the value (57.7%) typical for self-incompatible perennial herbaceous species (Sutherland et al., 1999). On the other hand, self-incompatible species with hermaphrodite flowers have similarly low fruit set (20.6%) relative to that of monoecious and dioecious species (Sutherland et al., 1999). This may indicate that, in addition to pollinator activity, fruit set in the self-incompatible *Oncocyclus* irises is affected by their mating system (i.e., self-incompatibility and prevention of auto/geitonogamy) more than by life form.

The results suggest that pollen is transferred among flowers by night-sheltering solitary male bees, mainly during their night shelter probing behaviour, when the male bees visit a sequence of flowers in search of a suitable night shelter. The pollen grains stay viable for several days and may be carried on the dorsal side of the male bees. Thus, pollen excreted from flowers in the morning and deposited on the stigma of other flowers on the following evening when the male bees enter them as a night shelter may also lead to compatible pollination and consequent fertilization. The reason for the probing behaviour is discussed elsewhere (Sapir, 2004).

Solitary male bees have also been reported to overnight within other flower species (Horovitz, 1976; Dafni et al., 1981; Danforth and Neff, 1992; Neff and Danforth, 1992; Gaglianone, 2000). We have also found several other flower species hosting night-sheltering male bees, such as: *Echium plantagineum* L. (Boraginaceae), *Acanthus syriacus* Boiss. (Acanthaceae), the red, bowl-shaped flowers of *Tulipa agenensis* DC. (Liliaceae), *Anemone coronaria* L. (Ranunculaceae), and *Ranunculus asiaticus* L. (Ranunculaceae). Most of these flowers are dark-coloured and have tubular or bowl-shaped flowers that close at night, creating a closed dark inner space. However, these flowers also have some other effective diurnal pollinators, thus we hypothesize that the role of the night-sheltering male bees in their pollination is only secondary or even negligible, playing no role in the natural selection of floral traits. In contrast, night-sheltering solitary male bees that are the main (or sole) pollinators of *Oncocyclus* irises might, by preference, have a major role in selection of floral traits, creating suitable night shelters (Sapir, 2004).

To summarize the presented evidence, we conclude that fertilization of *Oncocyclus* irises is highly dependent on night-sheltering male bees, due to complete self-incompatibility and the absence of visitors during the day. This makes the night-sheltering male bees the main or only visitors to the flowers and consequently their obligatory pollinators. This unique pollination system demands deeper exploration of the factors that attract night-sheltering male bees to the pollination tunnels and on the role of the male bees as agents of selection on floral traits of *Oncocyclus* irises.

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