Title:

Does floral herbivory reduce pollination-mediated fitness in shelter rewarding Royal Irises?

Authors:

Mahua Ghara (1*), Christina Ewerhardy (2),Gil Yardeni (1, 3), Mor Matzliach (1), Yuval Sapir (1)

(1) The Botanical Garden, School of Plant Science and Food Security, Tel Aviv University, Tel Aviv 69978, Israel

(2) Albrecht von Haller Institute of Plant Sciences, University of Göttingen, DE-37073 Göttingen, Germany

(3) Current address: Department of Botany and Biodiversity Research, University of Vienna, A-1030 Vienna, Austria

* Author for correspondence; mail: mahuaghara@gmail.com

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Abstract:

Florivory, the damage to flowers by herbivores, can make flowers less attractive to pollinators. Even when pollinated, flower consumed by florivores may fail to produce fruit or will produce lower seed set. Despite the widespread evidence of florivory across ecosystems and plant taxa, only a few studies tested experimentally the interaction of florivory and pollination by manipulative study. We studied the effect of two levels of florivory on both pollinator visitation and reproductive success over two flowering seasons in three *Iris* species. We hypothesized that florivory will reduce pollen deposition due to reduced attractiveness to pollinators, and that fruiting probability and seed set will depend on the extent of florivory. We performed artificial florivory treatments, representing high, low, and no florivory (control) in two experiments. In the first experiment, each of the three floral units of the same flower was subject to either low, high or no artificial florivory, after which we counted the number of pollen grains present on the stigma. In the second experiment, three flowers of the same plant were treated and were further recorded for fruit and seed production. Surprisingly, and against our hypotheses, in all three species no significant effect was found among and between florivory treatments and control, neither in pollen grain deposited nor in fruit and seed set. The results undermine the assumption that flower herbivory is necessarily antagonistic interaction and suggests that florivores are not strong selection agents on floral reproductive biology in the *Oncocyclus* irises.

Key words: herbivory, *Iris* section *Oncocyclus*, floral herbivory, florivory, mutualist-antagonist interaction, natural selection, pollen limitation.

Introduction

Flowers of animal-pollinated plants are the major mean of plants to advertise and attract pollinators. Floral traits serve as signals to the pollinators, usually fit to the best-efficient pollinator (Fenster et al., 2004). Flowers advertise through visual signals, fragrance cues, and even by acoustic signature, in order to stand out of the canopy or other flowering species (Schiestl and Johnson, 2013). Consequently, flower traits that increase attraction are selected by pollinators (Harder and Johnson, 2009). For example, floral size, contributing to the visibility of the flower, is positively selected by pollinators (Sletvold et al., 2010, Sletvold et al., 2016, Campbell et al., 1991, Conner and Rush, 1996, Harder and Johnson, 2009, Lavi and Sapir, 2015). However, flowers are costly organs that require investment in resources for production and maintenance. Other selection agents also act on floral traits, either in concert with, or in contrast to the selection exerted by pollinators (Strauss and Whittall, 2006). For example, floral size is under contrasting selection regimes, positive by pollinators and negative by drought and water loss (Galen, 2000, Carroll et al., 2001). Color polymorphism is also thought to be maintained by the combined effect of mutualists (pollinators) and antagonists (Carlson and Holsinger, 2010), or by opposing selection directions by herbivores and pathogens (Frey, 2004). Although many studies have examined floral adaptations to pollinators, the role of non-pollinator selection agents in shaping floral evolution and plant reproductive success is still underappreciated.

Florivory, the damage of flowers by herbivores, is widespread across plant taxa and ecosystems (Gonzáles et al., 2016, Burgess, 1991). Florivores comprises of various taxonomic orders of animals that consume the entire flower (or the buds) or floral parts (bracts, sepals, petals, nectaries, stamens, pistils or pollen). Florivory may affect plant fitness directly or indirectly, by reducing fruiting or seed-set, and can consequently affect population dynamics (Louda and Potvin, 1995). Florivory reduces fitness directly when reproductive parts are consumed, whereas indirect effect can be through effects on pollinator behavior and hence reduce pollination services. An increasing number of studies suggest that florivory can decrease pollinator visitation rate and pollination success (reviewed in Gonzáles et al., 2016, but see Zhu et al., 2017). Methods for studying the effect of florivory on pollination primarily focus on the effect of florivore presence or the extant of florivory on pollinator visits, using natural encounters and manipulative studies. For example, Kirk et al.(1995) placed black spots on flowers to simulate presence of florivore beetle and found that bees were likely to avoid flowers

with mimicry of florivore presence. Although artificial florivory performed in the field can provide an estimate to the direct effect of florivory on pollination, few studies implement this method. Moreover, while behavior of pollinators and/or measure of maternal fitness (measured as the number of visits or seed set, respectively) are often used to estimate the effect of florivory on pollination (e.g., Cardel and Koptur, 2010), direct evidence for pollination success, which is pollen deposition on the stigma, has rarely been incorporated in florivory studies. Although pollinator limitation and pollen limitation supply the ground for pollinator-mediated selection (Bartkowska and Johnston, 2015, Lavi and Sapir, 2015, Sletvold et al., 2017), it is unclear whether it affects florivore-mediated selection. Therefore, controlled manipulative experiments that measure pollen deposition and fitness, as well as incorporating pollen limitation, are needed to estimate directly the interaction of florivory and pollination success.

In a survey of the literature, we found only a small number of studies that used a controlled experimental florivory that mimic florivory by artificially manipulating or removing parts of the petals and tested for effect on pollination. Of these, seven studies found negative effect on pollination success , one study found mixed effects that depended on flower morph (Carper et al., 2016), and one study found no effect (Tsuji et al., 2016). Thus, it remains unclear whether florivory affects plant fitness indirectly through inducing negative effect on pollinators. As an example, McCall (2008) found that both natural and artificial petal damage indeed reduced fitness, and while it deterred pollinator activity, the effect was a result of petal physical damage rather than reduced pollinators activity, as pollen addition experiment did not recover fruit-set. Hence, for fully understanding the multiple effects of florivory on fitness and the interplay between direct and indirect effects it requires further experiments in diverse plant species.

We studied the effect of florivory on pollination success in three species of the Royal Irises (*Iris* section *Oncocyclus*) in their natural habitats in Israel (Figure 1 A–C). The Royal Irises comprise of about 30 species across the Middle East, most are narrow endemic (Rix, 1997, Mathew, 1989). This group was well studied as a system for pollination by shelter reward to male *Eucera* bees (Figure 1 D; Sapir et al., 2005, Sapir et al., 2006, Watts et al., 2013, Monty et al., 2006, Vereecken et al., 2013, Lavi and Sapir, 2015). Floral organs in irises are organized in three units consisting of a tunnel, in which the male *Eucera* bees seek shelter for the night or at low temperatures. A stigma and anther are located within each tunnel and pollen is deposited on stigmas by bees that move among flowers as they seek shelter (Sapir et al., 2005).

The Royal Irises are eaten by various florivores, from snails and true bugs to grasshoppers, birds and goats, and the intensity of damage ranges from a few superficial scratches or poke marks, up to >90% of damage to floral tissue (M. Ghara and Y. Sapir, per. observations; Figure 1 E–G). From our observations, it appears that all flower parts are potentially eaten, either bracts, petals, the petaloid style, anthers or ovaries. While damage to the reproductive organs may obviously reduce fitness directly, we asked whether florivory affects fitness indirectly through reduced attraction to pollinators. In order to estimate the effect of florivory on pollinators' visitation and pollination success we manipulated the flowers to simulate two levels of florivory, i.e., high (more than 50% damage) and low (10-30% damage), and compared to control flowers without damage. We asked the following questions: (1) Does florivory affect pollinator visitation? (2) Does florivory affect fruiting probability and seed set? We used pollen deposition on stigma as a surrogate for estimating pollinator visitation and used fruit- and seed-set to estimate overall effect of florivory on (maternal) fitness. Accounting for two measures of pollination success simultaneously is likely to reveal an indication of the possible effect of florivory on pollination-mediated fitness in the royal irises.

Methods

Study species and sites

We conducted the florivory experiment in two consecutive years on three species of the Royal Irises, namely *Iris atropurpurea* Baker, *I. petrana* Dinsm., and *I. lortetii* Barbey ex Boiss. Experiments were conducted in the natural environment at the largest population for each of the species. *Iris atropurpurea* was studied in Netanya Iris Reserve (32.28°N, 34.84°E, alt. 35 m), located on stable coastal sand dunes in Mediterranean climate and consisting of mostly low shrub vegetation. Population size is estimated >1,000 plants (Yardeni et al., 2016). Flowering season, and hence experiment time, is earlier compared to other species of the Royal Irises, starting as early as mid-January, and peaks in February. Experiments in *I. atropurpurea* in Netanya were conducted between February 12th and March 2nd, 2016, and between February 19th and March 7th, 2017. *Iris petrana* was studied in Yeruham Iris Reserve (31.02°N, 34.97°E, alt. 560 m), a large population (estimated >10,000 plants) growing on sandy loess hills over Neogene sandstone in arid climate. Vegetation is sparse desert shrubs, mostly *Retama raetam* (Forssk.) Webb and *Anabasis articulata* (Forssk.) Moq. Flowering season is in March, and experiments in *I. petrana* were conducted between March 5th and 14th, 2016, and between March 19th and April

2nd, 2017. The shift in dates in 2017 resulted due to about two weeks delay in flowering period in 2017. *Iris lortetii* was studied in two sub-populations near Malkiya in the upper Galilee (central coordinates: 33.09°N, 35.52°E, alt. 620 m). Populations of *I. lortetii* are sparse and relatively small, thus, two sub-populations at a distance of 3 km of each other were pooled to achieve a sufficient sample size. Plants are growing on Eocene limestone in mesic Mediterranean climate and vegetation is open woodland dominated by *Quercus calliprinos* Webb and *Pistacia atlantica* Desf. trees, accompanied by dense herbaceous vegetation. *Iris lortetii* is the late blooming species among the Israeli species of the Royal Irises; experiments were conducted between March 29th and April 7th, 2016. The experiments were not conducted on *Iris lortetii* in 2017 because of high herbivory in 2016 and therefore a potential decrease in sample size.

Flowers of the Royal Irises grow singly on a stem, but the plant (genet) comprises of one to hundreds of stems (ramets) in a well-defined patch. Plants for the experiments were randomly selected in a dense part of the population in Netanya, or along transects in Yeruham. In Malkiya the plants are sparse and plants in all genets located were used. The three experiments described below were conducted simultaneously in time with only a single experiment conducted in each genet to avoid the joint effect of several treatments.

Pollinator and pollen limitation

To assess the extent of pollinator and pollen limitations we took the approach of Lavi and Sapir (2015). In Netanya (*I. atropurpurea*) we supplemented 54 flowers in 2016 and 30 flowers in 2017 with additional pollen. In Yeruham (*I. petrana*) we supplemented additional pollen for 22 flowers in 2016 and 45 in 2017. Pollinator and pollen limitation were not quantified in *I. lortetii*.

Flowers were randomly chosen and were pollinated using a mixture of pollen collected from >20 flowers located at least 10 meters away from the supplemented flowers. The flowers were left open for natural pollination. At the end of the season, the fruits of all flowers were collected and seeds were counted after full ripening. Fraction of fruits produced and number of seeds were compared with flowers of the control treatment in the experiment testing for florivory effect on maternal fitness (see below).

Florivory manipulations – effect on pollination

Flowers of the Royal Irises comprise of three identical units, each bearing one upright and one downright petals (standard and fall, respectively), and one petaloid style, curved above the fall to

create a tunnel where the reproductive organs reside (Figure 1). The three stigmas, located at the top of the entrance of each of the pollination tunnels, are merged in the base of the flower to one style. A previous study showed that pollinating one style is sufficient to produce seeds in all three carpels in the ovary (Watts et al., 2013). To study the effect of florivory on pollen deposition, a single flower in a genet was selected and each flower unit (i.e., pollination tunnel) was given one of three treatments as follows. High damage – both lower petal and its opposite upper petal were manually damaged up to 50% or more of the petal's area, using a puncher of 6-8 mm in diameter. Low – 10-30% of the petals' area were removed using puncher. Control – petals were not damaged and left un-manipulated (Figure 2). To control for the possible effect of the contact between the metal hole-puncher and the flower the puncher was rubbed on the petal surface in the control treatments. In addition, because damaging the petals required holding a layer of tissue paper against the puncher, we also gently rubbed tissue paper under the surface of the petal in the control treatment.

Flowers were treated in the first day after anthesis and left open for two consecutive evenings following treatments to enable pollinators to visit naturally. In the morning of the third day, the stigmas of the three pollination units were collected in separate vials containing 1 ml of 70% ethanol. To prevent naturally occurring florivores from damaging the flowers, the stem of the treated flower was coated with a layer of double sided sticky paper tape, as well as a layer of Petroleum jelly (Vaseline). Occasionally we found insects trapped on the Vaseline layer, and in some rare cases, we found florivores that passed this barrier. Flowers found to be damaged naturally (mostly by flying insects, snails, or mammals) were discarded in order to account for the effect of controlled artificial florivory only.

Collected stigmas were brought to the laboratory for pollen counting and kept in room temperature. Pollen grains were stained using a drop of basic fucshin (Calberla's stain). Stigma was then dissected in a drop 70% aqueous glycerol (Dafni et al., 2005), mounted on microscope glass slide and the pollen present on the stigma was counted under dissecting microscope (WILD Heerbrugg Switzerland M5-72558).

Florivory manipulations – effect on maternal fitness

To study the effect of florivory on seed set, three buds of the same genet, roughly of the same developmental stage (i.e., before emerging from bracts) were selected and bagged to avoid bud florivory. Upon anthesis, each of the three flowers was randomly assigned to one of the florivory

treatments described above, i.e., high, low, or control. To control for the effect of visual attraction mediated by flower size (Lavi and Sapir, 2015) we measured flower length as a surrogate for display size of each flower before manipulation. Flower length was measured from the bottom of the lower petal to the top end of the upper one. The flowers were left open to enable naturally occurring pollination. At the end of the season, approximately three weeks after the end of the flowering in each site, the fruits were collected and brought to the laboratory. In 2016, fruits of *I. atropurpurea, I. petrana* and *I. lortetii* were collected on March 21st, April 2nd and April 27th, respectively. In 2017, fruits were collected on March 12th and 22nd for *I. atropurpurea* and on April 13th and 27th for *I. petrana*. Fruits were kept in paper bags in room temperature until seed maturation. Fitness was recorded as presence or absence of a fruit (binomial data), and as the number of viable seeds (count data).

Data analyses

The data was analyzed in R (R Development Core Team, 2014) using R-studio interface. To test for differences between control and supplemental pollination treatments, we used generalized linear models (GLMs) with year and treatment effects nested within site. For fruit-set, we used GLM with binomial distribution errors, and for number of seeds we used quasi-Poisson distribution errors due to over-dispersion of the data. Pollen and pollinator limitation indices were calculated as $PLI=1–O/S$, where O is fruit-set or seed-set in open flowers, and S is fruit-set or seed-set in supplementary pollination treatment. Number of pollen grains deposited on stigmas was analyzed using GLM with year and treatment effects nested within species (note that in *I. lortetii* experiment was done only in 2016). As in number of seeds, we used quasi-Poisson distribution errors due to over-dispersion of the data. Because the three treated floral units were on the same flower, for analysis of pollen deposition these were considered as non-independent. Thus, we initially tested models that included also the effect of flower ("block" effect). We found this effect to be non-significant (not shown), hence it was removed from the analysis. For analyses using fruit or seeds as explained variables, we incorporated flower size as a covariate. For seeds as a response variable, we used only the subset of flowers that set a fruit. In order to account for non-normal distribution, we used GLM with binomial distribution errors for fruits and GLM with quasi-Poisson distribution errors for seeds.

Results

Pollinator and pollen limitation

We found a significant effect of site and a significant effect of treatment, nested within year and within site, on both fruit-set and seed-set (Table 1). The effect of year was not significant. In Netanya, flowers supplemented with pollen produced significantly higher fruit-set than control flowers, open to natural pollinators only (48.1% versus 5.5% in 2016 and 60% versus 15% in 2017; P<0.001 in both years; Figure 3 A&B). Seed-set in Netanya in the two years was also significantly higher in flowers supplemented with excess pollen, compared to open flowers pollinated only naturally (mean number of seeds 11.5 versus 1.1 in 2016 and 10.3 versus 1.7 in 2017, P<0.005 in both years; Figure 3 C&D). Both pollinator limitation and pollen limitation were extremely high in Netanya in both years (PLI=0.88 for fruit-set and PLI=0.91 for seed-set in 2016; PLI=0.75 for fruit-set and PLI=0.84 for seed-set in 2017).

In Yeruham, fruit-set was similar between supplementary and open flowers in 2016 (40.9% versus 41.7%, respectively, P=0.96; Figure 3A). In 2017, fruit-set was significantly higher in flowers supplemented with pollen, compared to not-treated control flowers (73.3% versus 34.8%, respectively, P<0.001; Figure 3 B). Likewise, seed-set was not significantly different between supplementary and open flowers in 2016 (mean number of seeds 7.1 versus 9.4, respectively, P=0.675) but differ between treatments in 2017 (mean number of seeds 15.6 versus 5.8, respectively, P=0.003; Figure 3 C&D). Pollinator limitation and pollen limitation were below zero in Yeruham in 2016 (PLI= –0.02 for fruit-set and PLI= –0.32 for seed-set), but relatively high in 2017 (PLI= 0.53 for fruit-set and PLI= 0.64 for seed-set).

Pollen deposition on stigma

We found significant effect of year and species on number of pollen grains deposited on stigmas of flowers ($F_{2,691}$=4.38, P=0.013 for species and $F_{2,691}$=24.96, P<0.001 for year effect, nested within species). Nonetheless, treatment effect (nested within year and within species) was not significant ($F_{2,691}$=0.63, P=0.706). In *Iris atropurpurea*, 49 (26.3%) stigmas did not receive pollen at all in 2016 and 8 (5.3%) in 2017. Flowering units with high artificial florivory revealed lower number of pollen grains on stigma in 2016 and lower in 2017(Figure 4), but these differences were small and far from significance ($F_{2,183}$=0.67, P=0.515 in 2016 and $F_{2,135}$=0.16, P=0.855 in 2017). In *I. petrana*, all stigmas received pollen grains in 2016, but in 2017, 51 (34%) did not receive any pollen grain. Number of pollen grains deposited on the stigmas was an order

of magnitude larger than in *I. atropurpurea* in 2016 (Figure 4 A), but similar in 2017 (Figure 4 B). Nonetheless, as in *I. atropurpurea*, no significant treatment effect was found in both years ($F_{2,195}$=0.53, P=0.588 in 2016 and $F_{2,144}$=1.89, P=0.155 in 2017). In *Iris lortetii*, as in *I. petrana* in 2016, all stigmas received pollen and stigmas in units treated by high artificial florivory received pollen grains in a similar level as the control, untreated units, both higher than medium artificial florivory treatment (Figure 4 A). However, this difference was not significant as well ($F_{2,30}$=0.322, P=0.727).

Fruit and seed sets

In *Iris atropurpurea*, 80 flowers were included in the final analyses in 2016, because 16 of the treated flowers were either not found or damaged. Of these, only 13 flowers set fruits, indicating extreme pollinator limitation and lack of pollinator visitations. Treatment did not affect either fruit-set or number of seeds ($F_{2,75}$=0.69, P=0.501, and $F_{2,8}$=0.26, P=0.774, respectively; Figure 5 A & C). In both analyses flower size did not affect significantly (P=0.266 and P=0.554 for fruits and seeds, respectively). In 2017, 124 flowers were included in the experiment, of which four were damaged. Of the remaining 120 flowers, 23flowers set fruits. As in 2016, no effect of the treatment was found, neither on fruit-set, nor on number of seeds ($F_{2,116}$=0.37, P=0.695, and $F_{2,19}$=0.95, P=0.404, respectively; Figure 5 B & D). Similar to 2016, flower size as covariate did not affect fruit-set or seed-set (P=0.188 and P=0.857, respectively).

In *I. petrana* population in Yeruham, 132 flowers were treated in 2016, but 29 flowers of all treatments were eaten by goats that entered the reserve illegally and ate wilting flowers and young fruits in the pre-dispersal stage. Of the remaining 103 treated flowers, 31 flowers set fruits. Treatment effect on fruit-set was not significant ($F_{2,98}$=1.89, P=0.156 ;Figure 5 A). Interestingly, flower length (before treatment) significantly affected fruit set ($F_{1,98}$=5.09, P=0.026), but with no significant interaction with treatment ($F_{2,96}$=0.26, P=0.770). Although control flowers produced almost twice fraction of fruits compared to florivory treatment (34% vs. 18%), this difference was not significant when controlled for flower size. Number of seeds was not affected by treatment ($F_{2,98}$=1.89, P=0.156, n=31; Figure 5C). Flower size did not affect seed-set ($F_{1,26}$=1.23, P=0.278). In 2017, only six flowers were eaten or not found, out of 188 flowers treated. As in 2016, no effect of the treatment was found, neither on fruit-set, nor on number of seeds ($F_{2,133}$=1.64, P=0.199, and $F_{2,29}$=0.72, P=0.495, respectively; Figure 5 B & D). As opposed to 2016, flower size as covariate did not affect fruit-set but did affect seed-set

(P=0.472 and P<0.001, respectively). No interaction was found between flower size and florivory treatment in their effect on seed-set (P=0.463).

In the two sites of *Iris lortetii*, 61 out of 62 flowers treated (12 in Avivim and 50 in Malkiya) were found at the end of the season and included in the analyses. No significant difference was found among treatments ($F_{2,57}$=0.21, P=0.811; Figure 4 A). Flower size affected fruit-set ($F_{1,57}$=4.42, P=0.40). Number of seeds was not significantly affected by treatment ($F_{2,12}$=0.37, P=0.698; Figure 4 C), and neither by flower size ($F_{1,12}$=0.92, P=0.356).

Discussion

Florivory, namely the damage herbivores cause to floral organs, can affect fitness either directly by consuming pollen or ovules or physiological costs, or indirectly, by reducing plant attraction signal for the pollinators (Burgess, 1991, McCall and Irwin, 2006). Here we tested for both direct and indirect effects of florivory on fitness by executing artificial florivory and measuring both fitness and pollination. Our results do not support the hypothesis that florivory affects pollination success in the Royal Irises either directly or indirectly. Instead, we show that artificial damage to reproductive tissues in three species of the royal irises did not significantly reduce either pollen deposition or fitness of manipulated flowers, compared to un-treated ones (Figure 4 and Figure 5).

While numerous studies were concerned with the effect of florivory on plant fitness, controlled, artificial florivory was rarely performed. Most studies examined flowers that were naturally attacked by florivores (e.g., Meindl et al., 2013, Ruane et al., 2014, Eliyahu et al., 2015) or used experimental florivore removal or prevention (e.g., Krupnick et al., 1999, Theis and Adler, 2012, Althoff et al., 2013). Only a few studies implemented methods similar to ours, using cutting flowers to simulate florivory. These studies revealed mixed results. For example, Sõber et al. (2010) experimentally showed a correlation between extent of florivory and pollinator visitations at both population and plant level. On the other hand, Tsuji et al. (2016) found no evidence for pollinators discrimination against experimentally damaged flowers. Interestingly, mixed results can be found within the same system: Carper et al.(2016) found differences between heterostylous morphs in pollinator responses to artificial damage, and found no effect on fitness. Our study adds to the puzzle by providing yet another piece of evidence that florivory itself does not deter pollinators, nor reduces fitness.

Negative effect of florivory on pollination may act in two avenues. One possible effect of florivory on pollinator behavior is the deterring of pollinators from eaten flowers. This is achieved by either avoidance flowers where florivore is visually detected (Kirk et al., 2005) or by a change in volatile compounds (Kessler et al., 2013). Another possible effect of florivory on pollination is mediated by the effect of the overall advertisement size of the flower and reducing visual signaling for the pollinators (Sánchez-Lafuente, 2007). This may reduce number of visits and lead to pollinator limitation or pollen limitation, which in turn reduces fruit-set and seed-set, respectively (Sapir et al., 2015). This study was conducted in natural populations of which at least one (Netanya) indeed experience strong pollinator limitation. It is likely that the effect of pollen limitation in this population obscures the effect of florivory (McCall, 2010). Thus, we propose that selection mediated by pollinator/pollen limitation is stronger than selection pressure exerted by florivory. A previous study that tested for pollen limitation in two *Iris* species showed, indeed, that pollinators limitation provides conditions for pollinator-mediated selection (Lavi and Sapir, 2015). However, in Yeruham we found no evidence for pollen limitation, still no effect of florivory was found, which contradicts this hypothesis. Our mixed results suggest that while pollinators may be a selection agent on flower traits (as in Lavi & Sapir, 2015), florivores do not act as selection agents because pollen limitation balances their effect (cf. Jogesh et al., 2016), but this connection cannot be generalized beyond our specific system.

Pollinators are thought to be the major selection agent on floral traits through their positive effect on fitness; this, however, was challenged by observations on contrasting effects of abiotic conditions or antagonistic biotic interactions (Herrera, 1996, reviewed in Strauss and Whittall, 2006). Strauss and Whittall (2006) proposed two scenarios of such mutualistic-antagonistic effect, in which overall selection acts as either directional or stabilizing on floral traits. Based on our results, we propose a third scenario, where florivory affects fitness at the same level as the mean effect of pollinators and regardless floral trait. In this case, pollinator-mediated selection will govern trait evolution, but the presence of florivory reduces or diminishes effect size (Figure 6). We speculate that florivory may indeed not affect the direction of selection but the intensity of it. Thus, we suggest that testing for the net selection mediated by pollinators should control experimentally for the effect of florivores. In a follow-up experiment, we intend to test for net pollinator-mediated selection on floral color in the Royal Irises. Floral pigment concentration is expected to deter florivores and attract pollinators (de Jager and Ellis, 2014, McCall et al., 2013),

and given the results of the current study we assume that florivory may not necessarily reduce fitness; instead, it is expected that a weak directional pollinator-mediated selection on floral color will be detected after controlling for florivory.

While few previous studies have presented only slight (or no) effect of florivory on pollination, it may be further hypothesized that the Royal Irises are unique and thus do not represent a general rule relevant to other species. The Royal Irises present a unique pollination syndrome in which pollination is performed by night sheltering male bees (Sapir et al., 2005, Watts et al., 2013). Previous studies suggest that flower display itself is not necessarily the major attractant for these pollinators (Lavi and Sapir, 2015). While our florivory-like manipulations were performed on petals, it is likely that these manipulations did not affect pollinator choice because the shelter itself (the pollination tunnel) was not damaged. Because we have observed florivory of stigma and anthers (M. Ghara pers. observations), we suspect that florivory may affect male and female fitness through consumption of reproductive organs, but we have yet to assess the cost of florivory on reproductive organs. Nonetheless, while natural florivory is widespread in all species and most populations of the Royal Irises in Israel, the estimated proportion of flowers of which pollination tunnels were eaten is rather small (M. Ghara, manuscript in preparation). Thus, our manipulation on petals accurately mimics natural florivory and our conclusions on the role of florivory in selection on flowers of the Royal Irises are valid.

Finally, our study provides an application to conservation. Of the three species studied, two (*Iris atropurpurea* and *I. lortetii*) are rare and endangered species (Sapir, 2016a, Sapir, 2016b). Understanding the relative contribution of biotic interactions to population dynamics may shed light on the factors affecting species survival in a way that may contribute for evidence-based management. The study presented here suggests that reduced mutualistic interactions, namely, pollination services, rather than antagonistic florivory, threatens the maintenance of positive population growth in the Royal Irises.

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Tables

Table 1 – Analysis of variance table for the effects of site (Netanya and Yeruham), year (2016 and 2017) and treatment (supplementary or open pollination) on fruit-set and seed-set.

Source	DF	SS	F	Significance
Fruit-set				
Site	1	3.05	15.68	P<0.001
Site/year	2	0.72	1.84	P=0.161
Site/year/treatment	2	10.6	27.13	P<0.001
Residuals	309	60.2		
Seed-set				
Site	1	1063	5.52	P=0.794
Site/year	2	172	0.45	P=0.641
Site/year/treatment	2	5303	13.77	P<0.001
Residuals	305	58724		

Figure legends

Figure 1 –(A) *Iris atropurpurea* in Netanya; (B) *Iris petrana* in Yeruham; (C) *Iris lortetii* in Malkiya; (D) Male *Eucera* bee, the specific pollinator of the royal irises, sheltering within a pollination tunnel of *Iris petrana*; (E-G) Natural florivory in flowers of *Iris atropurpurea* (E), *I. petrana* (F) and *I. lortetii* (G).

Figure 2 –Artificial florivory manipulations exemplified in *Iris atropurpurea*. (A) Within flower manipulation – each floral unit treated as either high florivory (>50% petal cut), low florivory (10-30% petal removed by hole puncher), or control (no treatment). These treatments were used for testing the effect on pollination. (B–D) Flowers used for testing the effect of florivory on fitness. (B) Flower treated as high florivory. (C) Flower treated as low florivory. (D) Control flower.

Figure 3 – Fruit-set and seed-set in pollen limitation experiment in *Iris atropurpurea* in Netanya, and *I. petrana* in Yeruham. Bars are fractions ± standard errors. (A) Fraction of flowers that produced fruits in 2016 (B) and in 2017. (C) Mean number of seeds in 2016 (D) and in 2017. Open – flowers open to natural pollination. Supp. – flowers received supplementary pollen artificially.

Figure 4–Mean number of pollen grains counted on stigma (± standard errors) as a function of florivory treatment in three species.(A) Pollen grains in 2016 experiment; (B) Pollen grains in 2017 experiment. Bars are fractions ± standard errors.

Figure 5–Fitness as a function of artificial florivory manipulations. (A) Fruit-set (fraction of flowers that set fruits) in three species in 2017 and in 2017 (B). (C) Seed-set (mean number of seeds in a fruit) in 2016 and in 2017 (D). Bars are means ± standard errors.

Figure 6–Hypothetical floral trait evolution as a function of selection by both mutualists (pollinators) and antagonists (florivores). (A) When pollinators and florivores exert selection in the same direction, the concert selection favors the same trait optimum. (B) When pollinators and florivores exert opposing selection on a trait, an intermediate trait optimum is favored. A and B

adapted from figure 7.1 in Strauss &Whittall (2006). (C) When florivores have no preference, or their effect is similar to mean fitness derived from pollinators' effect, trait will have optimum fitness selected by pollinators like in A.

Figure 1

A B C D

E F G

Figure 2

Figure 3

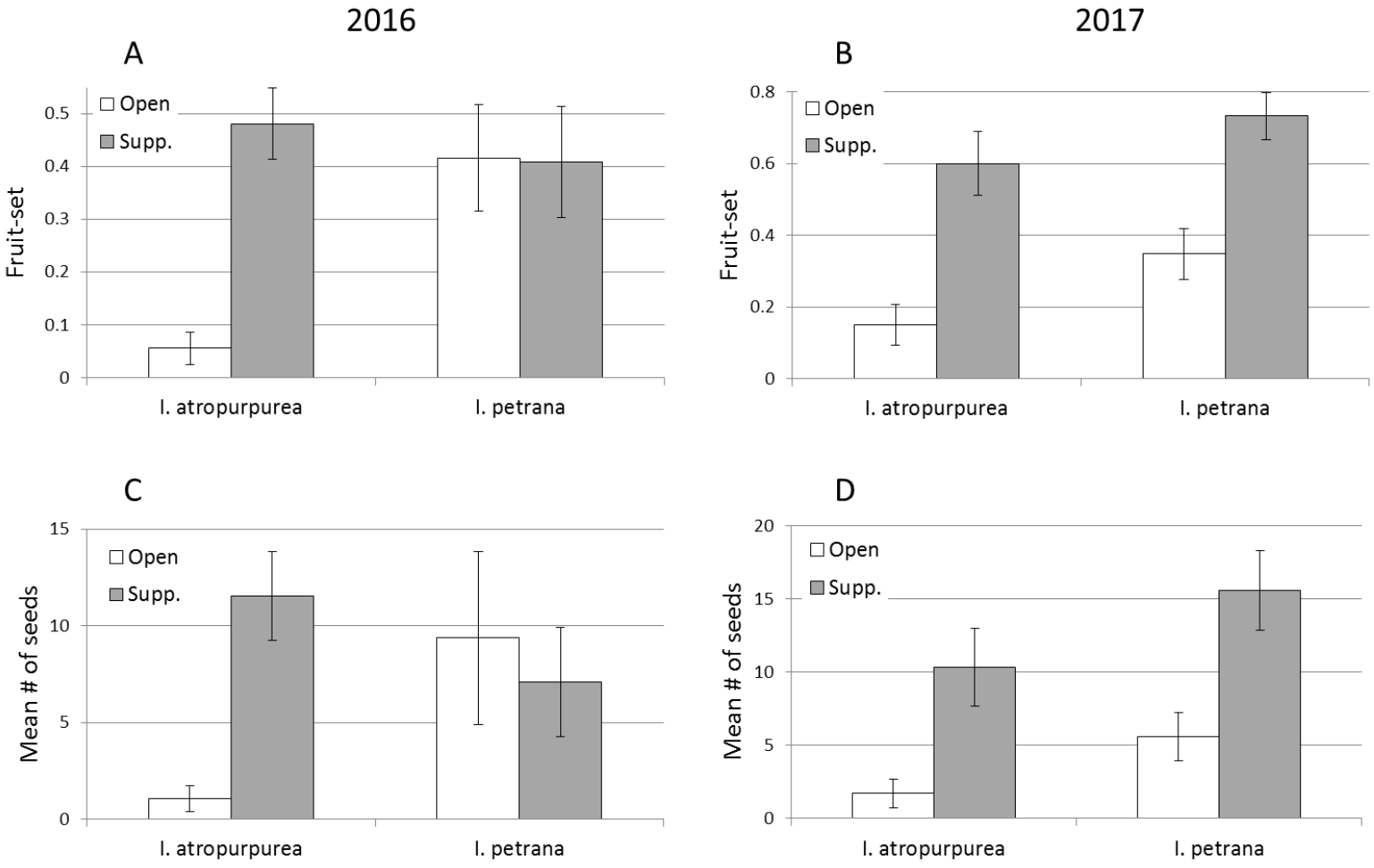

2016
2017
A
B
C
D
Open
Supp.
Fruit-set
Mean # of seeds
I. atropurpurea
I. petrana
0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
5
10
15
20

Figure 4

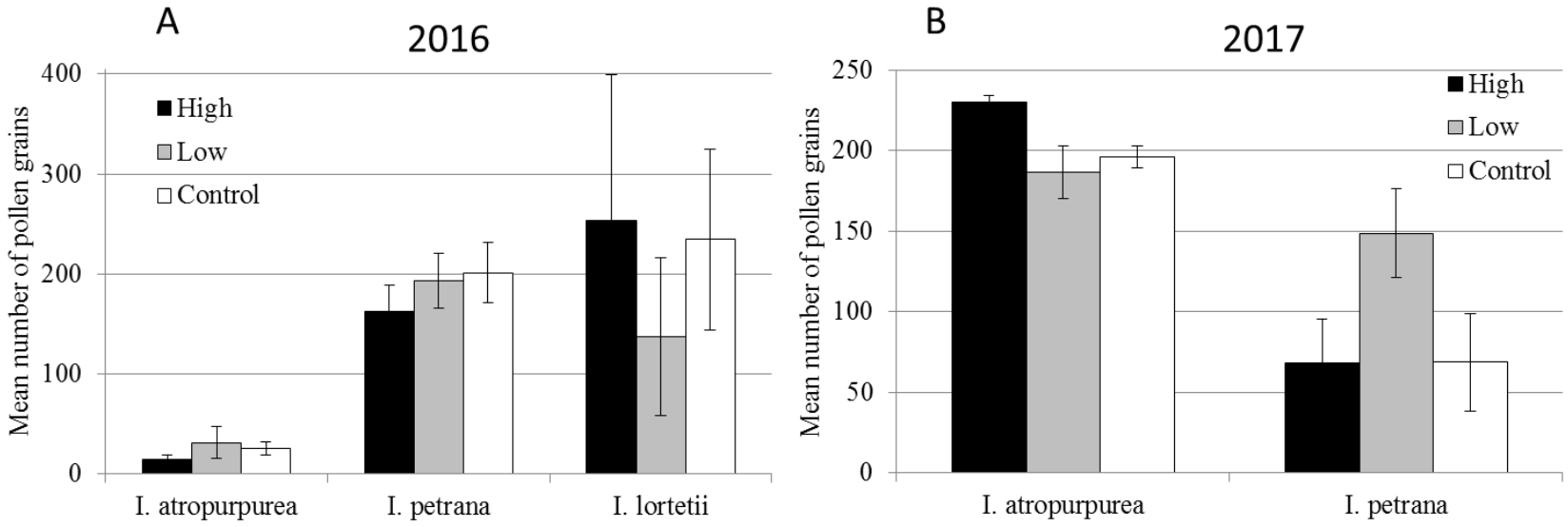

A
2016
Mean number of pollen grains
400
300
200
100
0
High
Low
Control
I. atropurpurea
I. petrana
I. lortetii
B
2017
Mean number of pollen grains
250
200
150
100
50
0
High
Low
Control
I. atropurpurea
I. petrana

Figure 5

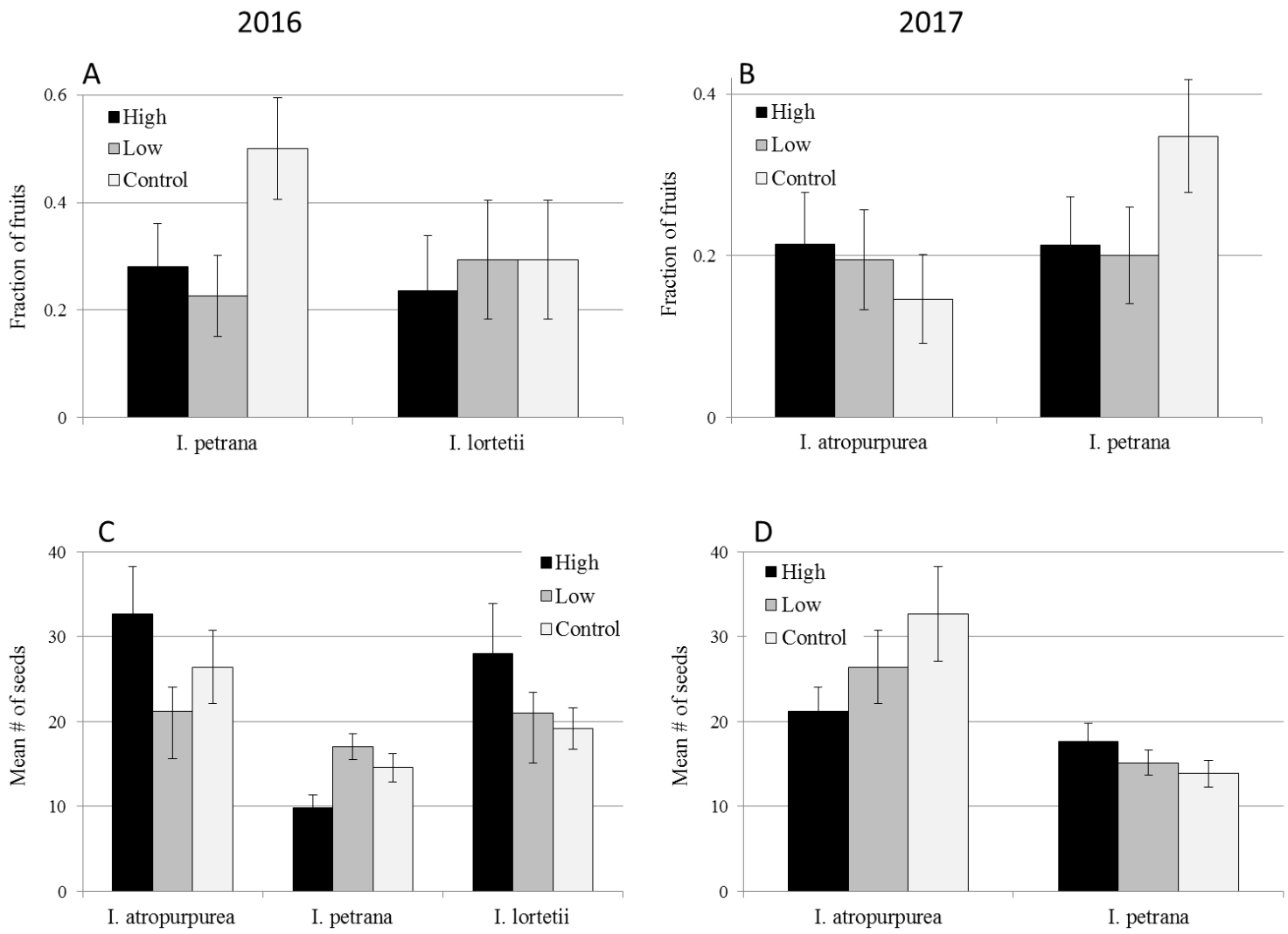
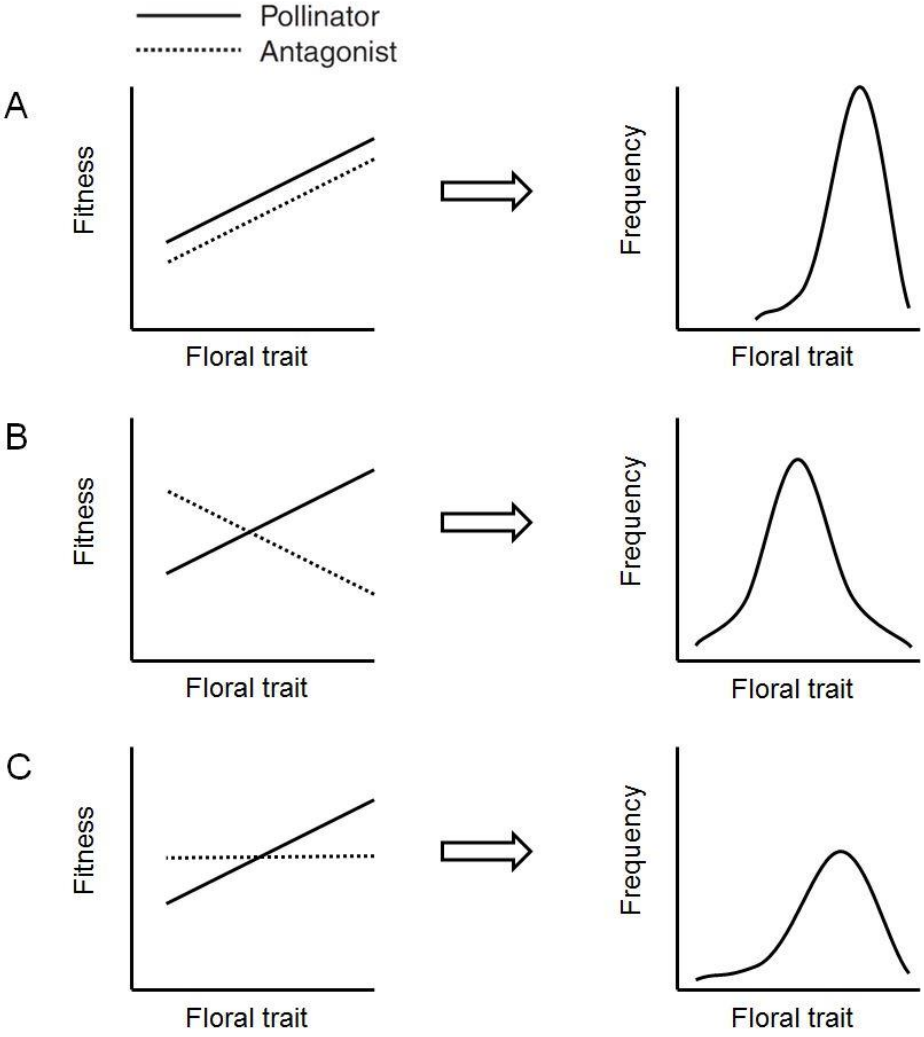

2016
2017
A
B
C
D
High
Low
Control
Fraction of fruits
Mean # of seeds
I. petrana
I. lortetii
I. atropurpurea
0
0.2
0.4
0.6
10
20
30
40

Figure 6


Pollinator
Antagonist
A
Fitness
Floral trait
Frequency
Floral trait
B
Fitness
Floral trait
Frequency
Floral trait
C
Fitness
Floral trait
Frequency
Floral trait