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2 **Pollination and reproduction of an invasive plant inside and outside its**
3 **ancestral range**

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23 Highlights

- 24 • Weedy *Solanum elaeagnifolium* is invasive outside its ancestral North America range.
- 25 • We compared its sexual reproduction in Arizona, USA (“AZ”) and Greece (“GR”).
- 26 • Pollination in GR was by native bees that resemble ancestral AZ pollinators.
- 27 • GR plants invest more in flowers and ovules but do not produce more seeds.
- 28 • These results suggest promising avenues for further research.

29 **Abstract**

30 Comparing traits of invasive species within and beyond their ancestral range may improve our
31 understanding of processes that promote aggressive spread. *Solanum elaeagnifolium*
32 (silverleaf nightshade) is a noxious weed in its ancestral range in North America and is
33 invasive on other continents. We compared investment in flowers and ovules, pollination
34 success, and fruit and seed set in populations from Arizona, USA (“AZ”) and Greece (“GR”).
35 In both countries, the populations we sampled varied in size and types of present-day
36 disturbance. Stature of plants increased with population size in AZ samples whereas GR
37 plants were uniformly tall. Taller plants produced more flowers, and GR plants produced
38 more flowers for a given stature and allocated more ovules per flower. Similar functional
39 groups of native bees pollinated in AZ and GR populations, but visits to flowers decreased
40 with population size and we observed no visits in the largest GR populations. As a result,
41 plants in large GR populations were pollen-limited, and estimates of fecundity were lower on
42 average in GR populations despite the larger allocation to flowers and ovules. These
43 differences between plants in our AZ and GR populations suggest promising directions for
44 further study. It would be useful to sample *S. elaeagnifolium* in Mediterranean climates
45 within the ancestral range (e.g., in California, USA), to study asexual spread via rhizomes,
46 and to use common gardens and genetic studies to explore the basis of variation in allocation
47 patterns and of relationships between visitation and fruit set.

48

49 **Keywords**

50 Ancestral range; Bees; Invaded range; Pollination success; Sexual allocation; *Solanum*
51 *elaeagnifolium*

52 **1. Introduction**

53

54 The introduction of species beyond their ancestral range often causes ecological damage, and
55 aggressive spread of invasive species threatens biodiversity worldwide (Elton 1958; Pimentel
56 et al. 2001; Traveset and Richardson 2006). Studies of species invasions suggest that they
57 depend both on attributes of the invaders and of the invaded systems, but much about what
58 promotes successful invasion remains a mystery (Richardson and Pysek 2006; Tanentzap et
59 al. 2010; van Kleunen et al. 2010).

60

61 Comparison across populations of invasive species within and beyond their ancestral range
62 may indicate ways in which individual traits have changed during invasion, thus offering
63 insight into factors that promote or accompany geographic spread. Few studies have
64 attempted such a comparison for plants, and most of these have focused on success in
65 recruitment and on genetic variance in invading populations (De los Santos et al. 2001;
66 Lafuma and Maurice 2007; Mandák et al. 2009; Colautti et al. 2011). Fewer have compared
67 aspects of pollination and reproduction (e.g., Caño et al. 2008, Li et al. 2012, Atlan et al.
68 2015), and to our knowledge only three such were carried out in the wild (Stout et al. 2006;
69 Petanidou et al. 2012; Montero-Castaño et al. 2014).

70

71 As a first step in exploring factors that might relate to the invasion success of *Solanum*
72 *elaeagnifolium*, a noxious weed, we examined components of its present-day sexual
73 reproduction in a sample of populations of different size and ecological context within and
74 beyond the ancestral range. Determining a potential invader's ability to reproduce is critical,
75 because propagule supply is essential for the founding and maintenance of populations
76 (Barrett 2011). We measured (1) traits related to plants' initial investment of resources

77 toward pistillate (female) sexual function (i.e., investment in flowers and ovules); (2)
78 pollinator visits and pollination success; and (3) components of realized fecundity (i.e., fruit
79 and seed production). The patterns that emerge indicate that plants sampled in the invaded
80 range allocated more resources to flowers and ovules than those in the ancestral range, but
81 received fewer visits from pollinators and did not produce more seeds. We suggest possible
82 reasons for these results and some avenues for further research.

83

84 **2. Materials and methods**

85

86 2.1. Study species

87

88 *Solanum elaeagnifolium* Cav. (silverleaf nightshade, Solanaceae) is a short-lived perennial
89 herb with an ancestral range in the southwest to west-central USA and northern Mexico. By
90 the 1970s the species had spread throughout the USA (Kearney et al. 1969; Munz 1974; Boyd
91 et al. 1984; Mekki 2007) and to all continents except Antarctica (Tscheulin et al. 2009; Brunel
92 et al. 2013). Plants often act as ruderals that colonize disturbed sites (USDA 2006; Tscheulin
93 et al. 2008, 2009). The blue-to-lilac hermaphroditic flowers are nectarless and are pollinated
94 mostly by bees that vibrate their wings to release pollen from the anthers (“buzz” pollination,
95 Buchmann and Cane 1989). Plants appear to be predominantly self-compatible in Arizona
96 (USA), within the ancestral range, and predominantly self-incompatible in Greece, part of the
97 invaded range (Petanidou et al. 2012). Mature fruits are small, dry, globose berries that can
98 contain >100 seeds (Tscheulin et al. 2009; Petanidou et al. 2012). Plants contain teratogenic
99 compounds that are toxic to livestock (Baker et al. 1989; Keeler et al. 1990), lower the yield
100 of many co-occurring crops (Boyd et al. 1984), and reduce the pollination success of native
101 plants (Tscheulin et al. 2009; Vilà et al. 2009; Tscheulin and Petanidou 2013).

102

103 2.2. Study populations

104

105 We studied *S. elaeagnifolium* in southeastern Arizona, USA, and in Greece, where it was first
106 recorded in 1927 (Krigas and Kokkini 2004). In each country, we sampled accessible
107 populations that ranged in size and in the presence of other plant species, and that varied in
108 type of disturbance they experience. In total we chose 27 populations (Table 1), 9 in
109 southeastern Arizona (“AZ”) near the town of Marana and the Santa Rita Mountains in Pima
110 County, and near the towns of Willcox and San Simon in Cochise County; and 18 in Greece
111 (“GR”) on the Island of Lesbos in the northeastern Aegean Sea and near the city of
112 Thessaloniki on the northern mainland.

113

114 To indicate different degrees of invasive spread we scored large monospecific stands with
115 >10,000 plants (see Fig. S1) as “2” on an ordinal scale, monospecific stands with 2,000–
116 10,000 plants as “1”, and small populations with < 2,000 plants intermixed with other non-
117 crop species as “0” (see Fig. S2). Because *S. elaeagnifolium* can propagate vegetatively as
118 well as sexually (Cooley and Smith 1971; Buchmann and Cane 1989; Tscheulin et al. 2008,
119 2009) some “plants” were ramets of the same genet (= clone). It should be kept in mind that
120 when we refer here to “plants” we may in some cases be describing different ramets of the
121 same genet. We also scored populations on roadsides, rangelands, and wastelands that were
122 exposed to periodic mowing or trampling as receiving “surface disturbance”, and those
123 associated with agricultural fields that were exposed to deeper soil turnover as being “tilled”.

124

125 Population sizes and disturbance regimes differed between AZ and GR samples

126 (Table 1). In AZ we studied 3 small and 2 medium-sized populations that

127 experienced surface disturbance, and one small, 2 medium-sized, and one large that
128 were tilled. In GR we studied 3 small, 2 medium-sized, and 3 large populations that
129 experienced surface disturbance and 9 large that were tilled. Thus there were more
130 large monospecific stands among GR populations, and more monospecific stands
131 among tilled populations.

132

133 2.3. Investment in flowers and ovules

134

135 In each study population we tagged 29-60 plants at random. We estimated the number of
136 flowers produced by each tagged plant based on the number of fruits it ultimately produced
137 (see Section 2.5) as:

138

139 $\# \text{ flowers per plant} = \# \text{ fruits per plant} \times (\text{mean } \# \text{ OP flowers} / \text{mean } \# \text{ OP fruits})$

140

141 where “#” signifies “number of” and “OP” refers to open-pollinated flowers and fruits from
142 an experimental study of pollen limitation (see Section 2.4). As a further measure of
143 investment in female function we collected ovaries from 1–2 randomly-chosen flowers on
144 each tagged plant and counted ovules. To estimate total per-plant ovule numbers we
145 multiplied mean per-flower ovule count for each population by estimated flowers per plant for
146 each tagged plant in that population.

147

148 2.4. Pollinator visits and pollination success

149

150 We studied pollinator visits during the peak flowering period of *S. elaeagnifolium* (June–
151 September) in 2006 in Arizona, 2006 and 2014 on Lesvos, and 2007 near Thessaloniki (Table

152 1). We surveyed each population several times (twice in Arizona and near Thessaloniki and
153 four times in Lesvos, all 20-30 days apart). During each survey we spent one or two days in
154 each population and took four 15-minute pollinator censuses on each day (total 60 or 120
155 minutes per population). During each census, we recorded the number of flower visits by
156 each insect that entered a transect 25m long × 2m wide. At noon each day we counted all
157 open flowers in the transect and from this calculated mean visits per flower per hour. All
158 observations were made on sunny calm days during peak insect activity, between 0600 and
159 1300 h in AZ, and 0830 and 1500 h in GR. Although all populations were surveyed more
160 than once, we based analyses only on the survey that yielded the highest visitation to flowers.
161 This approach allowed us to compare the local maximum of flowering and pollination across
162 populations. The additional surveys of each population added to our sample of pollinators, as
163 did an additional 15 minutes spent after each census netting flower visitors outside of
164 transects. Collected specimens were identified to species whenever possible and are
165 deposited in the Melissotheque of the Aegean, Laboratory of Biogeography and Ecology,
166 University of the Aegean (Petanidou et al. 2013).

167

168 As a measure of pollination success, we calculated a Pollen Limitation Index (PLI) for each
169 population (Tscheulin and Petanidou 2013):

170

171 $PLI = 1 - [(average \# \text{ seeds set after open pollination}) / (average \# \text{ of seeds set after pollen}$
172 $\text{ addition})]$

173

174 A value of 0 suggests that open pollination is maximally effective, whereas 1 represents
175 complete pollen limitation. We generated PLI values by marking 1-2 pairs of flower buds on
176 each of the 29-60 tagged plants in each population, and pollinating one of each pair chosen at

177 random (pollen addition, “PA”) while leaving the other untreated (open pollinated, “OP”).
178 Pollen was collected in the morning from several donor plants 5–20 m from each recipient
179 using a tuning fork or by shaking the anthers into a clean Petri dish, and was applied to
180 stigmas with a clean toothpick (Tscheulin et al. 2009; Petanidou et al. 2012). We counted
181 viable seeds in mature fruits 5–6 weeks later, and calculated the mean number of seeds per
182 fruit over all flowers receiving the same treatment in a population. In the few cases where
183 PLI was negative the value was set to zero before analysis.

184

185 2.5. Fruit and seed set

186

187 At the end of the flowering season we measured plant height of each of the 29-60 tagged
188 plants in each population to the nearest cm, as a proxy for overall size. At the same time we
189 counted all fruits produced by each tagged plant, and considered the mean of these counts for
190 each population as one component of average realized fecundity. Mean seeds per flower in
191 the OP treatment served as another component, and when multiplied by the average estimate
192 of flowers per plant for each population yielded a final component, the estimated mean total
193 seeds per plant for that population.

194

195 2.6. Data analysis

196

197 Some populations were destroyed or damaged before the study was completed; for these we
198 analyzed only data collected before destruction (populations 11, 16, 24; Table 1) or from
199 plants that escaped damage (populations 3, 7, 17). Seed set in GR population 11 was low
200 even after pollen was added by hand. Because GR populations tend to be self-incompatible
201 (Petanidou et al. 2012) this suggests low genetic diversity, and preliminary investigation

202 supports this hypothesis (R. Kariyat et al. unpublished). We excluded GR population 11 from
203 analyses of seed and fruit set and PLI, but included its fecundity values in Table 2 and figures.
204

205 Our AZ and GR populations are samples from those geographic areas. In this sense the
206 differences attributed to “country” in analyses cannot be taken to represent Arizona (or the
207 USA) vs. Greece overall, nor the overall ancestral vs. invaded ranges. Nonetheless it is
208 legitimate to compare how aspects of investment in female sexual function (i.e., flowers and
209 ovules), pollination, and fecundity varied in our samples with population size score and type
210 of disturbance, using ANOVA or ANCOVA. When multiple independent variables could be
211 expected *a priori* to contribute to variation in a response variable, we took a model-selection
212 approach based on minimizing AICc. Imbalance in our sample of AZ and GR populations
213 did not permit estimation of country \times population size \times disturbance interactions. For whole-
214 plant traits we included plant height as a covariate, since total flower, ovule, and fruit
215 production generally scale with plant stature, and AZ and GR samples might differ in scaling
216 relationships. For per-flower fecundity variables, we included pollinator visit rate and ovule
217 number as covariates. To help evaluate any apparent nonlinear relationships between
218 reproductive variables and population size we treated population size score as a continuous
219 variable and included second-order as well as first-order terms. With the exception of plant
220 height, analyses were based on grand means for populations, because we rarely obtained
221 multiple values per plant for other measured variables, so that plants nested within
222 populations served as the error term. In any event we are concerned here first and foremost
223 with patterns of variation among populations. For plant height, we could use plants nested
224 within populations as the error term and treat population nested within country as a random
225 effect. We transformed variables as needed to normalize model residuals. All analyses were

226 implemented in JMP Pro 11 (SAS Institute, Cary, North Carolina, USA). Table S1
227 summarizes models and variable transformations used in analyses.

228

229 **3. Results**

230

231 3.1. Investment in flowers and ovules

232

233 In our AZ sample mean plant height increased from small to medium-sized to large
234 populations (43.4 cm, 62.0 cm, 71.2 cm, respectively), whereas no such pattern appeared in
235 GR populations (67.6 cm, 66.8 cm, 64.7 cm, respectively; Fig. 1; $F_{1,19,17} = 4.252$, $P = 0.053$
236 for the interaction between country and population size from ANOVA using ln-transformed
237 height values). Similarly, tilled AZ populations supported taller plants on average than did
238 populations experiencing surface disturbance (67.8 cm and 43.4 cm, respectively), whereas
239 this was not true in GR populations (65.1 cm and 65.4 cm, respectively; $F_{1,19,14} = 4.664$, $P =$
240 0.044 for the interaction between country and disturbance type).

241

242 Estimated mean total flower production per plant was positively related to plant
243 height ($F_{1,18} = 10.077$, $P = 0.005$ from ANOVA with ln-transformed values of flowers
244 per plant). The slope of this relationship was not obviously different for AZ and GR
245 populations (Fig. 2; $F_{1,18} = 0.251$, $P = 0.623$ for the interaction between country and
246 height). Because AZ plants were smaller on average than GR plants, their estimated
247 total flower production also appeared somewhat smaller (mean \pm SE [N]: $309.5 \pm$
248 111.62 flowers [8] vs. 403.0 ± 89.11 flowers [14]).

249

250 Ovule number is a more precise proxy than flower number for initial female
251 investment. In both AZ and GR populations ovule number per flower increased
252 nonlinearly to an apparent plateau with increasing population size (positive linear and
253 negative second-order effects from polynomial ANCOVA with ln-transformed values
254 of ovules per flower), but the plateau was somewhat lower in AZ than in GR
255 populations (Fig. 3; $F_{1,17} = 4.666$, $P = 0.045$ for the interaction between country and
256 population size). Plant height had a slight negative effect because several small
257 populations had tall plants that produced flowers with few ovules (compare Figs. 1, 3).
258 Overall, AZ plants allocated fewer ovules per flower than GR plants (mean \pm SE [N]:
259 55.3 ± 4.66 ovules [9] vs. 95.3 ± 5.75 [19]; $F_{1,17} = 102.488$, $P < 0.0001$). Since GR
260 plants produced more flowers, they also allocated more to ovules on a per-plant basis.

261

262 3.2. Pollinator visits and pollination success

263

264 Flowers in all AZ populations and GR populations on Lesvos attracted up to six
265 species of buzz-pollinating bees (Table 3). AZ and GR populations shared no species
266 in common, but their bees belonged to equivalent functional groups and often to the
267 same genera (e.g., *Xylocopa* and *Bombus*, Apidae; *Nomia* and *Pseudapis*, Halictidae).
268 Several other insects, mainly small sweat bees (e.g., *Halictus resurgens*, Halictidae),
269 honeybees (*Apis mellifera*), and rarely small bees of the genus *Megachile*, visited
270 flowers on Lesvos without buzzing, collecting pollen that was shed on the surface of
271 anthers and the corolla after flowers were buzzed by other bees or shaken by winds
272 (see Section 4.2). We assumed that non-buzzers did not release new pollen from
273 anthers and had little impact on seed set, and so ignored them in visit rate estimates.

274

275 Observed rates of flower visits by buzzing pollinators decreased nonlinearly with
276 population size overall (Fig. 4; $F_{1,21} = 2.664$, $P = 0.118$ and $F_{1,21} = 13.507$, $P = 0.0014$,
277 respectively, from polynomial ANCOVA for first and second-order effects on square-
278 root transformed values of visit rate). The decrease was less pronounced in AZ than
279 GR populations ($F_{1,21} = 17.283$, $P = 0.0004$ for the interaction between country and
280 population size), but there was no clear difference in visit rates between AZ and GR
281 (mean \pm SE [N]: 0.070 ± 0.084 visits per flower per hour [9] vs. 0.113 ± 0.061 [17];
282 $F_{1,21} = 1.70$, $P = 0.685$). In the smallest populations, mean visit rates remained below
283 one per flower per hour, except for GR population 10 on Lesvos (Table 2). In the 13
284 large GR populations near Thessaloniki (13, 14, 16-18, 20-27) we observed no visits
285 at all, whereas this was not the case in the largest AZ population 7 (Table 2).

286

287 PLI provides one gauge of the effectiveness of pollination; indeed it decreased overall
288 with pollinator visit rate (Fig. 5; $F_{1,21} = 5.352$, $P = 0.031$ from ANCOVA using
289 square-root-transformed values of visit rate) in a similar fashion in populations from
290 AZ and GR ($F_{1,21} = 0.341$, $P = 0.566$ for the interaction between country and visit
291 rate). Overall, PLI was lower in our AZ than GR populations (mean \pm SE [N]: 0.447
292 ± 0.090 [9] vs. 0.878 ± 0.063 [17]; $F_{1,21} = 10.865$, $P = 0.0034$), but there was
293 considerable scatter, with some GR populations (notably 10) having high PLI in spite
294 of high visit rates, and others having lower visit rates but low PLI.

295

296 3.3. Fruit and seed set

297

298 Initial investment in flowers and ovules is expected to contribute to components of
299 realized fecundity such as fruit and seed set. Indeed, of alternative models including

300 ln (plant height), country, and pollinator visit rate, the best model indicated that total
301 number of fruits produced by a plant was positively related to plant height ($F_{1,17} =$
302 11.211, $P = 0.004$ from ANOVA using ln-transformed values of fruits per plant), a
303 relationship that did not obviously differ between AZ and GR populations (Fig. 6;
304 $F_{1,17} = 0.808$, $P = 0.381$ for the interaction between country and height). This result
305 seems logical given that flower number increases with plant height (see Section 3.1).
306 Nonetheless, even though AZ plants were on average slightly smaller than GR plants,
307 fruit production was higher in AZ than GR populations (mean fruits per plant \pm SE
308 [N]: 80.400 ± 17.196 [9] vs. 38.914 ± 13.490 [16]; $F_{1,17} = 12.243$, $P = 0.0027$).
309 Inferior pollination service in GR populations may have contributed to lower
310 fecundity. Whereas pollinator visit rate was excluded from the best model described
311 above (perhaps because it was an imperfect predictor of pollen limitation), the best
312 model when we included PLI as a predictor of total fruits per plant chose PLI, ln
313 (height), and $PLI \times \ln(\text{height})$, but excluded country. In this alternative model, ln
314 (fruits per plant) increased with ln (height) as before ($F_{1,17} = 9.026$, $P = 0.008$) and
315 decreased with PLI ($F_{1,17} = 14.008$, $P = 0.0016$). There was no indication that PLI
316 interacted with plant height.

317

318 Although per-plant fruit production was more strongly related to PLI than to visit rate,
319 the best-fit model for the number of seeds produced per marked, open-pollinated
320 flower did include visit rate in addition to country. Mean seeds per flower increased
321 with visit rate (Fig. 7; $F_{1,21} = 4.479$, $P = 0.046$ from ANCOVA using square-root-
322 transformed values for seeds per flower), and the increase was similar in AZ and GR
323 populations ($F_{1,21} = 0.012$, $P = 0.914$ for the interaction between country and visit
324 rate). Open-pollinated flowers (OP treatment) in AZ populations produced more

325 seeds than those in GR populations (mean seeds per flower \pm SE [N]: 13.611 ± 4.568
326 [9] vs. 4.888 ± 2.964 [16]; $F_{1,21} = 2.605$, $P = 0.121$). In contrast, flowers that had
327 received pollen by hand from distant donors in addition to open pollination (PA
328 treatment) showed the opposite pattern. Those in AZ populations produced fewer
329 seeds than those in GR populations (22.433 ± 6.872 [9] vs. 53.569 ± 4.706 [16]), as
330 expected if one considers only mean differences in numbers of ovules per flower (see
331 Section 3.1).

332

333 **4. Discussion**

334

335 This study was designed to compare aspects of reproduction of *S. elaeagnifolium* in replicate
336 populations within and outside of the ancestral range, in order to shed light on factors that
337 may affect invasion by this species. In what follows we first consider each aspect of
338 reproduction and pollination that we studied, then conclude with implications for future
339 studies.

340

341 4.1. Investment in flowers and ovules

342

343 Our GR populations allocated more than our AZ populations to female sexual function (i.e., to
344 flowers and ovules). Whereas plant stature in AZ populations increased in larger populations
345 and with deeper soil disturbance, this was not evident for GR populations, where plants
346 tended to be as tall as the tallest AZ plants. As a result, GR plants were taller on average, and
347 produced more flowers. They also allocated on average nearly twice as many ovules to each
348 flower.

349

350 Williams et al. (2016) documented evolution of increased plant stature across only 6
351 generations in a mesocosm invasion experiment. The tenure of *S. elaeagnifolium* in Greece
352 might suffice for similar changes, driven by a positive correlation of height and seed dispersal
353 distance (as Williams et al. 2016 speculate for their system) or by selection for greater flower
354 number. Burd (1995) presented a model that predicts that increased ovule number per flower
355 can be selected when pollination service is more variable. In this context, greater allocation to
356 flowers and ovules in GR populations might represent a “bet-hedging” response to greater
357 variation in the Greek pollination environment.

358

359 4.2. Pollinator visits and pollination success

360

361 Plants experienced high visit rates and good pollination success in some Greek populations,
362 such as those on Lesbos, due to the attention of native bees that resemble those in Arizona.
363 Both AZ and GR populations also hosted similar guilds of non-buzzing flower visitors. Thus
364 *S. elaeagnifolium* is successfully incorporated into networks of plant–pollinator interactions
365 that lack its ancestral pollinators (see also Memmott and Waser 2002; Stout et al. 2006;
366 Kaiser-Bunbury et al. 2009; Vilà et al. 2009). But pollination was far from assured in GR
367 populations, especially those around Thessaloniki, even though Greece is considered a “bee
368 paradise” (Petanidou and Ellis 1993, 1996; Petanidou and Lamborn 2005; Nielsen et al.
369 2011). Use of agricultural chemicals, habitat loss, and industrial development may contribute
370 to bee rarity around Thessaloniki, and frequent tilling may exclude ground-nesting bees (see
371 also Williams and Kremen 2007). Due to limited flight range (Greenleaf et al. 2007), many
372 bees cannot add large monospecific stands to a foraging itinerary that requires access to water
373 and a progression of flowers sufficient for a complete life cycle. Thessaloniki exemplifies

374 what we term an *invasive desert*—a monospecific stand that supports strikingly low
375 ecological diversity (see Fig. S1).

376

377 Although we recorded no buzzing pollinators in most Thessaloniki populations, flowers did
378 set some seeds. Without vibration, it is difficult to extract pollen from the anthers of *S.*
379 *elaegnifolium*, but it remains possible that pollen grains are small enough (< 30µm; Luna-
380 Cavazos and García-Moya 2002; Burkart et al. 2014) to be shaken out by local “etesian”
381 winds on hot summer days. The presence of such pollen might explain visits by honey bees
382 and other non-buzzing bees, which might cause some seed set, as appears to happen with
383 hover flies visiting (and not buzzing) *Solanum dulcamara* (Waser et al. 2011). If this
384 unexpected pollination occurs but is highly variable, its variation might contribute to greater
385 sexual allocation by the bet-hedging mechanism discussed above.

386

387 4.3. Fruit and seed set

388

389 Although GR plants produced more flowers and ovules than AZ plants, they did not achieve
390 greater fecundity in terms of fruit or seed set. This was true especially in large monospecific
391 stands, which had large PLI values. These results suggest that GR populations received
392 poorer pollination service, and perhaps also pollen of poorer “quality” (sensu Waser and Price
393 1983, p. 356; Aizen and Harder 2007). GR plants are less self-compatible than those in our
394 AZ populations (see Petanidou et al. 2012). Thus the availability of genetically-compatible
395 pollen donors may be lower, especially if some populations are formed by extensive
396 vegetative spread through rhizomes. We excluded one GR population from analyses because
397 we suspected that low genetic variability caused pollen-supplemented flowers to have very
398 low fecundity (see Section 2.6).

399

400 4.4. Implications for future studies

401

402 Our results suggest that increased investment in female sexual function accompanies
403 dispersal of *S. elaeagnifolium* beyond the ancestral range as well as transition from
404 small ruderal populations mixed with other species to large monospecific populations.
405 This greater investment occurs in concert with reduced self-compatibility (Petanidou
406 et al. 2012), and does not yield higher realized fecundity, at least in large GR
407 populations, perhaps because such environments are unfavorable for pollinators and
408 compatible mates are few.

409

410 Nonetheless, *S. elaeagnifolium* is obviously successful as an invasive. Its aggressive spread
411 appears to be favored by the same conditions in AZ and GR populations: deep soil
412 disturbance over large areas, which may facilitate spread by rhizome fragments. While this
413 does suggest some opportunities for control, such as replacement of tilling by mowing, many
414 questions remain for further study.

415

416 For closer comparison with Greece, a priority is to extend the study of pollination and
417 reproduction to additional populations in more Mediterranean climates within the ancestral
418 range, as opposed to the desert climates of Arizona. The species is found in Mediterranean-
419 climate southern California (Munz 1974), South Africa (Boyd et al. 1984), and Australia (Zhu
420 et al. 2013). Additional observations of pollinator visits in the largest GR populations (and
421 elsewhere) also would be useful, in part to document temporal variation. Also valuable would
422 be further investigation of the possibility of pollination via wind and non-buzzing visitors.

423

424 We have noted that low seed set and high PLI might be influenced by paucity of compatible
425 pollen even if pollinator visits are common. In our experimental pollen additions we chose
426 donors 5-20 m from recipient flowers. Perhaps on this spatial scale plant ramets usually
427 belong to different genetic individuals, whereas at shorter scales, over which pollinators
428 transfer most pollen in dense populations (Waser 1982), ramets often belong to the same
429 genet and so mates tend to be incompatible. This could be explored using genetic analysis to
430 characterize the spatial extent of clonal spread via rhizomes in populations of different sizes
431 and disturbance regimes (see Ellstrand and Roose 1987).

432

433 Our emphasis on sexual reproduction and aboveground allocation leaves unanswered
434 questions about belowground allocation to rhizomes. Analysis of the extent of individual
435 genets might be combined with measures of vegetative spread via rhizomes in populations
436 with different characteristics. In a preliminary study (R. Kariyat et al. unpublished), seeds
437 performed poorly in many respects (e.g., in plant establishment) compared to rhizome pieces
438 collected from the same plants in large Thessaloniki populations. Space filling by rhizomes
439 (along with seeds) may occur in tilled agricultural lands, where *S. elaeagnifolium* is
440 remarkably successful; seeds also might allow rapid colonization of new suitable habitat
441 fragments (see Williams et al. 2016).

442

443 Finally, any differences in the expression of sexual characteristics of *S.*
444 *elaegnifolium* plants documented here, as functions of population size, disturbance
445 regime, and country, might logically represent adaptive or neutral genetic differences
446 that trace back to those individuals that founded Greek populations and those in
447 Arizona landscapes that have been recently altered. Founder effects can be detected
448 through reduced genetic diversity at neutral or quasi-neutral marker loci (Dlugosch

449 and Parker 2008). Alternatively, any differences in character expression might
450 represent adaptations arising during decades of habitation in Greece or in altered
451 Arizona landscapes. Finally, they might be plastic responses to local conditions (Zhu
452 et al. 2013). The classic approach (Langlet 1971) to distinguishing genetic and plastic
453 mechanisms of phenotypic differentiation is to grow plants in common gardens, and
454 to do this reciprocally between ancestral and invaded ranges.

455

456 **Conflict of interest**

457 The authors state that they have no conflict of interest.

458

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468

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470

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635 TP designed the study with input from NW. TP and AK carried out fieldwork. MP and TT
636 analysed the data. TP, NW, and MP wrote the manuscript. All authors edited the manuscript.

Table 1. *Solanum elaeagnifolium* populations studied in Arizona (ancestral range) and Greece (invaded range). Criteria for evaluating type of disturbance and population size score are described in the text. Dates of observation of pollinators and of hand pollination to assess pollen limitation are given as days/month/year.

Population	Region	Coordinates	Habitat characteristics	Disturbance	Size (# of plants)	Size score	Dates of observation and hand pollination
<i>Arizona</i>							
1. Marana, Kai cotton	Pima	32° 26' 50"N, 111° 18' 12"W	within intensely cultivated cotton field	tilled	ca. 800	0	22–23, 25/07/2006
2. Marana, Kai fallow	Pima	32° 26' 55"N, 111° 18' 13"W	paved road verge	surface	>2000	1	22–23, 25/07/2006
3. Marana, Sanders cotton	Pima	32° 26' 45"N, 111° 14' 40"W	unpaved road verge	surface	>2000	1	22–23, 25/07/2006
4. Marana, Sanders fallow	Pima	32° 28' 03"N, 111° 14' 03"W	margins of intensely cultivated field	tilled	>2000	1	22–23, 25/07/2006
5. Santa Rita, lower	Pima	31° 46' 48"N, 110° 53' 16"W	paved road verge in a conservation area	surface	ca. 100	0	18–19/07/2006
6. Santa Rita, upper	Pima	31° 46' 12"N, 110° 53' 15"W	paved road verge in a conservation area	surface	ca. 500	0	18–19/07/2006
7. San Simon, huge	Cochise	32° 15' 05"N, 109° 11' 10"W	entire surface of a fallow field	tilled	>10,000	2	05–06/08/2006
8. San Simon, Noland	Cochise	32° 15' 19"N, 109° 10' 35"W	margins of intensely cultivated field	tilled	>2000	1	05–06/08/2006
9. Willcox, Moonlight	Cochise	32° 14' 19"N, 109° 46' 41"W	unpaved road verge; wild	surface	ca. 800	0	26/08/2006
<i>Greece</i>							
10. Arisvi	Lesvos	39° 14' 08"N, 26° 13' 31"E	unpaved road verge; naturalized	surface	ca. 1500	0	4-5/07/2014
11. Eressos	Lesvos	39° 08' 24"N, 25° 55' 19"E	residential lawn; naturalized	surface	ca. 150	0	13/08/2006
12. Plomari	Lesvos	38° 58' 17"N, 26° 22' 57"E	paved road verge; naturalized	surface	ca. 100	0	7–8/07/2014
13. Anchialos, Cemetery	Thessaloniki	40° 43' 05"N, 22° 47' 07"E	dry waste land	surface	>10,000	2	24/07/2007
14. Anchialos, Farmakis	Thessaloniki	40° 43' 25"N, 22° 48' 31"E	cultivated field (barley, vetch)	tilled	>10,000	2	01/08/2007
15. Anchialos, Ioannou	Thessaloniki	40° 42' 19"N, 22° 47' 18"E	waste land/yard of a factory	surface	>2000	1	31/07/2007
16. Anchialos, Narlis	Thessaloniki	40° 43' 34"N, 22° 47' 57"E	cultivated field (barley, vetch)	tilled	>10,000	2	02/08/2007
17. Anchialos, Toumba	Thessaloniki	40° 43' 07"N, 22° 49' 05"E	waste land	surface	>10,000	2	02/08/2007
18. Anchialos, Vaggelis	Thessaloniki	40° 43' 40"N, 22° 48' 28"E	wheat field left fallow	tilled	>10,000	2	03/08/2007
19. Panorama	Thessaloniki	40° 35' 22"N, 23° 02' 38"E	semi-natural meadow in urbanized area	surface	>2000	1	04, 11/08/07
20. Raidestos, Atlanta	Thessaloniki	40° 32' 14"N, 23° 02' 49"E	wheat field	tilled	>10,000	2	13/07/2007
21. Raidestos, onion	Thessaloniki	40° 31' 05"N, 23° 03' 23"E	onion field	tilled	>10,000	2	30/07/2007
22. Raidestos, wheat	Thessaloniki	40° 30' 59"N, 23° 04' 25"E	wheat field	tilled	>10,000	2	11/07/2007

23. Raideostos, Wind	Thessaloniki	40° 30' 56"N, 23° 04' 21"E	semi-natural Mediterranean scrub	surface	>10,000	2	14/07/2007
24. Sindos, TEI	Thessaloniki	40° 39' 12"N, 22° 48' 50"E	wheat field	tilled	>10,000	2	19/07/2007
25. Sindos, cultivated	Thessaloniki	40° 41' 47"N, 22° 48' 35"E	waste land, partly cultivated	tillage	>10,000	2	15/07/2007
26. Triadi, Kosmidis	Thessaloniki	40° 32' 33"N, 23° 02' 37"E	wheat field left fallow	tillage	>10,000	2	20/07/2007
27. Triadi, Namco	Thessaloniki	40° 32' 57"N, 23° 02' 09"E	wheat field left fallow	tillage	>10,000	2	26/07/2007

Table 2. Aspects of allocation to female sex function, pollination success, and realized fecundity in the study populations. The symbol # means “number of” and values are grand means for marked plants in each population \pm SE (sample size). Sample size is number of plants for plant height and fruits per plant; otherwise number of flowers. Visit rate includes only visits by pollinators that buzzed flowers. OP = open-pollinated; PA = cross pollen added by hand; PLI = Pollen Limitation Index. * = study population destroyed or damaged during the study.

Study populations	plant height (cm)	# ovules flower ⁻¹	# flowers per plant	# visits flower ⁻¹ h ⁻¹	# seeds/ flower (OP)	# seeds/ flower (PA)	PLI	# fruits per plant
<i>Arizona</i>								
1. Marana, Kai cotton	69.0 \pm 2.6 (21)	32.3 \pm 1.96 (35)	713	0.046	0.7 \pm 0.64 (56)	1.8 \pm 0.65 (42)	0.61	38.2 \pm 9.77 (19)
2. Marana, Kai fallow	62.3 \pm 1.7 (29)	71.0 \pm 2.27 (73)	-	0.000	0 (75)	2.7 \pm 0.96 (73)	1	2.2 \pm 1.05 (18)
3. Marana, Sanders cotton	54.7 \pm 1.3 (41)	69.1 \pm 3.38 (30)	1205	0.027	0.8 \pm 0.60 (57)	2.4 \pm 1.21 (49)	0.67	30.0 \pm 6.13 (20)
4. Marana, Sanders fallow	67.4 \pm 3.6 (21)	68.7 \pm 3.07 (38)	387	0.014	13.4 \pm 2.18 (60)	36.9 \pm 3.12 (61)	0.64	203.1 \pm 20.77 (19)
5. Santa Rita, lower	29.9 \pm 1.1 (33)	41.8 \pm 3.80 (24)	44	0.034	8.7 \pm 1.50 (60)	6.6 \pm 0.88 (51)	0.00	33.7 \pm 3.01 (61)
6. Santa Rita, upper	38.9 \pm 1.0 (41)	43.5 \pm 1.84 (24)	46	0.252	16.4 \pm 1.73 (47)	14.9 \pm 1.67 (45)	0.00	35.0 \pm 3.90 (29)
7. San Simon, huge	71.2 \pm 2.8 (20)	53.6 \pm 1.74 (37)	371	0.044	37.6 \pm 2.92 (77)	39.5 \pm 2.93 (74)	0.05	163.8 \pm 37.13 (20)
8. San Simon, Noland	63.4 \pm 2.0 (21)	64.5 \pm 3.19 (37)	1636	0.015	33.0 \pm 3.32 (68)	46.2 \pm 3.94 (60)	0.29	120.4 \pm 22.84 (30)
9. Willcox, Moonlight	31.1 \pm 0.9 (54)	52.9 \pm 2.39 (31)	40	0.195	11.9 \pm 3.33 (41)	50.9 \pm 4.70 (29)	0.77	17.5 \pm 3.76 (32)
<i>Greece</i>								
10. Arisvi	73.0 \pm 1.5 (30)	49.5 \pm 1.53 (30)	253	1.130	8.7 \pm 1.81 (57)	24.1 \pm 2.86 (58)	0.64	102.0 \pm 16.59 (30)
11. Eressos*	-	49.9 \pm 6.63	-	0.620	48.7 \pm 4.30	60.7 \pm 5.13	0.20	-

12. Plomari	63.7±1.7 (30)	(13) 52.5±1.79 (30)	157	0.170	(46) 0.1±0.03 (59)	(42) 0.1±0.05 (59)	0	15.9±2.01 (30)
13. Anchialos, Cemetery	68.4±1.9 (29)	94.6±4.39 (60)	1234	0.000	2.1±1.94 (58)	4.7±1.40 (58)	0.55	41.1±5.40 (25)
14. Anchialos, Farmakis	71.1±1.9 (30)	108.6±4.86 (56)	394	0.000	1.7±1.13 (60)	71.9±4.86 (60)	0.98	59.1±7.82 (26)
15. Anchialos, Ioannou	68.4±2.0 (30)	106.9±3.91 (60)	315	0.000	1.4±1.20 (60)	68.6±5.31 (60)	0.98	52.5±7.22 (26)
16. Anchialos, Narlis*	-	111.5±6.16 (60)	-	-	-	-	-	-
17. Anchialos, Toumba*	-	77.78±3.77 (60)	-	0.000	0 (30)	64.6±7.93 (30)	1	-
18. Anchialos, Vaggelis	76.1±1.7 (30)	113.3±5.15 (60)	647	0.000	0.3±0.16 (60)	66.6±6.29 (60)	1	43.1±5.51 (29)
19. Panorama	65.1±2.4 (30)	104.7±7.39 (60)	317	0.008	0.9±0.35 (60)	56.9±10.59 (60)	0.98	52.9±6.99 (30)
20. Raideostos, Atlanta	54.3±2.5 (30)	99.8±5.03 (60)	103	0.000	2.9±1.58 (60)	51.0±5.60 (60)	0.94	19.0±3.85 (24)
21. Raideostos, onion	52.5±1.8 (30)	98.2±5.26 (61)	195	0.000	2.5±1.68 (60)	61.8±4.21 (60)	0.96	22.0±2.94 (31)
22. Raideostos, wheat	65.2±1.6 (30)	109.6±3.90 (60)	75	0.000	0.7±0.38 (60)	28.3±4.37 (60)	0.98	6.2±2.36 (25)
23. Raideostos, Wind	58.3±1.5 (31)	117.3±5.09 (60)	109	0.000	0.5±0.20 (62)	63.8±4.80 (62)	0.99	14.1±3.15 (25)
24. Sindos, TEI*	-	119.3±5.44 (72)	-	0.000	2.3±1.65 (70)	67.0±6.55 (70)	0.97	-
25. Sindos, cultivated	84.3±2.3 (37)	91.1±3.90 (73)	416	0.000	2.7±1.25 (72)	64.1±4.17 (72)	0.96	56.2±8.77 (33)
26. Triadi, Kosmidis	51.5±1.8 (30)	113.1±5.25 (60)	311	0.000	2.0±1.22 (58)	54.9±5.00 (58)	0.96	20.7±5.75 (15)
27. Triadi, Namco	63.4±1.4 (30)	114.1±4.61 (60)	1022	0.000	0.8±0.80 (60)	48.1±3.99 (60)	0.98	17.0±1.95 (29)

Table 3. Bee taxa recorded on *Solanum elaeagnifolium* flowers in our study populations, as indicated by “+”. All taxa “buzzed” flowers, and thus are considered major pollinators, except those marked with *. Lesvos populations were regularly visited by non-buzzing honeybees that collected spilled pollen.

Population	<i>Apidae</i>							<i>Andrenidae</i>			<i>Halictidae</i>			
	<i>Amegilla</i> spp.	<i>Bombus terrestris</i>	<i>Exomalopsis solani</i>	<i>Melissodes</i> spp.	<i>Xylocopa californica</i> ssp. <i>arizonensis</i>	<i>Xylocopa iris</i>	<i>Xylocopa violacea</i>	<i>Centris atripes</i> , <i>C. rodopis</i>	<i>Apis mellifera</i> *	<i>Protandrena mexicanorum</i>	<i>Protoxaea gloriosa</i>	<i>Pseudapis bispinosa</i> (= <i>Nomia bispinosa</i>)	<i>Nomia. tetrazonata</i>	unidentified small bees*
Arizona														
1. Marana, Kai cotton	-	-	+	+	+	-	-	-	-	-	+	-	+	+
2. Marana, Kai fallow	-	-	+	+	+	-	-	-	-	-	+	-	+	+
3. Marana, Sanders cotton	-	-	+	+	+	-	-	-	-	-	+	-	+	+
4. Marana, Sanders fallow	-	-	+	+	+	-	-	-	-	-	+	-	+	+
5. Santa Rita, lower	-	-	-	-	+	-	-	+	-	+	+	-	+	+
6. Santa Rita, upper	-	-	-	-	+	-	-	+	-	+	+	-	+	+
7. San Simon, huge	-	-	-	-	-	-	-	-	-	-	+	-	-	+
8. San Simon, Noland	-	-	-	-	-	-	-	-	-	-	+	-	-	+
9. Willcox, Moonlight	-	-	+	-	+	-	-	-	-	-	-	-	-	+
Greece														
10. Arisvi	-	+	-	-	-	+	-	-	+	-	-	+	-	+
11. Eressos	+	+	-	-	-	+	-	-	-	-	-	+	-	+
12. Plomari	-	-	-	-	-	+	-	-	+	-	-	+	-	+
13. Anchialos, Cemetery	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14. Anchialos, Farmakis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15. Anchialos, Ioannou	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16. Anchialos, Narlis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17. Anchialos, Toumba	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18. Anchialos, Vaggelis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19. Panorama	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20. Raidestos, Atlanta	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21. Raidestos, onion	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22. Raidestos, wheat	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23. Raidestos, Wind	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24. Sindos, TEI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25. Sindos, cultivated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26. Triadi, Kosmidis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27. Triadi, Namco	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 1. Relationship between mean plant height and population size score. Plant stature increased with population size in our US (open circles) but not in our GR (filled circles) populations.

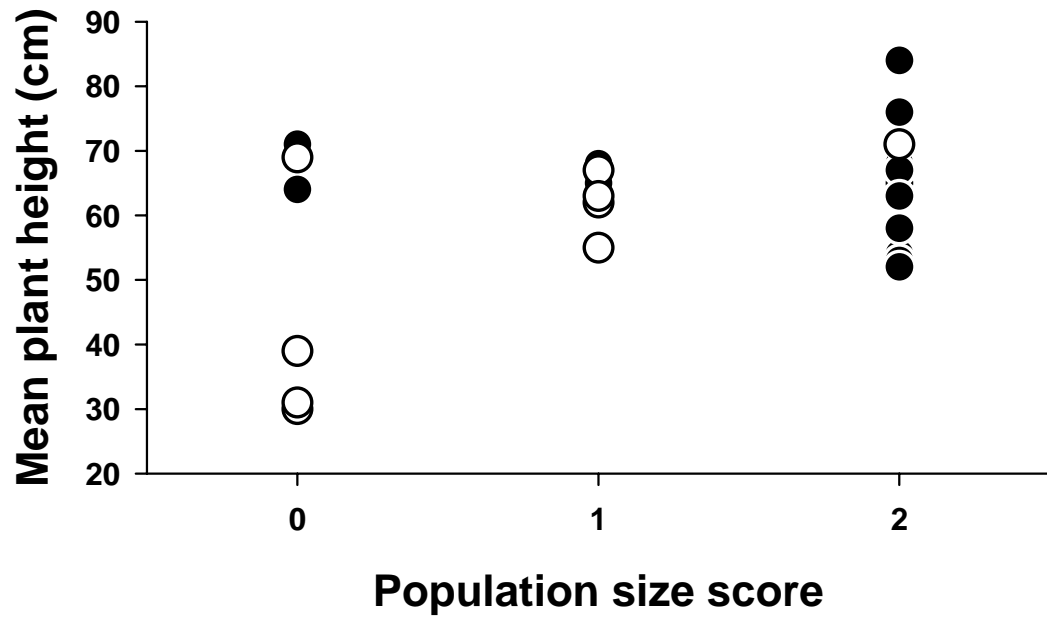


Fig. 2. Estimated total flowers per plant as a function of plant stature. Flower number increased with plant height in both our US (open circles) and in our GR (filled circles) populations; US plants were smaller on average and produced fewer flowers on average.

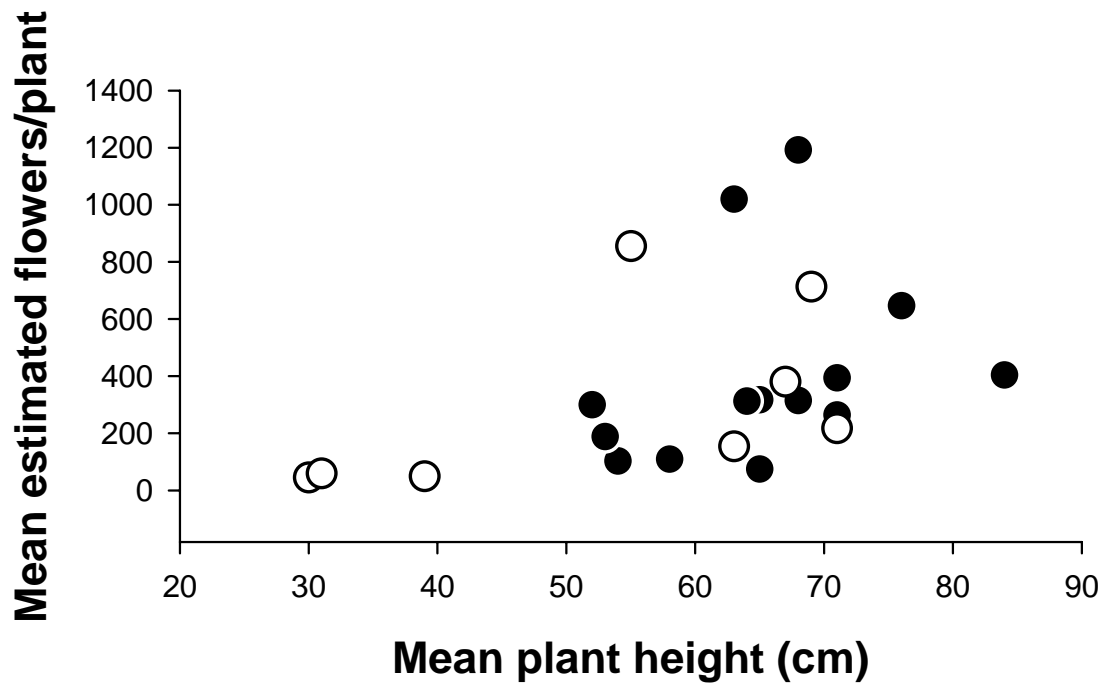


Fig. 3. Nonlinear increase in ovule number per flower with population size. In both our US (open circles) and in our GR (filled circles) populations, ovule number increased to an apparent plateau, which was lower in the US sample.

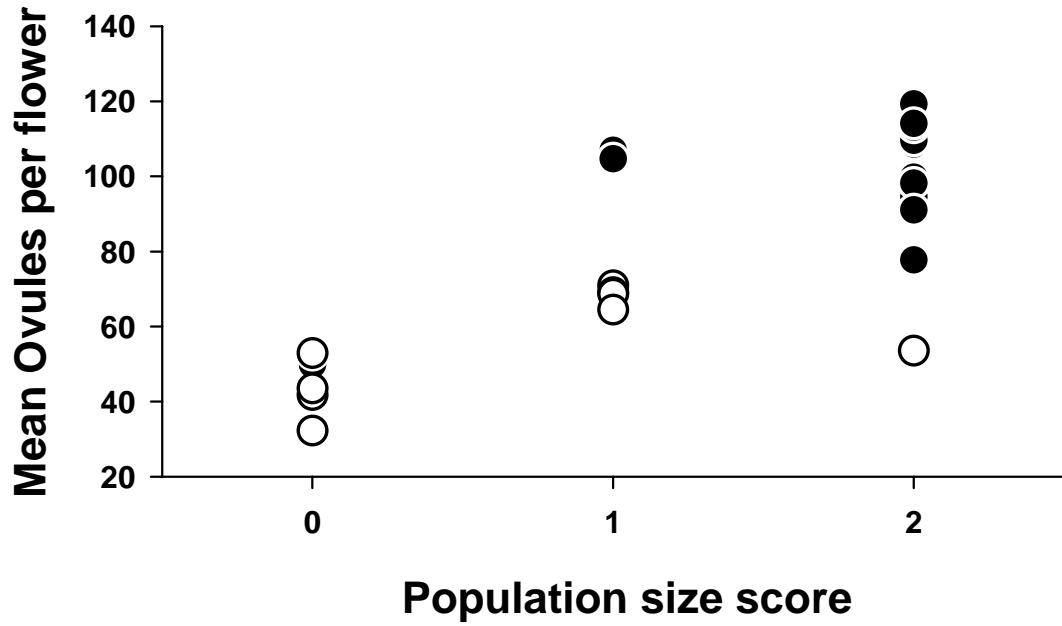


Fig. 4. Nonlinear decrease in pollinator visits with size of our US (open circles) and GR (filled circles) populations. Observed visitation rates were higher in smaller populations, and highest in two populations on Lesvos; they declined to very low values or to zero in the largest populations, especially those around Thessaloniki.

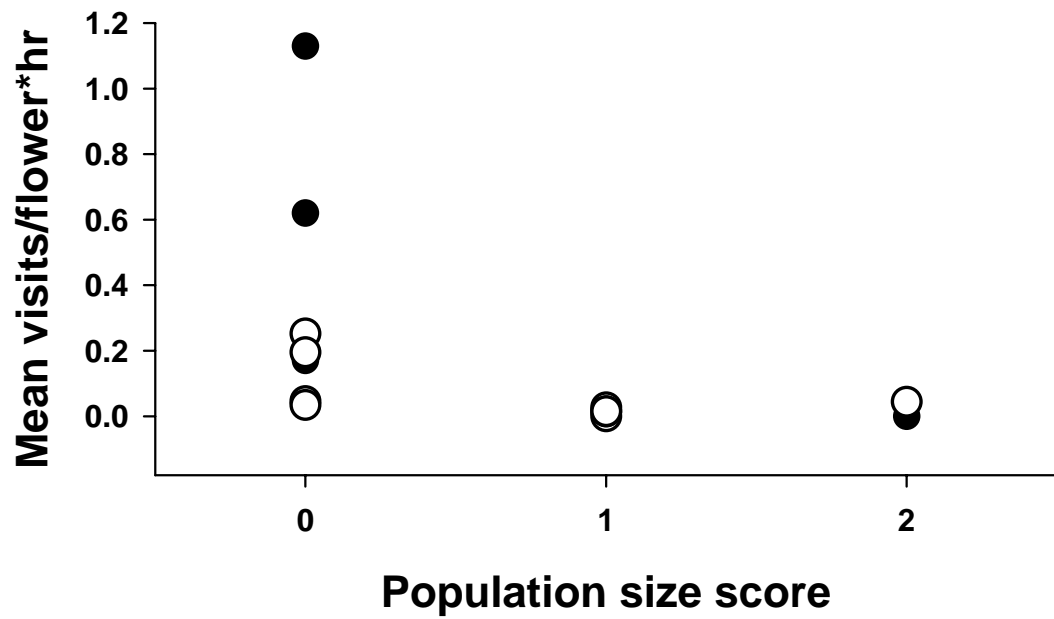


Fig. 5. Pollen Limitation Index vs. pollinator visit rate. PLI decreased with pollinator visit rate in both our US (open circles) and in our GR (filled circles) populations, although the range of visit rates was far greater in GR.

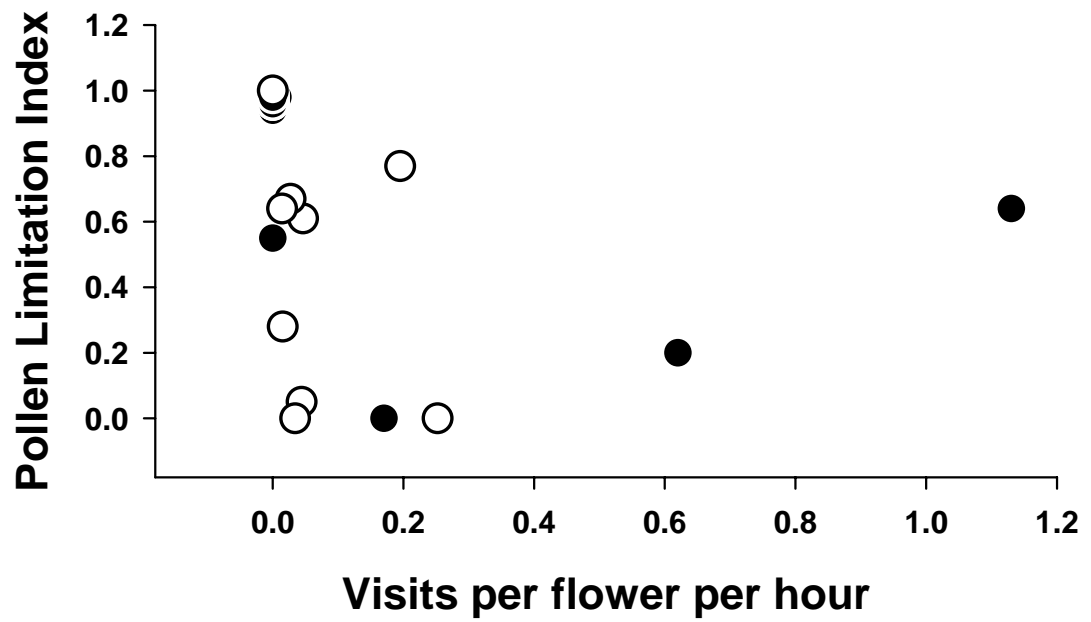


Fig. 6. Total fruits per plant vs. plant stature. Fruit production increased close to linearly with plant height in both our US (open circles) and in our GR (filled circles) populations, but the overall mean was greater for US plants.

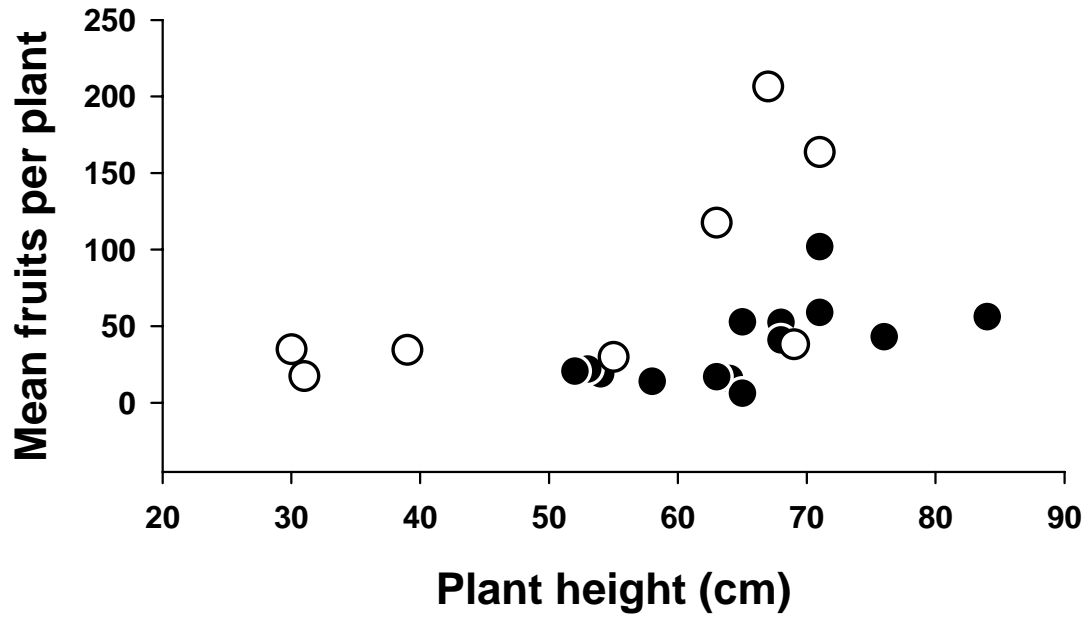


Fig. 7. Seeds per flower vs. pollinator visit rate. Mean seed set increased with visits in both our US (open circles) and in our GR (filled circles) populations. Flowers produced more seeds on average in US than in GR populations; it is not obvious from the figure that 15 GR populations are clustered near the origin (0,0), as Table 2 shows.

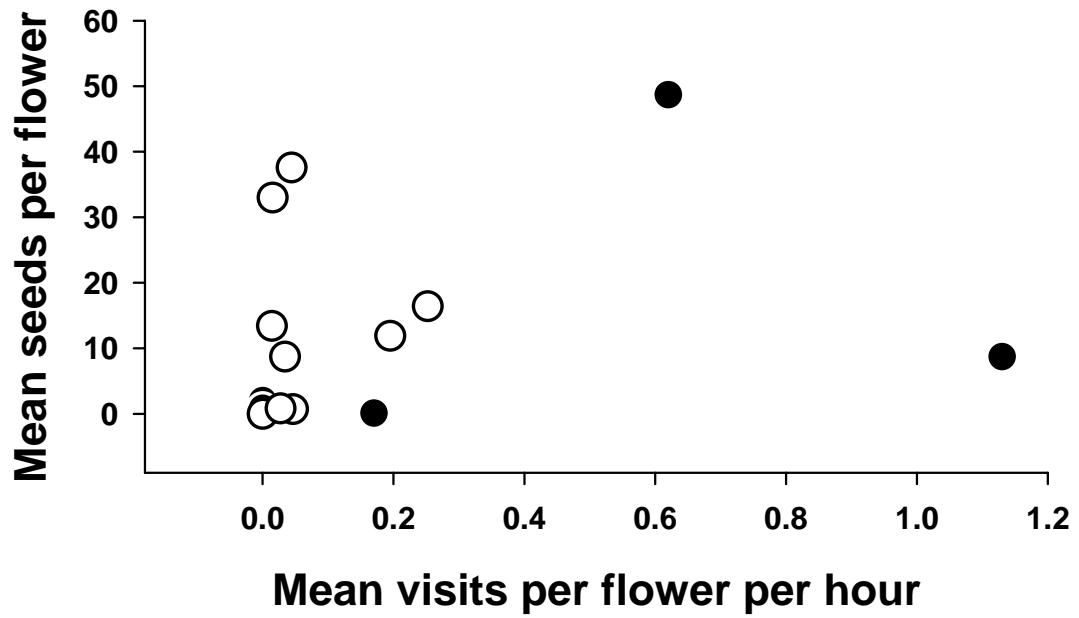


Table S1. Details of statistical models from which results reported in the text are derived.

“VARIABLE” = response variable; SOURCE = predictor variable; “Country” = country of origin of the sample of study populations; “Pop size” = population size score (0, 1, or 2) treated as a continuous variable; “Disturbance” = type of soil disturbance (surface vs. tilled) experienced by a population. Two alternative analyses are included for fruits per plant, as described in the text. The analysis of seeds per flower refers to marked flowers in the open-pollinated (OP) treatment.

VARIABLE	SOURCE	Df	F	P	COMMENTS
ln (Plant height) Model $R^2_{adj} = 0.724$	Country	1,19.20	0.821	0.376	REML ANCOVA
	Pop size	1,19.17	2.554	0.126	based on 775
	Disturbance	1,19.14	4.875	0.040	individual values;
	Country \times pop size	1,19.17	4.252	0.053	only fixed effect
	Country \times disturbance	1,19.14	4.664	0.044	tests given
ln (Flowers/plant) Model $R^2_{adj} = 0.364$	Country	1,18	0.034	0.879	ANCOVA
	Plant height	1,18	10.077	0.005	based on
	Country \times plant height	1,18	0.251	0.623	population means
ln (Ovules/flower) Model $R^2_{adj} = 0.951$	Country	1,17	102.488	<0.0001	Polynomial
	Plant height	1,17	9.537	0.007	ANCOVA
	Pop size	1,17	9.227	0.007	based on
	Pop size ²	1,17	62.645	<0.0001	population means
	Country \times pop size	1,17	4.666	0.045	
$\sqrt{}$ (Visits/flower/hour) Model $R^2_{adj} = 0.786$	Country	1,21	0.170	0.685	Polynomial
	Pop size	1,21	2.664	0.118	ANCOVA
	Pop size ²	1,21	13.507	0.0014	based on
	Country \times pop size	1,21	17.283	0.0001	population means
PLI Model $R^2_{adj} = 0.490$	Country	1,21	10.865	0.0034	ANCOVA
	$\sqrt{}$ (Visits/flower/hour)	1,21	5.352	0.031	based on
	Country \times	1,21	0.341	0.566	population means
	$\sqrt{}$ (Visits/flower/hour)				
ln (Fruits/plant) Model $R^2_{adj} = 0.409$	Country	1,17	12.243	0.0027	ANCOVA
	ln (Plant height)	1,17	11.211	0.0038	based on
	Country \times ln (plant height)	1,17	0.808	0.381	population means
ln (Fruits/plant) Model $R^2_{adj} = 0.449$	PLI	1,17	14.008	0.0016	ANCOVA
	ln (Plant height)	1,17	9.026	0.008	based on
	PLI \times ln (plant height)	1,17	0.148	0.706	population means
$\sqrt{}$ (Seeds/flower) Model $R^2_{adj} = 0.338$	Country	1,21	2.605	0.121	ANCOVA
	$\sqrt{}$ Visits/flower/hour	1,21	4.479	0.046	based on
	Country \times	1,21	0.012	0.914	population means
	$\sqrt{}$ (Visits/flower/hour)				

Fig. S1. An *invasive desert* near Anchialos, in the western metropolitan area of Thessaloniki—a large area taken over by a very aggressive monospecific stand of *S. elaeagnifolium* (image: T. Petanidou).



Fig. S2. A smaller, less aggressive population of *S. elaeagnifolium* in the countryside near Willcox, Arizona (image: T. Petanidou).

