No evidence that seed predators constrain pollinator-mediated trait evolution in a tropical vine

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Running head: Weak effects of seed predators on floral evolution

Abstract

Premise of The Study: Turnover in biotic communities across heterogeneous landscapes is expected to lead to variation in interactions among plants, their mutualists, and their antagonists. Across a fragmented landscape in northern Costa Rica, populations of the euphorb vine *Dalechampia scandens* vary widely in mating systems and associated blossom traits. Previous work suggests that populations are well adapted to the local reliability of pollination by apid and megachilid bees. Here, we test whether variation in the intensity of predispersal seed predation by *Nanobaris* seed weevils also contributes to the observed variation in blossom traits.

Methods: We studied spatio-temporal variation in the relationships between floral advertisement and the probability of seed predation within three focal populations. Then, we assessed among-population covariation of predation rate, pollination reliability, mating system, and blossom traits across 20 populations.

Key Results: The probability of seed predation was largely unrelated to variation in floral advertisement both within focal populations, and among the larger sample of populations. The rate of seed predation was only weakly associated with the rate of cross-pollination (allogamy) in each population but tended to be proportionally greater in populations experiencing less reliable pollination.

Conclusions: These results suggest that geographic variation in the intensity of antagonistic interactions have had only minor modifying effects on the evolutionary trajectories of floral advertisement in plant populations in this system. Thus, pollinator-driven floral trait evolution in *Dalechampia scandens* in the study area appears not to be influenced by conflicting seed-predator-mediated selection.

Key words: conflicting selection; *Dalechampia*; Euphorbiaceae; herkogamy; interaction turnover; phenotypic selection; plant mating systems; seed predation

INTRODUCTION

Changes in biotic assemblages and disruption of species interactions are important biological consequences of global climate change, habitat destruction, and other anthropogenic disruptions of the environment (Magurran, 2016; Urban et al., 2016). Plant species occurring across fragmented or otherwise heterogeneous landscapes often experience variation in communities of competitors, mutualists, and antagonists, and provide excellent opportunities for understanding plant responses to turnover in interactor communities. For example, the ongoing decline of pollinators is expected to reduce the reliability of pollination in plant populations worldwide (Aguilar et al., 2006; Eckert et al., 2010; Potts et al., 2010; Thomann et al., 2013). The most commonly observed plant evolutionary response to pollinator declines is the evolution of greater autonomous selfing rates as a mechanism of reproductive assurance (Moeller, 2006; Eckert et al., 2010; Brys and Jacquemyn, 2012; Opedal et al., 2016a; but see Koski et al., 2017). However, the evolution of floral traits and mating systems in response to changing pollinator communities may not necessarily occur independently from other biotic interactions, such as herbivory and seed predation. Indeed, many studies have demonstrated antagonist-mediated selection on floral traits, and that this selection can sometimes run counter to pollinator-mediated selection (e.g. Strauss and Armbruster, 1997; Gómez and Zamora, 2000; Adler and Bronstein, 2004; Cariveau et al., 2004; Rey et al., 2006; Strauss and Whittall, 2006; Gómez et al., 2009; Kolb and Ehrlen, 2010; Pérez-Barrales et al., 2013; Sun et al., 2016). Therefore, the outcome of plant adaptation to changes in the abundance of one interacting species may be modified by conflicting selective pressures generated by other interactors.

The net strength and direction of selection on floral traits is expected to depend on the relative intensities of mutualistic and antagonistic interactions (Benkman, 2013; Vanhoenacker et al., 2013). All else being equal, the opportunity for mutualist-mediated selection is expected to decrease with increasing interaction intensity, while the opportunity for antagonist-mediated selection is expected to increase with increasing interaction intensity. Therefore, much of the observed variation in selection acting on plant phenotypes may result from spatial and temporal variation in the intensities of species interactions (Thompson, 2005). Spatial turnover in species interactions may be particularly common in anthropogenically disturbed landscapes, because pollinators and other interactors often respond differently to habitat destruction and fragmentation (Cunningham, 2000; Steffan-Dewenter et al., 2001; Garcia and Chacoff, 2007; Magrach et al., 2014; Brudvig et al., 2015).

75 For example, hawthorn trees in northern Spain occurring in more fragmented habitats

76 experienced less reliable pollination by bees and flies, and less frugivory by birds, but more

77 intense seed predation by mice (Garcia and Chacoff, 2007). Similarly, both the bee pollinators

78 and lepidopteran and dipteran seed predators of *Centaurea jacea* in Germany were less

79 abundant in experimental populations located at sites containing less semi-natural habitat, but

80 the effect of landscape structure on interactions were species-specific and depended on the

81 spatial scale analyzed (Steffan-Dewenter et al., 2001). We may therefore expect both the

82 absolute and relative intensities of mutualistic and antagonistic interactions to vary across

83 heterogeneous landscapes, but whether this leads to systematic differences in selection

84 remains an empirical question.

85   The neotropical vine *Dalechampia scandens* L. (Euphorbiaceae) is pollinated by

86 female apid and megachilid bees (Armbruster and Webster, 1982; Armbruster, 1985). A

87 recent study in Costa Rica showed that the floral traits and mating systems of populations

88 varied predictably along a gradient of pollination reliability (Opedal et al., 2016a):

89 populations at pollinator-poor sites had evolved blossom traits associated with greater rates of

90 autonomous selfing, including smaller involucral bracts (a floral-advertisement trait;

91 Armbruster et al., 2005) and reduced herkogamy. While these results suggest that population-

92 mean floral trait values have tracked variation in the reliability of pollination, pollinators may

93 not be the only biotic interactors affecting the evolution of *D. scandens* blossom traits. This

94 was demonstrated by a study in a Mexican population, where the net selection on blossom

95 traits was determined by interactions with both pollinators and predispersal seed predators

96 (Pérez-Barrales et al., 2013). While pollinators selected for larger floral bracts, seed predators

97 apparently responded to the same cues, generating conflicting selection. If seed predators

98 consistently select for reduced floral advertisement, seed-predator-mediated selection might

99 shift trait values away from the mating-system-related optimum determined by the local

100 reliability of pollination. Specifically, if the strength of selection increases with the intensity

101 of predation (Vanhoenacker et al., 2013), more intensively predated populations would be

102 expected to have smaller floral bracts than expected from their historical pollination

103 environment.

104   To assess whether predispersal seed predators modify pollinator-mediated floral trait

105 evolution in *D. scandens*, we first studied spatio-temporal variation in seed-predator-mediated

106 selection, i.e. the relationships between floral advertisement and the probability of seed

107 predation within populations. Second, we assessed the joint influence of mating system and

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108   seed predation rate on among-population divergence in floral advertisement. Specifically, we

109   asked (1) whether seed predators exert selection on floral advertisement, and (2) whether

110   selection varies in time and space depending on the rate of seed predation. Using data from a

111   larger number of populations, we tested (3) whether predation rates covary with pollination

112   reliability among populations, and (4) whether the rate of attack by seed predators predicts

113   population-mean floral trait values after controlling for the effect of mating systems.

114   **MATERIALS AND METHODS**

115   *Study system*

116   *Dalechampia scandens* L. (s.l.) (Euphorbiaceae) is a species complex of perennial woody

117   vines native to the lowland Neotropics (Armbruster, 1985). It occurs in naturally open areas

118   and anthropogenic disturbances, including limestone outcrops, open shrublands, light gaps,

119   and roadsides. Male and female flowers are aggregated into bisexual blossom inflorescences

120   (pseudanthia – 'false flowers'), which function as pollination units. Blossoms are

121   protogynous, with a female phase of c. 2-3 days followed by a bisexual phase. The blossoms

122   are visited and pollinated by female apid and megachilid bees, which collect floral resin from

123   a gland-like structure associated with the male flowers (Armbruster, 1985). Outcrossing rates

124   in four natural populations in Costa Rica ranged from 0.16 to 0.49, indicating a mixed mating

125   system with a tendency towards selfing (Opedal et al., 2016a). The male and female

126   subinflorescences are together subtended by a pair of involucral bracts that open during the

127   day to allow pollination, and close at night to protect the floral tissues (Armbruster, 1985;

128   Armbruster et al., 1997). The bracts are normally creamy white during anthesis, when they

129   function as an advertisement towards pollinators. During fruit maturation, the bracts change

130   color to dark green and increase their rates of photosynthesis, becoming more cryptic and

131   providing carbon for the developing seeds (Pélabon et al., 2015b). A maximum of nine seeds

132   per blossom is dispersed by explosive dehiscence of capsules (Armbruster, 1982). Developing

133   seeds are subject to predation by seed weevils (Curculionidae: Baridinae). The weevils

134   oviposit on blossoms prior to seed development, and may thus use the bracts to find receptive

135   inflorescences and/or the bract size as a cue to determine the future availability of resources

136   for their larvae (Pérez-Barrales et al., 2013).

137   During the peak blooming seasons (Oct.-Dec.) of 2014 and 2015, we studied 20

138   populations in north-western Costa Rica (see Appendix S1 in the Supplementary Data with

139   this article for exact locations), belonging to the 'large-glanded' taxon of the *D. scandens*

140   complex (Bolstad et al., 2014). Neighboring populations are separated by 1.8 – 36.5 km, and

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141   contemporary gene flow between populations is uncommon or absent (Opedal et al., 2017b).

142   Further details about the populations are provided in Opedal et al. (2016).

143   *Effects of floral advertisement on seed predation within populations*

144   We studied the effect of a floral-advertisement trait on the probability of seed predation in

145   each of three populations, one of which was studied in two consecutive years, as part of a

146   long-term study of spatio-temporal variation in selection on *Dalechampia scandens* blossom

147   traits (see also Pérez-Barrales et al., 2013). During each study, we marked distinct patches

148   comprising one or sometimes several intertwined individuals. In each patch, we recorded

149   daily the number of pollen grains deposited onto the stigmas of individually-marked

150   blossoms. On the first day of the bisexual phase (the day the first male flower opened), we

151   measured a set of blossom traits involved in interactions with pollinators and predispersal

152   seed predators. We also measured the height of each blossom above ground. In this study, we

153   focused on the area of the upper floral bract, computed as the product of bract length and

154   width. Bract area is positively correlated with the size of the resin gland and thus represents

155   an honest signal of the quantity of reward offered to pollinators (Armbruster et al., 2005;

156   Pélabon et al., 2012). Previous work suggests that bees preferentially visit blossoms with

157   larger bracts (Armbruster et al., 2005; Pérez-Barrales et al., 2013). However, bract area may

158   also be shaped by interactions with seed predators (Pérez-Barrales et al., 2013). We collected

159   developing infructescences approximately four weeks after measurements were made and

160   recorded the number of viable and predated seeds. Predated seeds are easily identified as

161   empty seed coats, often with exit holes of adult weevils. Undehisced capsules were dissected

162   to ascertain whether the seeds had been eaten.

163   *Population-level comparative study: relationships among mating system, seed-predation rate,*

164   *and upper bract area*

165   Our previous work has shown that blossom traits vary predictably along a gradient of

166   pollination reliability and mating systems. Here, we focus on the potential of seed predators to

167   modify the outcome of pollinator-mediated floral-trait evolution. To assess among-population

168   covariation of predation rate, pollination reliability, and blossom traits, we recorded all or a

169   subset of these variables in 20 populations (Appendix S1). To quantify realized pollination

170   reliability, we recorded allogamous pollen loads on the stigmas of female-phase blossoms (*n*

171    $= 14 - 101$ blossoms, mean $= 43.2$, median $= 36$), when autogamous selfing is not yet

172   possible. We measured upper bract area and anther-stigma distance (herkogamy), a key floral

173   trait mediating mating-system variation among populations and species (Opedal et al., 2017a;

174   Opedal, 2018), on randomly selected blossoms in early bisexual condition ($n = 4 - 55$

175   blossoms, mean = 23.9, median = 24). Trait differences among populations are largely

176   genetically determined, as demonstrated by the correlation of phenotypic traits among

177   populations measured in the wild and under common-environment greenhouse conditions

178   (Opedal et al., 2016a). We collected developing infructescences in 20 populations ($n = 3 -$

179   101 infructescences, mean = 22.9, median = 13). Following explosive dehiscence of capsules,

180   we recorded the number of seeds produced, and whether each seed had been eaten. Adult

181   weevils emerging from predated infructescences were identified as *Nanobaris plumbata*

182   (Curculionidae: Baridinae), a species distributed apparently from southern Mexico to Panama

183   (Champion, 1909; J. Prena, pers. com.).

184   Analyses

185   *Effects of floral advertisement on seed predation within populations*

186   We modelled the effect of upper bract area on the probability of seed predation at the blossom

187   level by fitting a generalized linear mixed-effect model with binomial error distribution and

188   logit link function (glmmADMB; Fournier et al., 2012). We included only those blossoms

189   that set seeds in the analysis ($n = 155$). The response variable included the number of seeds

190   eaten and the number of seeds surviving for each blossom, thus weighing the probability of

191   predation by the total number of seeds produced. We also included blossom height above

192   ground as a covariate, and patch as a random effect. This approach treats blossoms nested

193   within patches as the unit of study, which is justified by the fact that seed predators are

194   unlikely to differentiate between blossoms on individual vines when these grow intertwined in

195   a patch (Bolstad et al., 2010; Pérez-Barrales et al., 2013). Thus, we interpret any significant

196   relationship between floral advertisement and the probability of seed predation at the blossom

197   level as evidence for seed-predator-mediated phenotypic selection. To test for differences in

198   seed-predator oviposition patterns among populations, we compared a full model including

199   interactions between population and population mean-centered upper bract area and blossom

200   height to a simpler model excluding the bract area $\times$ population interaction using AICc

201   (Burnham and Anderson, 2002). Mean-centering was done by subtracting the population

202   mean from individual trait values. Because environmental factors vary between years and the

203   insect seed predators in different years are different individuals, we treated the observations of

204   the Palo Verde population in two consecutive years as different populations.

205   *Population-level comparative analysis*

206   We computed population-level predation rates as the percentage of infructescences with one
207   or more seeds eaten, and cross-pollination (allogamy) rates as the percentage of blossoms
208   receiving pollen during the female phase. These measures estimate the intensity of
209   interactions at the population level and thus the potential for selection (Vanhoenacker et al.,
210   2013), rather than the absolute abundances of pollinators and seed predators. Substituting
211   these measures with the average proportion of seeds predated per infructescence and average
212   stigmatic pollen loads yielded qualitatively identical results (not shown). Because this
213   analysis concerned long-term evolutionary trends, we pooled data across years to obtain the
214   best possible population-level estimate.

215   We used path analysis (Shipley, 2016) to assess the independent effects of mating
216   system and seed predation rate on the evolutionary divergence of upper bract area among
217   populations. In our study system, female-phase stigmatic pollen loads is a strong predictor of
218   current outcrossing rate (Opedal et al., 2016a). However, because the current analysis is at the
219   level of evolved relationships among populations, we used population-mean herkogamy as a
220   proxy of the long-term mating system of each population, assuming that this would average
221   out annual fluctuations in outcrossing rates (Opedal, 2018). This approach is justified by the
222   observation that, across the study populations, herkogamy is positively correlated with
223   pollination reliability (stigmatic pollen loads), bee abundance on perfume baits, outcrossing
224   rate, and allelic diversity at microsatellite loci (Opedal et al., 2016a). By treating herkogamy
225   as a proxy of the mating history of each population, we were able to 'remove' the mating-
226   system related variation in upper bract area when testing the effect of predation rate on upper
227   bract area. In other words, we tested whether predation rates explained variation in the
228   residuals of the previously observed relationship between pollination reliability and floral
229   traits. To achieve this, we obtained path coefficients from a multiple-regression model with
230   population-mean upper bract area as response variable, and herkogamy and predation rate as
231   explanatory variables. All variables were standardized to zero mean and unit variance in order
232   to obtain standardized regression coefficients interpretable as effect sizes in units of standard
233   deviations. Statistical analyses were conducted in R 3.3.1 (R Core Team, 2018).

234   **RESULTS**

235   *Effects of floral advertisement on seed predation within populations*
236   Both the absolute and relative intensities of mutualistic and antagonistic interactions differed
237   among the three focal populations, and between years at Palo Verde (Table 1). At Horizontes
238   in 2015 and at Palo Verde in 2014, most blossoms were visited by pollinators, and seed

239 predators attacked a substantial proportion of infructescenses. At Puente la Amistad in 2014

240 predation was of comparable magnitude, but pollination was unreliable. Conversely, at Palo

241 Verde in 2015, pollination was reliable but seed predation was rare.

242   The effect of upper bract area on the probability of seed predation (Fig. 1), after

243 controlling for effects of blossom height, was population specific (the full model was

244 supported over the simpler model with no trait × population interaction, ΔAICc = 4.67, and

245 over an intercept-only model, ΔAICc = 3.90). At Palo Verde in 2015, blossoms with smaller

246 bracts were more likely to suffer seed predation. At Palo Verde and Puente la Amistad in

247 2014, and at Horizontes in 2015, the probability of seed predation was independent of upper

248 bract area. Thus, seed-predator-mediated selection was detected only in the least heavily

249 predated population (Fig. 1b).

250 *Population-level comparative study: relationships among mating system, seed-predation rate,*

251 *and upper bract area*

252 Across 20 populations, 409 (13.9%) of the 2933 seeds scored were eaten by seed weevils. The

253 percentage of predated infructescences at the population level ranged from 0% to 75% (mean

254 = 34.5%, median = 32.5%, n = 20 populations, Appendix S1). Populations suffering greater

255 predation rates produced fewer viable seeds (r = -0.71, 95% CI = -0.88, -0.39). Predation rates

256 were similar between years across populations (30.6% of infructescences were predated in

257 2014, and 27.9% in 2015, respectively).

258   Predation rates covaried positively yet non-significantly with rates of allogamous

259 pollination across populations (r = 0.48, 95% CI = -0.10, 0.81, Fig. 2). The regression slope of

260 predation rate on allogamous pollination rate (β = 0.38 ± 0.21, Fig. 2) was less than one,

261 corresponding to a tendency for seed predation to be relatively more intense in populations

262 experiencing less reliable pollination.

263   Herkogamy and upper bract area covaried positively across populations, placing

264 populations along an axis of increasing trait values (Fig. 3b). After controlling for assumed

265 mating system (herkogamy), the relationship between predation rate and population-mean

266 upper bract area was negative yet non-significant (Fig. 3). Hence, there was a weakly

267 supported tendency for more intensively predated populations to have smaller floral bracts

268 than expected from their mating-system history.

269 **DISCUSSION**

9

270   Variation in interactor communities across heterogeneous, fragmented landscapes may lead to

271   variation in the selective pressures acting on plant populations, i.e. creating geographic

272   selection mosaics (Thompson, 2005; Gómez et al., 2009; Sun et al., 2016). Two important

273   parameters needed for predicting the long-term consequences of anthropogenic habitat

274   destruction and fragmentation for plant populations are therefore i) whether different

275   interactors (e.g. pollinators vs. antagonists) differ in their response to environmental change

276   and ii) the degree to which spatial and temporal variation in interaction intensities lead to

277   differences in selection (Benkman, 2013; Vanhoenacker et al., 2013). Across a fragmented

278   landscape in north-western Costa Rica, *D. scandens* populations experience contrasting levels

279   of pollination reliability, and have apparently adapted to the resulting reproductive

280   environment (Opedal et al., 2016a). Here, we showed that those populations also experience

281   contrasting intensities of seed predation, with a tendency for greater predation intensities in

282   populations with more reliable pollination. We also observed a tendency for the intensity of

283   seed predation relative to pollination to be higher at sites experiencing unreliable pollination

284   (Fig. 2). For example, the pollinator-poor Puente la Amistad population experienced seed

285   predation at a rate comparable to or even greater than that observed in the more pollinator-rich

286   Palo Verde and Horizontes populations (Table 1). However, variation in the intensity of seed

287   predation has not detectably influenced the evolution of floral advertisement (involucral-bract

288   size) in *D. scandens* populations.

289   Predispersal seed predation by *Nanobaris* seed weevils occurred independently of

290   variation in floral advertisement in all populations except Palo Verde in 2015, where the

291   probability of predation decreased with increasing bract area (Fig. 1). These result are

292   inconsistent with the expectations that seed predators use increasing floral advertisement as a

293   cue indicating greater availability of resources for their offspring in the future (Brody, 1992;

294   Strauss and Irwin, 2004; Strauss and Whittall, 2006; Parachnowitsch and Caruso, 2008;

295   Pérez-Barrales et al., 2013; Sun et al., 2016), and that the strength of antagonist-mediated

296   selection increases with increasing interaction intensity (Benkman, 2013; Vanhoenacker et al.,

297   2013). Although the negative effect detected in the Palo Verde 2015 study was statistically

298   significant, the low number of infructescenses attacked in that study suggests that this effect

299   could be a false positive (Type I error). We can think of no obvious direct mechanism

300   explaining weevil preference for smaller floral bracts, although one possibility would be that

301   bract area correlates negatively with some other trait attractive to weevils, such as color

302   (Carlson and Holsinger, 2010) or fragrance (Theis and Adler, 2012). These results also

303   contrast with a study conducted in a Mexican *D. scandens* population, where 30% of

304   infructescenses were affected by seed predation, and seed weevils were more likely to

305   oviposit on blossoms with larger bracts (Pérez-Barrales et al., 2013). This pattern was not

306   detected in the same population in the following year, however, despite a predation rate of

307   18% (R. Pérez-Barrales, unpublished data). Overall, in six studies conducted in four different

308   *D. scandens* populations in two different regions, relationships between floral advertisement

309   and the probability of seed predation have been detected only twice (including the Palo Verde

310   2015 study), and in opposite directions. These observations lead us to conclude that, while

311   seed predators may occasionally mediate phenotypic selection on *D. scandens* blossom traits,

312   it occurs infrequently and is not predictable from population-mean seed predation rates within

313   the range observed in the *D. scandens* study populations.

314   The hypothesis that joint attraction of pollinators and seed predators generate

315   conflicting selection on floral advertisement has received reasonably strong empirical support

316   (Brody, 1992; Brody and Waser, 1995; Brody and Mitchell, 1997; Cariveau et al., 2004;

317   Pérez-Barrales et al., 2013; Sun et al., 2016), yet the likelihood of detecting seed-predator-

318   mediated selection may depend on several factors. Working in a large set of *Primula farinosa*

319   populations, Vanhoenacker et al. (2013) demonstrated stronger seed-predator-mediated

320   selection at greater intensities of predation, yet the relationship tended to be non-linear and

321   accelerating at greater predation intensities. Furthermore, the strength of seed-predator-

322   mediated selection may depend not only on the intensity of seed predation, but also on the

323   reliability of pollination (Brody, 1992; Vanhoenacker et al., 2013). This effect arises in part

324   because the reliability of pollination may affect the degree to which seed set differs between

325   attractive vs. non-attractive phenotypes, and hence the reliability of floral advertisements as a

326   cue indicating seed production. While pollen limitation on seed set has been demonstrated in

327   at least one study detecting predator-mediated selection (Brody, 1992), others have detected

328   selection in the absence of apparent pollen limitation (Cariveau et al., 2004; Parachnowitsch

329   and Caruso, 2008). Furthermore, Bartkowska and Johnston (2012) found that pollinators, but

330   not seed predators, mediate selection in a pollen-limited population of *Lobelia cardinalis*. In

331   the current study, we failed to detect seed-predator-mediated selection across focal

332   populations that differed both in the rate of predation and in the reliability of pollination.

333   Thus, while further work is needed to resolve these contrasting results, one possible

334   explanation for the lack of weevil choosiness within *D. scandens* populations is that variation

335   in seed set is too limited to generate strong preferences for floral phenotypes associated with

336   larger seed sets (see Brody, 1992). Such effects could perhaps be expected when pollination is

337   reliable, or when a self-compatible species is capable of effective autonomous self-

338   pollination.

339   The general lack of detectable effects of upper bract area on seed predation within

340   populations was mirrored in the patterns observed among populations. If seed predators

341   preferentially oviposit on large-bracted blossoms, we would expect a negative relationship

342   between predation rate and the size of advertisement traits among populations. After

343   controlling for mating-system-related variation in upper bract area by including herkogamy in

344   the path analysis, the relationship between predation rate and upper bract area was indeed

345   negative, but statistically non-significant (Fig. 3). This result argues against our causal

346   hypothesis, that the intensity of seed predation determines the strength of predator-mediated

347   selection and thus contributes to the evolutionary divergence of floral traits. Weak

348   relationships between current predation intensity, strength of selection, and floral traits also

349   argue against the alternative hypothesis that seed predators are differentially attracted to

350   populations with different mean trait values (Dart and Eckert, 2015). There are at least two

351   non-mutually-exclusive explanations for this finding. First, the weak relationships between

352   the phenotypic traits and current predation intensity may be due to recent changes in predation

353   intensity. Indeed, while variation in herkogamy and upper bract area represents the outcome

354   of long-term interactions with pollinators, antagonists, and other selective factors, our data on

355   predation intensity were collected over only two years. Second, the current and/or long-term

356   relative abundance of seed weevils across populations may depend on other factors not

357   included in our analysis.

358   Dart and Eckert (2015) have suggested that florivores are attracted to large-flowered

359   populations of *Camissoniopsis cheiranthifolia* due to the greater quantity of resources

360   available. Similarly, seed weevils benefit from laying eggs on blossoms producing many

361   seeds, and seed predation rates might therefore depend on the average seed set in each

362   population. While the average number of outcrossed seeds produced in *D. scandens*

363   populations is likely to increase with the rate of allogamous pollination, and indirectly with

364   blossom size, the opposite may be true for the number of selfed seeds. In *D. scandens*, seeds

365   resulting from selfing are the same size as those resulting from outcrossing (Opedal et al.,

366   2015; Pélabon et al., 2015a), suggesting they are equally valuable as resources for seed

367   predators. Indeed, mating-system-related local adaptation in mixed-mating plant species may

368   tend to reduce among-population variation in seed set, and hence the amount of resources

369   available for seed predators. If seed predators respond to mean resource availability at the

370   population level, seed predation rates would then be expected to vary independently from

371   pollination environments and floral traits, as observed in *D. scandens*. We lack data on

372   average open-pollinated seed set for most of our study populations, precluding a strong test of

373   this hypothesis. However, the range observed across our focal populations was indeed limited

374   (Table 1), and across all populations the range of seed sets of blossoms developing fruits (and

375   hence collected for the purpose of this study) was also relatively limited (mean = 6.45 seeds,

376   s.d. = 0.82, range = 4.23 - 8.25, Appendix S1).

377   Judging from their rates of interaction with *D. scandens* populations, the relative

378   abundances of bee pollinators and coleopteran seed predators are largely decoupled across our

379   study area, suggesting that these species groups respond differently to landscape-scale

380   environmental heterogeneity. While pollination tended to be more reliable in populations

381   occurring in less-disturbed habitats (along gravel roads through forested areas), predation

382   tended to be more intense in heavily disturbed habitats along highways (Appendix S1). The

383   primary pollinators of *D. scandens* in the study area are female euglossine bees (Table 1, Fig.

384   3). These forest-associated bees appear largely to avoid highway roadsides and other heavily

385   disturbed habitats (Brosi, 2009; Briggs et al., 2013; Opedal et al., 2017b), suggesting that

386   habitat destruction may be an indirect driver of mating-system and floral-trait evolution in this

387   system. In contrast, *Nanobaris* seed weevils (Fig. 3) seem less affected by habitat type in our

388   study area and were relatively abundant at several pollinator-poor sites. If low population

389   densities of euglossine bees at heavily disturbed sites is indeed caused by habitat destruction,

390   this finding adds to previous studies suggesting stronger effects of habitat destruction and

391   fragmentation on mutualistic than antagonistic interactions (Magrach et al., 2014; Brudvig et

392   al., 2015).

393   The main conclusion of this study is that, although predispersal seed predation may

394   reduce the average fitness of *D. scandens* populations and thus be ecologically important

395   (Kolb et al., 2007), seed-predator-mediated selection is unlikely to have been an important

396   driver of floral evolution, at least for the traits we measured. By excluding a possible

397   confounding effect, this observation strengthens our previous conclusion that pollination-

398   related selection drives the evolutionary divergence of blossom traits among *D. scandens*

399   populations (Opedal et al., 2016a). Specifically, the study populations appear to have tracked

400   variation in adaptive landscapes generated by pollinator communities, but seed predators had

401   only minor modifying effects on the outcome of pollinator-mediated evolution of blossom

traits. Hence, seed predators are unlikely to constrain pollinator-mediated floral-trait evolution in this system.

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Author contributions

ØHO, EA, RPB, and WSA conducted field work. ØHO conducted lab work, analyzed data, and wrote the manuscript with contributions from all authors.

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Data Accessibility

564 Population-level predation and pollination data are available in Appendix S1 in the

565 Supplementary Data with this article. Trait measurements are available in the Dryad Digital

566 Repository: https://doi.org/10.5061/dryad.8ph35 (Opedal et al., 2016b).

567

Figure legends

Fig. 1. (a) Effects of population-mean centered upper bract area (UBA) on the probability of seed predation within *Dalechampia scandens* populations at Puente la Amistad (yellow curve), Palo Verde (blue curve, 2014; green curve, 2015) and Horizontes (red curve). (b) Relationship between population-level predation rate and β_{UBA}, the slope of the logistic regression of predation probability (P) on upper bract area. Circle sizes in (a) are proportional to blossom seed set, and error bars in (b) indicate standard errors.

Fig. 2. Relationship between allogamy rate (percentage of inflorescences receiving allogamous pollen) and seed predation rate (percentage of inflorescences suffering seed predation) across 13 *D. scandens* populations in Costa Rica. The solid line indicates the 1:1 relationship, where mutualistic and antagonistic interactions are equally intense, and the dashed line indicates the estimated regression slope.

Fig. 3. Path diagram and scatterplots showing effects of mating system (population-mean herkogamy) and predation rate (proportion of infructescences attacked by *Nanobaris* seed weevils) on population-mean upper bract area (UBA, a floral advertisement trait). The double-headed curved arrow indicates the correlation (a) between herkogamy and predation rate. Single-headed arrows indicate direct effects of mating system (b) and predation rate (c) on upper bract area and are given with standardized regression coefficients interpretable as effect size in units of standard deviations. U = unexplained variation, computed as $\sqrt{1-r^2}$. Circle sizes in panels (a) and (c) are proportional to the square root of the sample size for predation rates, and error bars in (b) indicate standard errors. Photographs by Ø. H. Opedal.

Table 1. Summary of biotic interactions in *Dalechampia scandens* populations in Costa Rica. Pollinator visitation rates are the percentages of observed pollinator visits made by members of each pollinator genus, pollination rate is the percentage of blossoms receiving allogamous pollen, predation rate is the percentage of infructescenses that developed seeds and were then predated, and seed set is population-mean open-pollinated seed number (out of a maximum of 9 seeds), including those seeds scored as predated.

Population: Year	Coordinates	Pollinator visitation rates (%)			Pollination rate (%)	Predation rate (%)	Seed set (s.d)
		Hypanthidium	*Euglossa*	*Eufriesea*			
Puente la Amistad: 2014	10°14' N, 85°15' W	13.0	26.1	60.9	30.3	31.4	2.32 (3.27)
Palo Verde: 2014	10°23' N, 85°19' W	22.7	77.3		88.1	37.5	3.58 (3.46)
Palo Verde: 2015	10°23' N, 85°19' W	19.1	30.9	50.0	78.6	9.1	2.32 (3.30)
Horizontes: 2015	10°42' N, 85°36' W	43.2	56.8		82.8	40.4	2.75 (3.68)

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