1   Title:

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

3   Authors:

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

5   (1)

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

7   Tel Aviv 69978, Israel

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

9   Göttingen, Germany

10   (3) Current address: Department of Botany and Biodiversity Research, University of Vienna,

11   A-1030 Vienna, Austria

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

13

14

15

16

17   Author Contributions: MG conceived and designed the experiments. YS supervised research.

18   MG, CE, GY and MM performed the experiments. MG and YS analyzed the data. YS wrote the

19   manuscript with contributions of all other authors.

20   **Abstract:**

21   Florivory, the damage to flowers by herbivores, can make flowers less attractive to pollinators.

22   Even when pollinated, flower consumed by florivores may fail to produce fruit or will produce

23   lower seed set. Despite the widespread evidence of florivory across ecosystems and plant taxa,

24   only a few studies tested experimentally the interaction of florivory and pollination by

25   manipulative study. We studied the effect of two levels of florivory on both pollinator visitation

26   and reproductive success over two flowering seasons in three *Iris* species. We hypothesized that

27   florivory will reduce pollen deposition due to reduced attractiveness to pollinators, and that

28   fruiting probability and seed set will depend on the extent of florivory. We performed artificial

29   florivory treatments, representing high, low, and no florivory (control) in two experiments. In the

30   first experiment, each of the three floral units of the same flower was subject to either low, high

31   or no artificial florivory, after which we counted the number of pollen grains present on the

32   stigma. In the second experiment, three flowers of the same plant were treated and were further

33   recorded for fruit and seed production. Surprisingly, and against our hypotheses, in all three

34   species no significant effect was found among and between florivory treatments and control,

35   neither in pollen grain deposited nor in fruit and seed set. The results undermine the assumption

36   that flower herbivory is necessarily antagonistic interaction and suggests that florivores are not

37   strong selection agents on floral reproductive biology in the *Oncocyclus* irises.

38

41

42   **Introduction**

43   Flowers of animal-pollinated plants are the major mean of plants to advertise and attract

44   pollinators. Floral traits serve as signals to the pollinators, usually fit to the best-efficient

45   pollinator (Fenster et al., 2004). Flowers advertise through visual signals, fragrance cues, and

46   even by acoustic signature, in order to stand out of the canopy or other flowering species

47   (Schiestl and Johnson, 2013). Consequently, flower traits that increase attraction are selected by

48   pollinators (Harder and Johnson, 2009). For example, floral size, contributing to the visibility of

49   the flower, is positively selected by pollinators (Sletvold et al., 2010, Sletvold et al., 2016,

50   Campbell et al., 1991, Conner and Rush, 1996, Harder and Johnson, 2009, Lavi and Sapir, 2015).

51   However, flowers are costly organs that require investment in resources for production and

52   maintenance. Other selection agents also act on floral traits, either in concert with, or in contrast

53   to the selection exerted by pollinators (Strauss and Whittall, 2006). For example, floral size is

54   under contrasting selection regimes, positive by pollinators and negative by drought and water

55   loss (Galen, 2000, Carroll et al., 2001). Color polymorphism is also thought to be maintained by

56   the combined effect of mutualists (pollinators) and antagonists (Carlson and Holsinger, 2010), or

57   by opposing selection directions by herbivores and pathogens (Frey, 2004). Although many

58   studies have examined floral adaptations to pollinators, the role of non-pollinator selection

59   agents in shaping floral evolution and plant reproductive success is still underappreciated.

60   Florivory, the damage of flowers by herbivores, is widespread across plant taxa and

61   ecosystems (Gonzáles et al., 2016, Burgess, 1991). Florivores comprises of various taxonomic

62   orders of animals that consume the entire flower (or the buds) or floral parts (bracts, sepals,

63   petals, nectaries, stamens, pistils or pollen). Florivory may affect plant fitness directly or

64   indirectly, by reducing fruiting or seed-set, and can consequently affect population dynamics

65   (Louda and Potvin, 1995). Florivory reduces fitness directly when reproductive parts are

66   consumed, whereas indirect effect can be through effects on pollinator behavior and hence

67   reduce pollination services. An increasing number of studies suggest that florivory can decrease

68   pollinator visitation rate and pollination success (reviewed in Gonzáles et al., 2016, but see Zhu

69   et al., 2017). Methods for studying the effect of florivory on pollination primarily focus on the

70   effect of florivore presence or the extant of florivory on pollinator visits, using natural

71   encounters and manipulative studies. For example, Kirk et al.(1995) placed black spots on

72   flowers to simulate presence of florivore beetle and found that bees were likely to avoid flowers

73   with mimicry of florivore presence. Although artificial florivory performed in the field can

74   provide an estimate to the direct effect of florivory on pollination, few studies implement this

75   method. Moreover, while behavior of pollinators and/or measure of maternal fitness (measured

76   as the number of visits or seed set, respectively) are often used to estimate the effect of florivory

77   on pollination (e.g., Cardel and Koptur, 2010), direct evidence for pollination success, which is

78   pollen deposition on the stigma, has rarely been incorporated in florivory studies. Although

79   pollinator limitation and pollen limitation supply the ground for pollinator-mediated selection

80   (Bartkowska and Johnston, 2015, Lavi and Sapir, 2015, Sletvold et al., 2017), it is unclear

81   whether it affects florivore-mediated selection. Therefore, controlled manipulative experiments

82   that measure pollen deposition and fitness, as well as incorporating pollen limitation, are needed

83   to estimate directly the interaction of florivory and pollination success.

84   In a survey of the literature, we found only a small number of studies that used a controlled

85   experimental florivory that mimic florivory by artificially manipulating or removing parts of the

86   petals and tested for effect on pollination. Of these, seven studies found negative effect on

87   pollination success , one study found mixed effects that depended on flower morph (Carper et al.,

88   2016), and one study found no effect (Tsuji et al., 2016). Thus, it remains unclear whether

89   florivory affects plant fitness indirectly through inducing negative effect on pollinators. As an

90   example, McCall (2008) found that both natural and artificial petal damage indeed reduced

91   fitness, and while it deterred pollinator activity, the effect was a result of petal physical damage

92   rather than reduced pollinators activity, as pollen addition experiment did not recover fruit-set.

93   Hence, for fully understanding the multiple effects of florivory on fitness and the interplay

94   between direct and indirect effects it requires further experiments in diverse plant species.

95   We studied the effect of florivory on pollination success in three species of the Royal Irises

96   (*Iris* section *Oncocyclus*) in their natural habitats in Israel (Figure 1 A–C). The Royal Irises

97   comprise of about 30 species across the Middle East, most are narrow endemic (Rix, 1997,

98   Mathew, 1989). This group was well studied as a system for pollination by shelter reward to

99   male *Eucera* bees (Figure 1 D; Sapir et al., 2005, Sapir et al., 2006, Watts et al., 2013, Monty et

100 al., 2006, Vereecken et al., 2013, Lavi and Sapir, 2015). Floral organs in irises are organized in

101 three units consisting of a tunnel, in which the male *Eucera* bees seek shelter for the night or at

102 low temperatures. A stigma and anther are located within each tunnel and pollen is deposited on

103 stigmas by bees that move among flowers as they seek shelter (Sapir et al., 2005).

104   The Royal Irises are eaten by various florivores, from snails and true bugs to grasshoppers,

105   birds and goats, and the intensity of damage ranges from a few superficial scratches or poke

106   marks, up to >90% of damage to floral tissue (M. Ghara and Y. Sapir, per. observations; Figure 1

107   E–G). From our observations, it appears that all flower parts are potentially eaten, either bracts,

108   petals, the petaloid style, anthers or ovaries. While damage to the reproductive organs may

109   obviously reduce fitness directly, we asked whether florivory affects fitness indirectly through

110   reduced attraction to pollinators. In order to estimate the effect of florivory on pollinators'

111   visitation and pollination success we manipulated the flowers to simulate two levels of florivory,

112   i.e., high (more than 50% damage) and low (10-30% damage), and compared to control flowers

113   without damage. We asked the following questions: (1) Does florivory affect pollinator

114   visitation? (2) Does florivory affect fruiting probability and seed set? We used pollen deposition

115   on stigma as a surrogate for estimating pollinator visitation and used fruit- and seed-set to

116   estimate overall effect of florivory on (maternal) fitness. Accounting for two measures of

117   pollination success simultaneously is likely to reveal an indication of the possible effect of

118   florivory on pollination-mediated fitness in the royal irises.

119   **Methods**

120   *Study species and sites*

121   We conducted the florivory experiment in two consecutive years on three species of the Royal

122   Irises, namely *Iris atropurpurea* Baker, *I. petrana* Dinsm., and *I. lortetii* Barbey ex Boiss.

123   Experiments were conducted in the natural environment at the largest population for each of the

124   species. *Iris atropurpurea* was studied in Netanya Iris Reserve (32.28°N, 34.84°E, alt. 35 m),

125   located on stable coastal sand dunes in Mediterranean climate and consisting of mostly low shrub

126   vegetation. Population size is estimated >1,000 plants (Yardeni et al., 2016). Flowering season,

127   and hence experiment time, is earlier compared to other species of the Royal Irises, starting as

128   early as mid-January, and peaks in February. Experiments in *I. atropurpurea* in Netanya were

129   conducted between February 12th and March 2nd, 2016, and between February 19th and March

130   7th, 2017. *Iris petrana* was studied in Yeruham Iris Reserve (31.02°N, 34.97°E, alt. 560 m), a

131   large population (estimated >10,000 plants) growing on sandy loess hills over Neogene

132   sandstone in arid climate. Vegetation is sparse desert shrubs, mostly *Retama raetam* (Forssk.)

133   Webb and *Anabasis articulata* (Forssk.) Moq. Flowering season is in March, and experiments in

134   *I. petrana* were conducted between March 5th and 14th, 2016, and between March 19th and April

135   2nd, 2017. The shift in dates in 2017 resulted due to about two weeks delay in flowering period in

136   2017. *Iris lortetii* was studied in two sub-populations near Malkiya in the upper Galilee (central

137   coordinates: 33.09°N, 35.52°E, alt. 620 m). Populations of *I. lortetii* are sparse and relatively

138   small, thus, two sub-populations at a distance of 3 km of each other were pooled to achieve a

139   sufficient sample size. Plants are growing on Eocene limestone in mesic Mediterranean climate

140   and vegetation is open woodland dominated by *Quercus calliprinos* Webb and *Pistacia atlantica*

141   Desf. trees, accompanied by dense herbaceous vegetation. *Iris lortetii* is the late blooming

142   species among the Israeli species of the Royal Irises; experiments were conducted between

143   March 29th and April 7th, 2016. The experiments were not conducted on *Iris lortetii* in 2017

144   because of high herbivory in 2016 and therefore a potential decrease in sample size.

145   Flowers of the Royal Irises grow singly on a stem, but the plant (genet) comprises of one to

146   hundreds of stems (ramets) in a well-defined patch. Plants for the experiments were randomly

147   selected in a dense part of the population in Netanya, or along transects in Yeruham. In Malkiya

148   the plants are sparse and plants in all genets located were used. The three experiments described

149   below were conducted simultaneously in time with only a single experiment conducted in each

150   genet to avoid the joint effect of several treatments.

151   *Pollinator and pollen limitation*

152   To assess the extent of pollinator and pollen limitations we took the approach of Lavi and

153   Sapir (2015). In Netanya (*I. atropurpurea*) we supplemented 54 flowers in 2016 and 30 flowers

154   in 2017 with additional pollen. In Yeruham (*I. petrana*) we supplemented additional pollen for

155   22 flowers in 2016 and 45 in 2017. Pollinator and pollen limitation were not quantified in *I.*

156   *lortetii*.

157   Flowers were randomly chosen and were pollinated using a mixture of pollen collected from

158   >20 flowers located at least 10 meters away from the supplemented flowers. The flowers were

159   left open for natural pollination. At the end of the season, the fruits of all flowers were collected

160   and seeds were counted after full ripening. Fraction of fruits produced and number of seeds were

161   compared with flowers of the control treatment in the experiment testing for florivory effect on

162   maternal fitness (see below).

163   *Florivory manipulations – effect on pollination*

164   Flowers of the Royal Irises comprise of three identical units, each bearing one upright and one

165   downright petals (standard and fall, respectively), and one petaloid style, curved above the fall to

166   create a tunnel where the reproductive organs reside (Figure 1). The three stigmas, located at the

167   top of the entrance of each of the pollination tunnels, are merged in the base of the flower to one

168   style. A previous study showed that pollinating one style is sufficient to produce seeds in all

169   three carpels in the ovary (Watts et al., 2013). To study the effect of florivory on pollen

170   deposition, a single flower in a genet was selected and each flower unit (i.e., pollination tunnel)

171   was given one of three treatments as follows. High damage – both lower petal and its opposite

172   upper petal were manually damaged up to 50% or more of the petal's area, using a puncher of 6-

173   8 mm in diameter. Low – 10-30% of the petals' area were removed using puncher. Control –

174   petals were not damaged and left un-manipulated (Figure 2). To control for the possible effect of

175   the contact between the metal hole-puncher and the flower the puncher was rubbed on the petal

176   surface in the control treatments. In addition, because damaging the petals required holding a

177   layer of tissue paper against the puncher, we also gently rubbed tissue paper under the surface of

178   the petal in the control treatment.

179   Flowers were treated in the first day after anthesis and left open for two consecutive evenings

180   following treatments to enable pollinators to visit naturally. In the morning of the third day, the

181   stigmas of the three pollination units were collected in separate vials containing 1 ml of 70%

182   ethanol. To prevent naturally occurring florivores from damaging the flowers, the stem of the

183   treated flower was coated with a layer of double sided sticky paper tape, as well as a layer of

184   Petroleum jelly (Vaseline). Occasionally we found insects trapped on the Vaseline layer, and in

185   some rare cases, we found florivores that passed this barrier. Flowers found to be damaged

186   naturally (mostly by flying insects, snails, or mammals) were discarded in order to account for

187   the effect of controlled artificial florivory only.

188   Collected stigmas were brought to the laboratory for pollen counting and kept in room

189   temperature. Pollen grains were stained using a drop of basic fucshin (Calberla's stain). Stigma

190   was then dissected in a drop 70% aqueous glycerol (Dafni et al., 2005), mounted on microscope

191   glass slide and the pollen present on the stigma was counted under dissecting microscope (WILD

192   Heerbrugg Switzerland M5-72558).

193   *Florivory manipulations – effect on maternal fitness*

194   To study the effect of florivory on seed set, three buds of the same genet, roughly of the same

195   developmental stage (i.e., before emerging from bracts) were selected and bagged to avoid bud

196   florivory. Upon anthesis, each of the three flowers was randomly assigned to one of the florivory

197   treatments described above, i.e., high, low, or control. To control for the effect of visual

198   attraction mediated by flower size (Lavi and Sapir, 2015) we measured flower length as a

199   surrogate for display size of each flower before manipulation. Flower length was measured from

200   the bottom of the lower petal to the top end of the upper one. The flowers were left open to

201   enable naturally occurring pollination. At the end of the season, approximately three weeks after

202   the end of the flowering in each site, the fruits were collected and brought to the laboratory. In

203   2016, fruits of *I. atropurpurea, I. petrana* and *I. lortetii* were collected on March 21st, April 2nd

204   and April 27th, respectively. In 2017, fruits were collected on March 12th and 22nd for *I.*

205   *atropurpurea* and on April 13th and 27th for *I. petrana*. Fruits were kept in paper bags in room

206   temperature until seed maturation. Fitness was recorded as presence or absence of a fruit

207   (binomial data), and as the number of viable seeds (count data).

208   *Data analyses*

209   The data was analyzed in R (R Development Core Team, 2014) using R-studio interface. To

210   test for differences between control and supplemental pollination treatments, we used

211   generalized linear models (GLMs) with year and treatment effects nested within site. For fruit-

212   set, we used GLM with binomial distribution errors, and for number of seeds we used quasi-

213   Poisson distribution errors due to over-dispersion of the data. Pollen and pollinator limitation

214   indices were calculated as *PLI=1–O/S*, where *O* is fruit-set or seed-set in open flowers, and *S* is

215   fruit-set or seed-set in supplementary pollination treatment. Number of pollen grains deposited

216   on stigmas was analyzed using GLM with year and treatment effects nested within species (note

217   that in *I. lortetii* experiment was done only in 2016). As in number of seeds, we used quasi-

218   Poisson distribution errors due to over-dispersion of the data. Because the three treated floral

219   units were on the same flower, for analysis of pollen deposition these were considered as non-

220   independent. Thus, we initially tested models that included also the effect of flower ("block"

221   effect). We found this effect to be non-significant (not shown), hence it was removed from the

222   analysis. For analyses using fruit or seeds as explained variables, we incorporated flower size as

223   a covariate. For seeds as a response variable, we used only the subset of flowers that set a fruit.

224   In order to account for non-normal distribution, we used GLM with binomial distribution errors

225   for fruits and GLM with quasi-Poisson distribution errors for seeds.

226

227

228   **Results**

229   *Pollinator and pollen limitation*

230   We found a significant effect of site and a significant effect of treatment, nested within year

231   and within site, on both fruit-set and seed-set (Table 1). The effect of year was not significant. In

232   Netanya, flowers supplemented with pollen produced significantly higher fruit-set than control

233   flowers, open to natural pollinators only (48.1% versus 5.5% in 2016 and 60% versus 15% in

234   2017; P<0.001 in both years; Figure 3 A&B). Seed-set in Netanya in the two years was also

235   significantly higher in flowers supplemented with excess pollen, compared to open flowers

236   pollinated only naturally (mean number of seeds 11.5 versus 1.1 in 2016 and 10.3 versus 1.7 in

237   2017, P<0.005 in both years; Figure 3 C&D). Both pollinator limitation and pollen limitation

238   were extremely high in Netanya in both years (PLI=0.88 for fruit-set and PLI=0.91 for seed-set

239   in 2016; PLI=0.75 for fruit-set and PLI=0.84 for seed-set in 2017).

240   In Yeruham, fruit-set was similar between supplementary and open flowers in 2016 (40.9%

241   versus 41.7%, respectively, P=0.96; Figure 3A). In 2017, fruit-set was significantly higher in

242   flowers supplemented with pollen, compared to not-treated control flowers (73.3% versus

243   34.8%, respectively, P<0.001; Figure 3 B). Likewise, seed-set was not significantly different

244   between supplementary and open flowers in 2016 (mean number of seeds 7.1 versus 9.4,

245   respectively, P=0.675) but differ between treatments in 2017 (mean number of seeds 15.6 versus

246   5.8, respectively, P=0.003; Figure 3 C&D). Pollinator limitation and pollen limitation were

247   below zero in Yeruham in 2016 (PLI= −0.02 for fruit-set and PLI= −0.32 for seed-set), but

248   relatively high in 2017 (PLI= 0.53 for fruit-set and PLI= 0.64 for seed-set).

249   *Pollen deposition on stigma*

250   We found significant effect of year and species on number of pollen grains deposited on

251   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,

252   nested within species). Nonetheless, treatment effect (nested within year and within species) was

253   not significant ($F_{2,691}$=0.63, P=0.706). In *Iris atropurpurea*, 49 (26.3%) stigmas did not receive

254   pollen at all in 2016 and 8 (5.3%) in 2017. Flowering units with high artificial florivory revealed

255   lower number of pollen grains on stigma in 2016 and lower in 2017(Figure 4), but these

256   differences were small and far from significance ($F_{2,183}$=0.67, P=0.515 in 2016 and $F_{2,135}$=0.16,

257   P=0.855 in 2017). In *I. petrana*, all stigmas received pollen grains in 2016, but in 2017, 51 (34%)

258   did not receive any pollen grain. Number of pollen grains deposited on the stigmas was an order

259 of magnitude larger than in *I. atropurpurea* in 2016 (Figure 4 A), but similar in 2017 (Figure 4

260 B). Nonetheless, as in *I. atropurpurea*, no significant treatment effect was found in both years

261 ($F_{2,195}=0.53$, P=0.588 in 2016 and $F_{2,144}=1.89$, P=0.155 in 2017). In *Iris lortetii*, as in *I. petrana*

262 in 2016, all stigmas received pollen and stigmas in units treated by high artificial florivory

263 received pollen grains in a similar level as the control, untreated units, both higher than medium

264 artificial florivory treatment (Figure 4 A). However, this difference was not significant as well

265 ($F_{2,30}=0.322$, P=0.727).

266 *Fruit and seed sets*

267   In *Iris atropurpurea*, 80 flowers were included in the final analyses in 2016, because 16 of the

268 treated flowers were either not found or damaged. Of these, only 13 flowers set fruits, indicating

269 extreme pollinator limitation and lack of pollinator visitations. Treatment did not affect either

270 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

271 A & C). In both analyses flower size did not affect significantly (P=0.266 and P=0.554 for fruits

272 and seeds, respectively). In 2017, 124 flowers were included in the experiment, of which four

273 were damaged. Of the remaining 120 flowers, 23flowers set fruits. As in 2016, no effect of the

274 treatment was found, neither on fruit-set, nor on number of seeds ($F_{2,116}=0.37$, P=0.695, and

275 $F_{2,19}=0.95$, P=0.404, respectively; Figure 5 B & D). Similar to 2016, flower size as covariate did

276 not affect fruit-set or seed-set (P=0.188 and P=0.857, respectively).

277   In *I. petrana* population in Yeruham, 132 flowers were treated in 2016, but 29 flowers of all

278 treatments were eaten by goats that entered the reserve illegally and ate wilting flowers and

279 young fruits in the pre-dispersal stage. Of the remaining 103 treated flowers, 31 flowers set

280 fruits. Treatment effect on fruit-set was not significant ($F_{2,98}=1.89$, P=0.156 ;Figure 5 A).

281 Interestingly, flower length (before treatment) significantly affected fruit set ($F_{1,98}=5.09$,

282 P=0.026), but with no significant interaction with treatment ($F_{2,96}=0.26$, P=0.770). Although

283 control flowers produced almost twice fraction of fruits compared to florivory treatment (34%

284 vs. 18%), this difference was not significant when controlled for flower size. Number of seeds

285 was not affected by treatment ($F_{2,98}=1.89$, P=0.156, n=31; Figure 5C). Flower size did not affect

286 seed-set ($F_{1,26}=1.23$, P=0.278). In 2017, only six flowers were eaten or not found, out of 188

287 flowers treated. As in 2016, no effect of the treatment was found, neither on fruit-set, nor on

288 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).

289 As opposed to 2016, flower size as covariate did not affect fruit-set but did affect seed-set

290 (P=0.472 and P<0.001, respectively). No interaction was found between flower size and

291 florivory treatment in their effect on seed-set (P=0.463).

292 In the two sites of *Iris lortetii*, 61 out of 62 flowers treated (12 in Avivim and 50 in Malkiya)

293 were found at the end of the season and included in the analyses. No significant difference was

294 found among treatments ($F_{2,57}$=0.21, P=0.811; Figure 4 A). Flower size affected fruit-set

295 ($F_{1,57}$=4.42, P=0.40). Number of seeds was not significantly affected by treatment ($F_{2,12}$=0.37,

296 P=0.698; Figure 4 C), and neither by flower size ($F_{1,12}$=0.92, P=0.356).

297

298 **Discussion**

299 Florivory, namely the damage herbivores cause to floral organs, can affect fitness either

300 directly by consuming pollen or ovules or physiological costs, or indirectly, by reducing plant

301 attraction signal for the pollinators (Burgess, 1991, McCall and Irwin, 2006). Here we tested for

302 both direct and indirect effects of florivory on fitness by executing artificial florivory and

303 measuring both fitness and pollination. Our results do not support the hypothesis that florivory

304 affects pollination success in the Royal Irises either directly or indirectly. Instead, we show that

305 artificial damage to reproductive tissues in three species of the royal irises did not significantly

306 reduce either pollen deposition or fitness of manipulated flowers, compared to un-treated ones

307 (Figure 4 and Figure 5).

308 While numerous studies were concerned with the effect of florivory on plant fitness,

309 controlled, artificial florivory was rarely performed. Most studies examined flowers that were

310 naturally attacked by florivores (e.g., Meindl et al., 2013, Ruane et al., 2014, Eliyahu et al.,

311 2015) or used experimental florivore removal or prevention (e.g., Krupnick et al., 1999, Theis

312 and Adler, 2012, Althoff et al., 2013). Only a few studies implemented methods similar to ours,

313 using cutting flowers to simulate florivory. These studies revealed mixed results. For example,

314 Sõber et al. (2010) experimentally showed a correlation between extent of florivory and

315 pollinator visitations at both population and plant level. On the other hand, Tsuji et al. (2016)

316 found no evidence for pollinators discrimination against experimentally damaged flowers.

317 Interestingly, mixed results can be found within the same system: Carper et al.(2016) found

318 differences between heterostylous morphs in pollinator responses to artificial damage, and found

319 no effect on fitness. Our study adds to the puzzle by providing yet another piece of evidence that

320 florivory itself does not deter pollinators, nor reduces fitness.

321   Negative effect of florivory on pollination may act in two avenues. One possible effect of

322   florivory on pollinator behavior is the deterring of pollinators from eaten flowers. This is

323   achieved by either avoidance flowers where florivore is visually detected (Kirk et al., 2005) or

324   by a change in volatile compounds (Kessler et al., 2013). Another possible effect of florivory on

325   pollination is mediated by the effect of the overall advertisement size of the flower and reducing

326   visual signaling for the pollinators (Sánchez-Lafuente, 2007). This may reduce number of visits

327   and lead to pollinator limitation or pollen limitation, which in turn reduces fruit-set and seed-set,

328   respectively (Sapir et al., 2015). This study was conducted in natural populations of which at

329   least one (Netanya) indeed experience strong pollinator limitation. It is likely that the effect of

330   pollen limitation in this population obscures the effect of florivory (McCall, 2010). Thus, we

331   propose that selection mediated by pollinator/pollen limitation is stronger than selection pressure

332   exerted by florivory. A previous study that tested for pollen limitation in two *Iris* species

333   showed, indeed, that pollinators limitation provides conditions for pollinator-mediated selection

334   (Lavi and Sapir, 2015). However, in Yeruham we found no evidence for pollen limitation, still

335   no effect of florivory was found, which contradicts this hypothesis. Our mixed results suggest

336   that while pollinators may be a selection agent on flower traits (as in Lavi & Sapir, 2015),

337   florivores do not act as selection agents because pollen limitation balances their effect (cf. Jogesh

338   et al., 2016), but this connection cannot be generalized beyond our specific system.

339   Pollinators are thought to be the major selection agent on floral traits through their positive

340   effect on fitness; this, however, was challenged by observations on contrasting effects of abiotic

341   conditions or antagonistic biotic interactions (Herrera, 1996, reviewed in Strauss and Whittall,

342   2006). Strauss and Whittall (2006) proposed two scenarios of such mutualistic-antagonistic

343   effect, in which overall selection acts as either directional or stabilizing on floral traits. Based on

344   our results, we propose a third scenario, where florivory affects fitness at the same level as the

345   mean effect of pollinators and regardless floral trait. In this case, pollinator-mediated selection

346   will govern trait evolution, but the presence of florivory reduces or diminishes effect size (Figure

347   6). We speculate that florivory may indeed not affect the direction of selection but the intensity

348   of it. Thus, we suggest that testing for the net selection mediated by pollinators should control

349   experimentally for the effect of florivores. In a follow-up experiment, we intend to test for net

350   pollinator-mediated selection on floral color in the Royal Irises. Floral pigment concentration is

351   expected to deter florivores and attract pollinators (de Jager and Ellis, 2014, McCall et al., 2013),

352 and given the results of the current study we assume that florivory may not necessarily reduce

353 fitness; instead, it is expected that a weak directional pollinator-mediated selection on floral color

354 will be detected after controlling for florivory.

355   While few previous studies have presented only slight (or no) effect of florivory on

356 pollination, it may be further hypothesized that the Royal Irises are unique and thus do not

357 represent a general rule relevant to other species. The Royal Irises present a unique pollination

358 syndrome in which pollination is performed by night sheltering male bees (Sapir et al., 2005,

359 Watts et al., 2013). Previous studies suggest that flower display itself is not necessarily the major

360 attractant for these pollinators (Lavi and Sapir, 2015). While our florivory-like manipulations

361 were performed on petals, it is likely that these manipulations did not affect pollinator choice

362 because the shelter itself (the pollination tunnel) was not damaged. Because we have observed

363 florivory of stigma and anthers (M. Ghara pers. observations), we suspect that florivory may

364 affect male and female fitness through consumption of reproductive organs, but we have yet to

365 assess the cost of florivory on reproductive organs. Nonetheless, while natural florivory is

366 widespread in all species and most populations of the Royal Irises in Israel, the estimated

367 proportion of flowers of which pollination tunnels were eaten is rather small (M. Ghara,

368 manuscript in preparation). Thus, our manipulation on petals accurately mimics natural florivory

369 and our conclusions on the role of florivory in selection on flowers of the Royal Irises are valid.

370   Finally, our study provides an application to conservation. Of the three species studied, two

371 (*Iris atropurpurea* and *I. lortetii*) are rare and endangered species (Sapir, 2016a, Sapir, 2016b).

372 Understanding the relative contribution of biotic interactions to population dynamics may shed

373 light on the factors affecting species survival in a way that may contribute for evidence-based

374 management. The study presented here suggests that reduced mutualistic interactions, namely,

375 pollination services, rather than antagonistic florivory, threatens the maintenance of positive

376 population growth in the Royal Irises.

377

Acknowledgement

383   Ben-Ami and Zion Awaskar. Prof. D. Eisikowitch provided lab support and fruitful discussions

384   and ideas. The study was partially supported by the Israeli Nature-Parks Authority and a grant

385   from Israel Science Foundation #336/16 to YS. MG was supported by Israel Planning and

386   Budgeting Commission (PBC) post-doctoral fellowship.

387

388   **References**

389   **Althoff DM, Xiao W, Sumoski S, Segraves KA. 2013**. Florivore impacts on plant reproductive

390   success and pollinator mortality in an obligate pollination mutualism. *Oecologia,* **173**:

391   1345-1354.

392   **Bartkowska MP, Johnston MO. 2015**. Pollen limitation and its influence on natural selection

393   through seed set. *Journal of Evolutionary Biology,* **28**: 2097-2105.

394   **Botto-Mahan C, Ramírez PA, Ossa CG, Medel R, Ojeda-Camacho M, González AV. 2011**.

395   Floral herbivory affects female reproductive success and pollinator visitation in the

396   perennial herb *Alstroemeria ligtu* (Alstroemeriaceae). *International Journal of Plant*

397   *Sciences,* **172**: 1130-1136.

398   **Burgess K. 1991**. *Florivory: The ecology of flower feeding insects and their host plants,* PhD,

399   Harvard University.

400   **Campbell DR, Waser NM, Price MV, Lynch EA, Mitchell RJ. 1991**. Components of

401   phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*.

402   *Evolution,* **45**: 1458-1467.

403   **Cardel YJ, Koptur S. 2010**. Effects of Florivory on the Pollination of Flowers: An

404   Experimental Field Study with a Perennial Plant. *International Journal of Plant Sciences,*

405   **171**: 283-292.

406   **Carlson JE, Holsinger KE. 2010**. Natural selection on inflorescence color polymorphisms in

407   wild Protea populations: The role of pollinators, seed predators, and intertrait

408   correlations. *American Journal of Botany,* **97**: 934-944.

409   **Carper AL, Adler LS, Irwin RE. 2016**. Effects of florivory on plant-pollinator interactions:

410   Implications for male and female components of plant reproduction. *American Journal of*

411   *Botany,* **103**: 1061-1070.

412 **Carroll AB, Pallardy SG, Galen C. 2001**. Drought stress, plant water status, and floral trait
413   expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of*
414   *Botany,* **88**: 438-446.

415 **Conner JK, Rush S. 1996**. Effects of flower size and number on pollinator visitation to wild
416   radish, *Raphanus raphanistrum. Oecologia,* **105**: 509-516.

417 **Dafni A, Kevan PG, Husband BC. 2005.** Practical Pollination Biology. Ontario: Enviroquest.

418 **de Jager ML, Ellis AG. 2014**. Floral polymorphism and the fitness implications of attracting
419   pollinating and florivorous insects. *Annals of Botany,* **113**: 213-222.

420 **Eliyahu D, McCall AC, Lauck M, Trakhtenbrot A. 2015**. Florivory and nectar-robbing
421   perforations in flowers of pointleaf manzanita *Arctostaphylos pungens* (Ericaceae) and
422   their effects on plant reproductive success. *Arthropod-Plant Interactions,* **9**: 613-622.

423 **Fenster CB, Armbuster WS, Wilson P, Dudash MR, Thomson JD. 2004**. Pollination
424   syndromes and floral specialization. *Annual Review of Ecology, Evolution and*
425   *Systematics,* **35**: 375-403.

426 **Frey FM. 2004**. Opposing natural selection from herbivores and pathogens may maintain floral-
427   color variation in *Claytonia virginica* (Portulacacea). *Evolution,* **58**: 2426-2437.

428 **Galen C. 2000**. High and dry: drought stress, sex-allocation trade-offs, and selection on flower
429   size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *The American*
430   *Naturalist,* **156**: 72-83.

431 **Gonzáles WL, Suárez LH, Gianoli E. 2016**. Genetic variation in the reduction of attractive
432   floral traits of an annual tarweed in response to drought and apical damage. *Journal of*
433   *Plant Ecology,* **9**: 629-635.

434 **Harder LD, Johnson SD. 2009**. Darwin's beautiful contrivances: evolutionary and functional
435   evidence for floral adaptation. *New Phytologist,* **183**: 530-545.

436 **Herrera CM. 1996.** Floral traits and plant adaptation to insect pollinators: a devil's advocate
437   approach. In: Lloyd DG, Barrett SCH, eds. *Floral biology: studies on floral evolution in*
438   *animal-pollinated plants.* New York: Chapman & Hall.

439 **Jogesh T, Overson RP, Raguso RA, Skogen KA. 2016**. Herbivory as an important selective
440   force in the evolution of floral traits and pollinator shifts. *AoB Plants*.

441 **Kessler D, Diezel C, Clark DG, Colquhoun TA, Baldwin IT. 2013**. Petunia flowers solve the
442   defence/apparency dilemma of pollinator attraction by deploying complex floral blends.
443   *Ecology Letters,* **16**: 299-306.

444 **Kirk H, Vrieling K, Klinkhamer PGL. 2005**. Maternal effects and heterosis influence the
445   fitness of plant hybrids. *The New Phytologist,* **166**: 685-694.

446 **Kirk WDJ, Ali M, Breadmore KN. 1995**. The effects of pollen beetles on the foraging
447   behaviour of honey bees. *Journal of Apicultural Research,* **34**: 15-22.

448 **Krupnick GA, Weis AE, Campbell DR. 1999**. The consequences of floral herbivory for
449   pollinator service to *Isomeris arborea. Ecology,* **80**: 125-134.

450 **Lavi R, Sapir Y. 2015**. Are pollinators the agents of selection for the extreme large size and
451   dark color in Oncocyclus irises? *New Phytologist,* **205**: 369-377.

452 **Louda SM, Potvin MA. 1995**. Effect of Inflorescence-Feeding Insects on the Demography and
453   Lifetime of a Native Plant. *Ecology,* **76**: 229-245.

454 **Mathew B. 1989**. *The Iris.* London: Batsford.

455 **McCall AC. 2008**. Florivory affects pollinator visitation and female fitness in *Nemophila*
456   *menziesii. Oecologia,* **155**: 729-737.

457 **McCall AC. 2010**. Does dose-dependent petal damage affect pollen limitation in an annual
458   plant? *Botany,* **88**: 601-606.

459 **McCall AC, Irwin RE. 2006**. Florivory: the intersection of pollination and herbivory. *Ecology*
460   *Letters,* **9**: 1351-1365.

461 **McCall AC, Murphy SJ, Venner C, Brown M. 2013**. Florivores prefer white versus pink petal
462   color morphs in wild radish, *Raphanus sativus. Oecologia,* **172**: 189-195.

463 **Meindl GA, Bain DJ, Ashman T-L. 2013**. Edaphic factors and plant–insect interactions: direct
464   and indirect effects of serpentine soil on florivores and pollinators. *Oecologia,* **173**: 1355-
465   1366.

466 **Monty A, Saad L, Mahy Gg. 2006**. Bimodal pollination system in rare endemic Oncocyclus
467   irises (Iridaceae) of Lebanon. *Canadian Journal of Botany,* **84**: 1327–1338.

468 **Muola A, Weber D, Malm LE, Egan PA, Glinwood R, Parachnowitsch AL, Stenberg JA.**
469   **2017**. Direct and Pollinator-Mediated Effects of Herbivory on Strawberry and the
470   Potential for Improved Resistance. *Frontiers in Plant Science,* **8**: 823.

471   **Pohl N, Carvallo G, Botto-Mahan C, Medel R. 2006**. Nonadditive effects of flower damage

472   and hummingbird pollination on the fecundity of Mimulus luteus. *Oecologia,* **149**: 648-

473   655.

474   **R Development Core Team. 2014.** R: A Language and Environment for Statistical Computing.

475   Vienna: R Foundation for Statistical Computing.

476   **Rix M. 1997.** Section *Oncocyclus* (Siemssen) Baker. In: The species group of the British Iris

477   Society, ed. *A Guide to Species Irises*: Cambridge University Press.

478   **Ruane LG, Rotzin AT, Congleton PH. 2014**. Floral display size, conspecific density and

479   florivory affect fruit set in natural populations of *Phlox hirsuta*, an endangered species.

480   *Annals of Botany,* **113**: 887-893.

481   **Sánchez-Lafuente AM. 2007**. Corolla Herbivory, Pollination Success and Fruit Predation in

482   Complex Flowers: An Experimental Study with *Linaria lilacina* (Scrophulariaceae).

483   *Annals of Botany,* **99**: 355-364.

484   **Sapir Y. 2016a**. *Iris atropurpurea. The IUCN Red List of Threatened Species 2016*:

485   e.T13161450A18611400.

486   **Sapir Y. 2016b**. *Iris lortetii. The IUCN Red List of Threatened Species 2016*:

487   e.T13161743A18612235.

488   **Sapir Y, Dorchin A, Mandelik Y. 2015.** Indicators of Pollinator Decline and Pollen Limitation.

489   In: Armon RH, Hänninen O, eds. *Environmental Indicators*: Springer Netherlands.

490   **Sapir Y, Shmida A, Ne'eman G. 2005**. Pollination of the *Oncocyclus* irises (*Iris*: Iridaceae) by

491   night-sheltering male bees. *Plant Biology,* **7**: 417-424.

492   **Sapir Y, Shmida A, Ne'eman G. 2006**. Morning floral heat as a reward to the pollinators of the

493   *Oncocyclus* irises. *Oecologia,* **147**: 53-59.

494   **Schiestl FP, Johnson SD. 2013**. Pollinator-mediated evolution of floral signals. *Trends in*

495   *Ecology and Evolution,* **28**: 307-315.

496   **Sletvold N, Grindeland JM, Ågren J. 2010**. Pollinator-mediated selection on floral display,

497   spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*.

498   *New Phytologist,* **188**: 385–392.

499   **Sletvold N, Tye M, Ågren J. 2016**. Resource- and pollinator-mediated selection on floral traits.

500   *Functional Ecology*: doi: 10.1111/1365-2435.12757.

501 **Sletvold N, Tye M, Ågren J. 2017**. Resource- and pollinator-mediated selection on floral traits.

502   *Functional Ecology,* **31**: 135-141.

503 **Sõber V, Moora M, Teder T. 2010**. Florivores decrease pollinator visitation in a self-

504   incompatible plant. *Basic and Applied Ecology,* **11**: 669-675.

505 **Strauss SY, Whittall JB. 2006.** Non-pollinator agents of selection on floral traits. In: Harder

506   LD, Barrett SCH, eds. *Ecology and Evolution of Flowers*. Oxford Oxford University

507   Press.

508 **Theis N, Adler LS. 2012**. Advertising to the enemy: enhanced floral fragrance increases beetle

509   attraction and reduces plant reproduction. *Ecology,* **93**: 430-435.

510 **Tsuji K, Dhami MK, Cross DJR, Rice CP, Romano NH, Fukami T. 2016**. Florivory and

511   pollinator visitation: a cautionary tale. *AoB Plants,* **8**.

512 **Vereecken NJ, Dorchin A, Dafni A, Hötling S, Schulz S, Watts S. 2013**. A pollinators' eye

513   view of a shelter mimicry system. *Annals of Botany,* **111**: 1155-1165.

514 **Watts S, Sapir Y, Segal B, Dafni A. 2013**. The endangered *Iris atropurpurea* (Iridaceae) in

515   Israel: honey-bees, night-sheltering male bees and female solitary bees as pollinators.

516   *Annals of Botany,* **111**: 395-407.

517 **Yardeni G, Tessler N, Imbert E, Sapir Y. 2016**. Reproductive isolation between populations of

518   *Iris atropurpurea* is associated with ecological differentiation. *Annals of Botany,* **118**:

519   971-982.

520 **Zhu Y-R, Yang M, Vamosi JC, Armbruster WS, Wan T, Gong Y-B. 2017**. Feeding the

521   enemy: loss of nectar and nectaries to herbivores reduces tepal damage and increases

522   pollinator attraction in *Iris bulleyana*. *Biology Letters,* **13**: 20170271.

523

524

525 **Tables**

526   Table 1 – Analysis of variance table for the effects of site (Netanya and Yeruham), year (2016

527 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		

528

529 **Figure legends**

530 Figure 1 –(A) *Iris atropurpurea* in Netanya; (B) *Iris petrana* in Yeruham; (C) *Iris lortetii* in

531 Malkiya; (D) Male *Eucera* bee, the specific pollinator of the royal irises, sheltering within a

532 pollination tunnel of *Iris petrana*; (E-G) Natural florivory in flowers of *Iris atropurpurea* (E), *I.*

533 *petrana* (F) and *I. lortetii* (G).

534

535 Figure 2 –Artificial florivory manipulations exemplified in *Iris atropurpurea*. (A) Within flower

536 manipulation – each floral unit treated as either high florivory (>50% petal cut), low florivory

537 (10-30% petal removed by hole puncher), or control (no treatment). These treatments were used

538 for testing the effect on pollination. (B–D) Flowers used for testing the effect of florivory on

539 fitness. (B) Flower treated as high florivory. (C) Flower treated as low florivory. (D) Control

540 flower.

541

542 Figure 3 – Fruit-set and seed-set in pollen limitation experiment in *Iris atropurpurea* in Netanya,

543 and *I. petrana* in Yeruham. Bars are fractions ± standard errors. (A) Fraction of flowers that

544 produced fruits in 2016 (B) and in 2017. (C) Mean number of seeds in 2016 (D) and in 2017.

545 Open – flowers open to natural pollination. Supp. – flowers received supplementary pollen

546 artificially.

547

548 Figure 4–Mean number of pollen grains counted on stigma (± standard errors) as a function of

549 florivory treatment in three species.(A) Pollen grains in 2016 experiment; (B) Pollen grains in

550 2017 experiment. Bars are fractions ± standard errors.

551

552 Figure 5–Fitness as a function of artificial florivory manipulations. (A) Fruit-set (fraction of

553 flowers that set fruits) in three species in 2017 and in 2017 (B). (C) Seed-set (mean number of

554 seeds in a fruit) in 2016 and in 2017 (D). Bars are means ± standard errors.
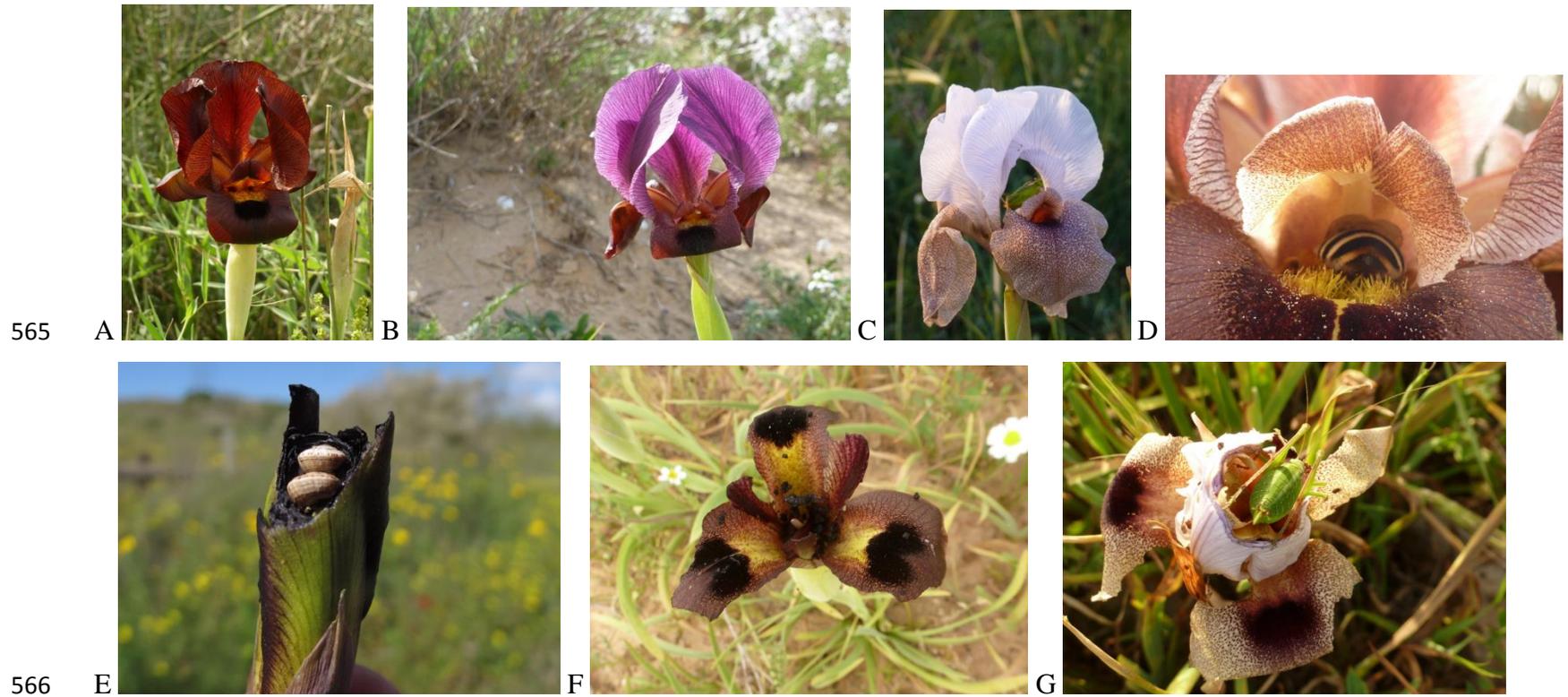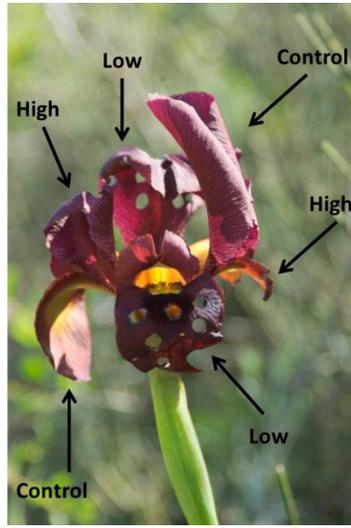
555

556 Figure 6–Hypothetical floral trait evolution as a function of selection by both mutualists

557 (pollinators) and antagonists (florivores). (A) When pollinators and florivores exert selection in

558 the same direction, the concert selection favors the same trait optimum. (B) When pollinators and

559 florivores exert opposing selection on a trait, an intermediate trait optimum is favored. A and B

560   adapted from figure 7.1 in Strauss &Whittall (2006). (C) When florivores have no preference, or

561   their effect is similar to mean fitness derived from pollinators' effect, trait will have optimum

562   fitness selected by pollinators like in A.

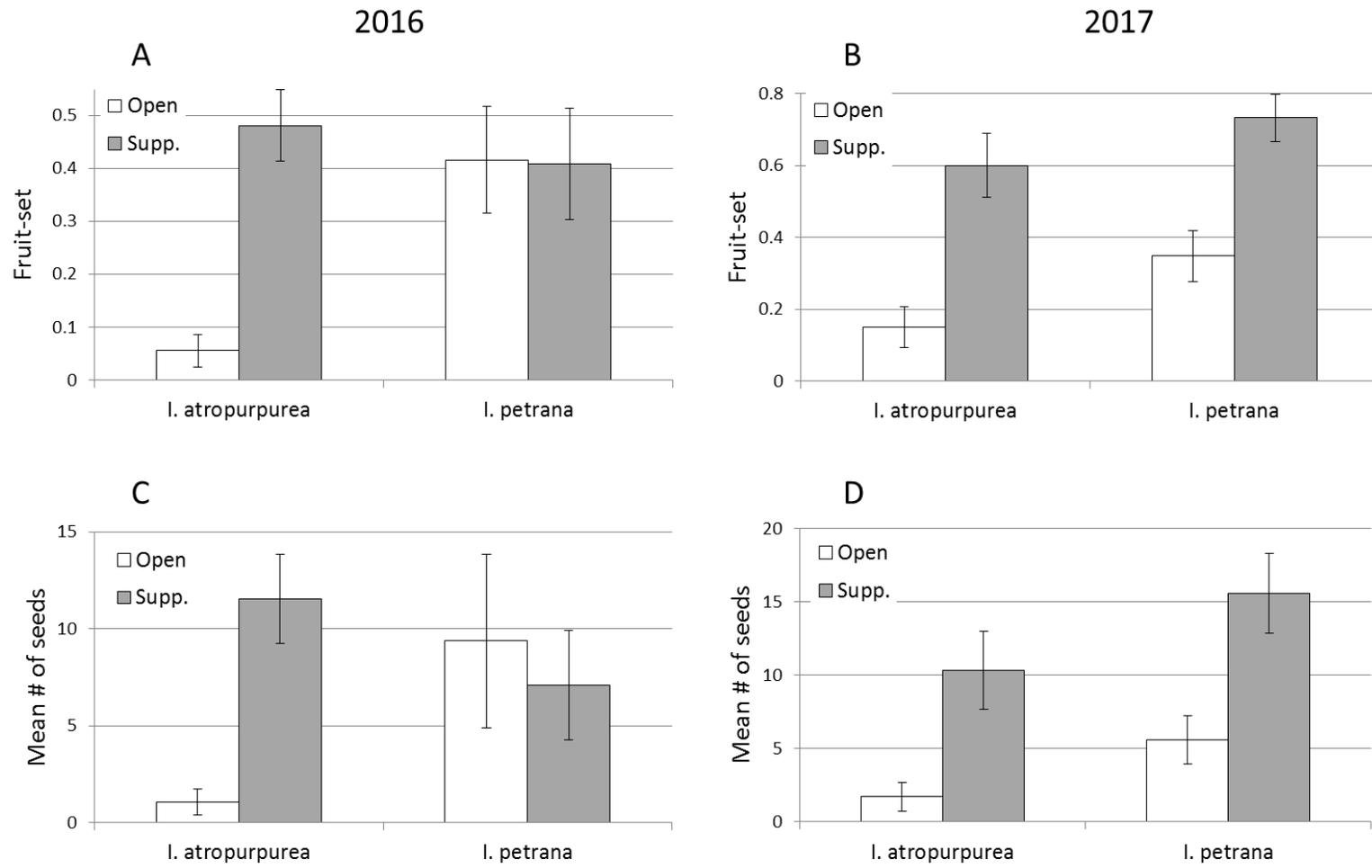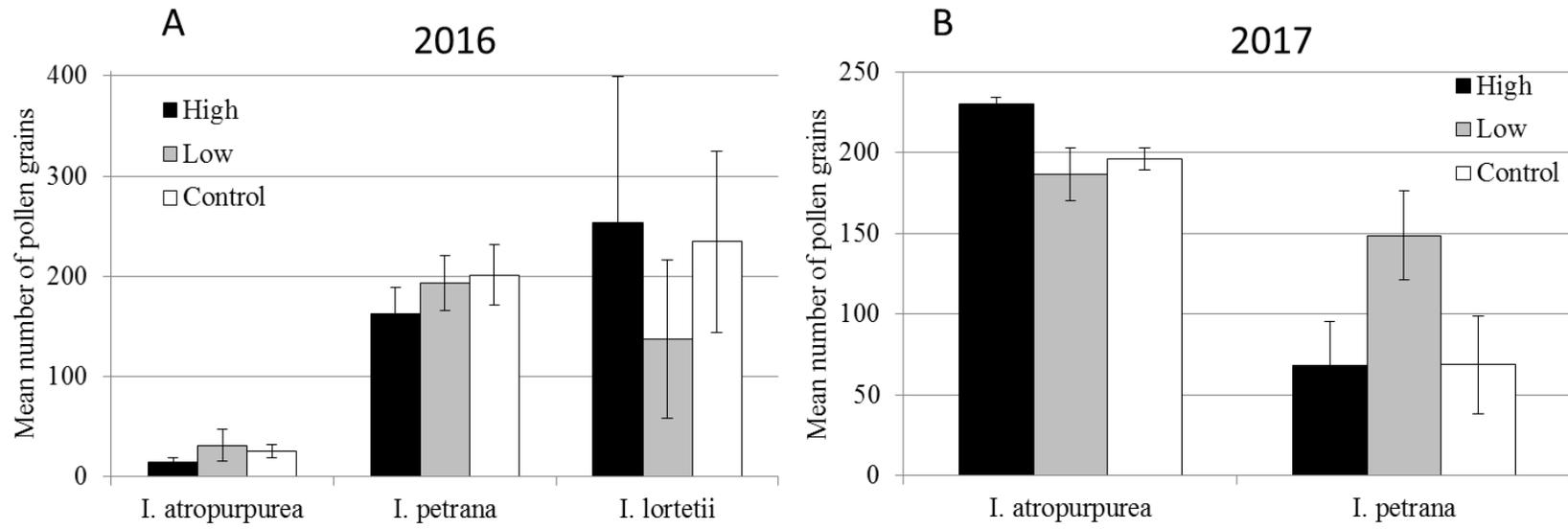563

564   Figure 1

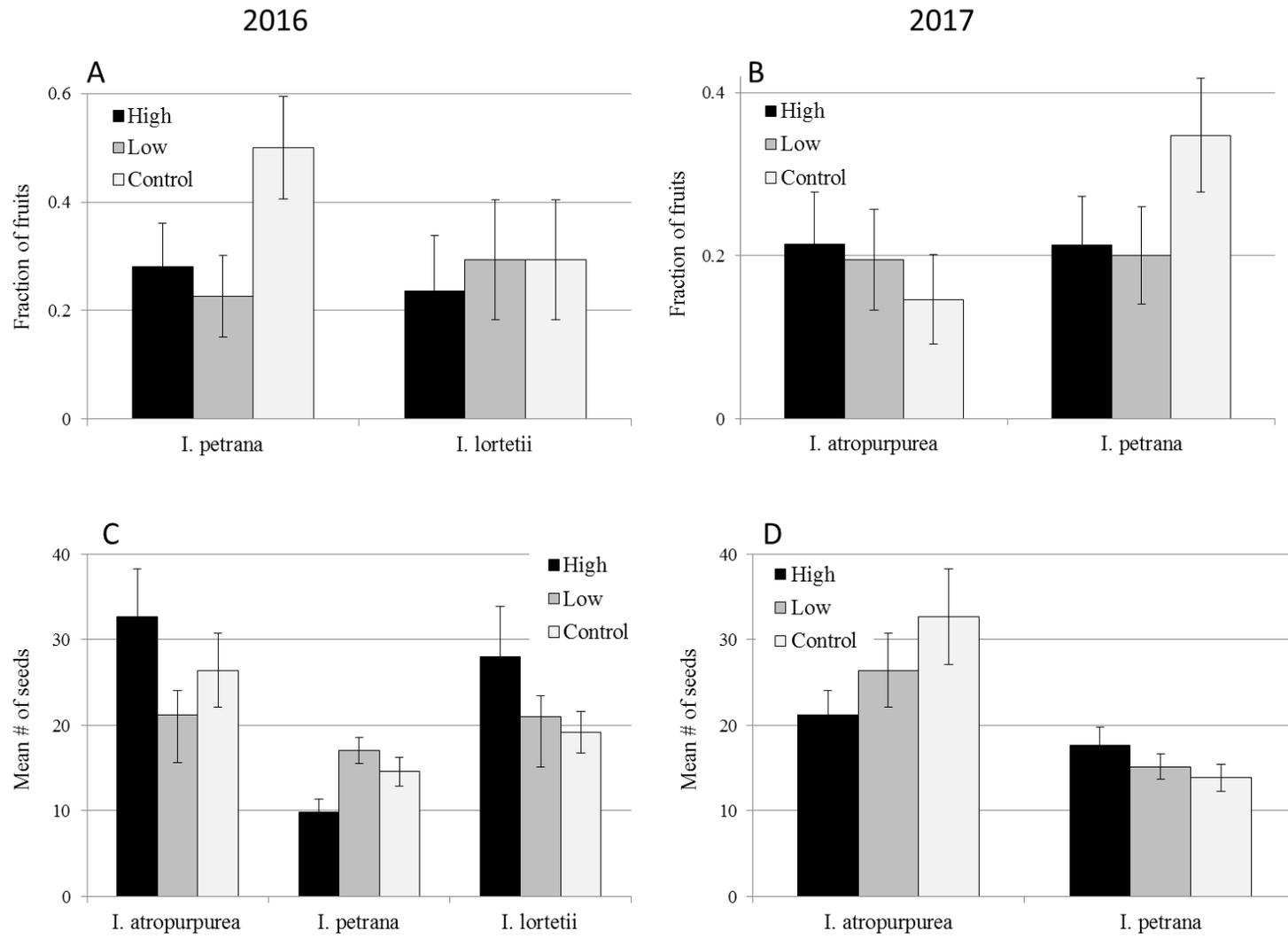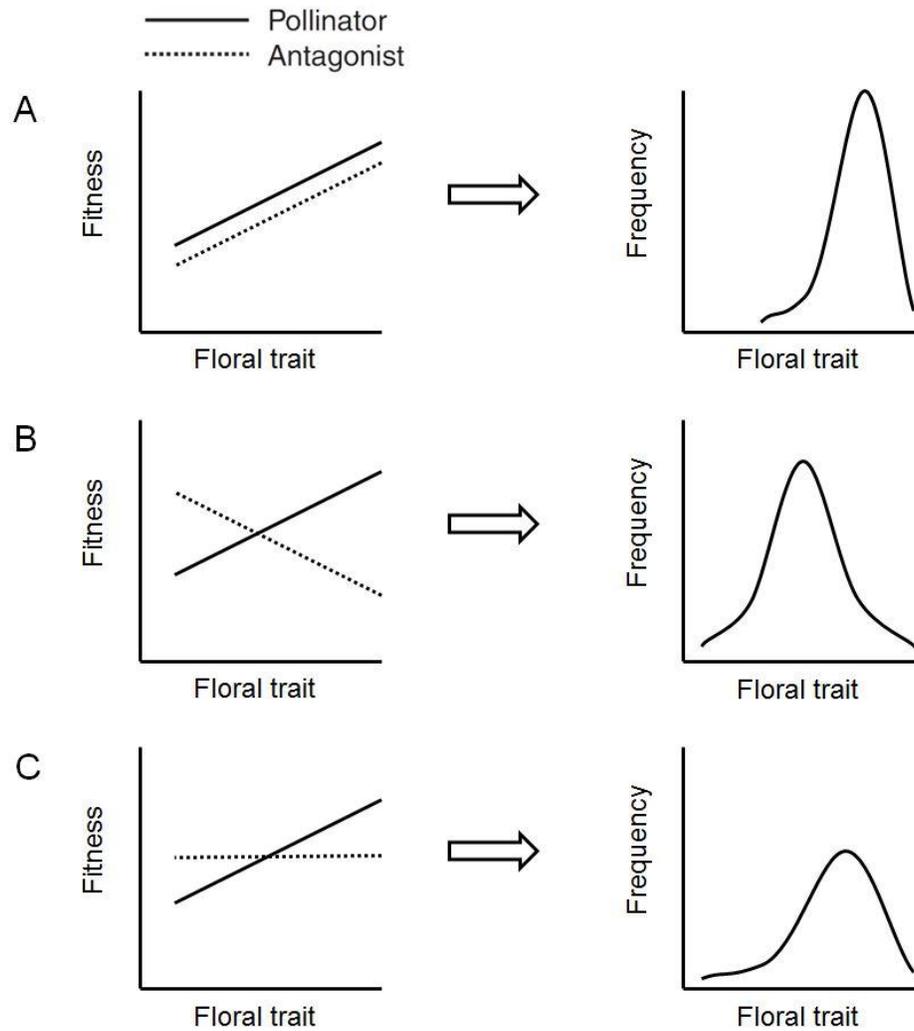565   A   B   C   D

566   E   F   G

567

568   Figure 2

569

570

C   D

Figure 3

2016

A

C

2017

B

D

573   Figure 4

A
2016

Mean number of pollen grains

High
Low
Control

I. atropurpurea   I. petrana   I. lortetii

B
2017

Mean number of pollen grains

High
Low
Control

I. atropurpurea   I. petrana

574

575   Figure 5

577   Figure 6

578

579