

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

3 Øystein H. Opedal^{1,*}, Elena Albertsen¹, Rocío Pérez-Barrales², W. Scott Armbruster^{2,3} & Christophe
4 Pélabon¹

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6 ¹Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and
7 Technology, NTNU, 7491 Trondheim, Norway.

8 ²School of Biological Sciences, King Henry Building, King Henry I Street, University of Portsmouth,
9 Portsmouth PO1 2DY, UK.

10 ³Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775, USA.

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12 *Author for correspondence: oystein.opedal@helsinki.fi

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16 Running head: Weak effects of seed predators on floral evolution

17 **Abstract**

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

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

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

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

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

42 INTRODUCTION

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

64 The net strength and direction of selection on floral traits is expected to depend on the
65 relative intensities of mutualistic and antagonistic interactions (Benkman, 2013;
66 Vanhoenacker et al., 2013). All else being equal, the opportunity for mutualist-mediated
67 selection is expected to decrease with increasing interaction intensity, while the opportunity
68 for antagonist-mediated selection is expected to increase with increasing interaction intensity.
69 Therefore, much of the observed variation in selection acting on plant phenotypes may result
70 from spatial and temporal variation in the intensities of species interactions (Thompson,
71 2005). Spatial turnover in species interactions may be particularly common in
72 anthropogenically disturbed landscapes, because pollinators and other interactors often
73 respond differently to habitat destruction and fragmentation (Cunningham, 2000; Steffan-
74 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

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 involucre 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

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 infructescences. 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 \times population interaction, $\Delta\text{AICc} = 4.67$, and
245 over an intercept-only model, $\Delta\text{AICc} = 3.90$). 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 ($\beta = 0.38 \pm 0.21$, 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**

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 results 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 infructescences 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 infructescences 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

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

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412 **Author contributions**

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

415 **Literature Cited**

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562

563 **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

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 infructescences 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)
		<i>Hypanthidium</i>	<i>Euglossa</i>	<i>Eufriesea</i>			
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)

568

569 **Figure legends**

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

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

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

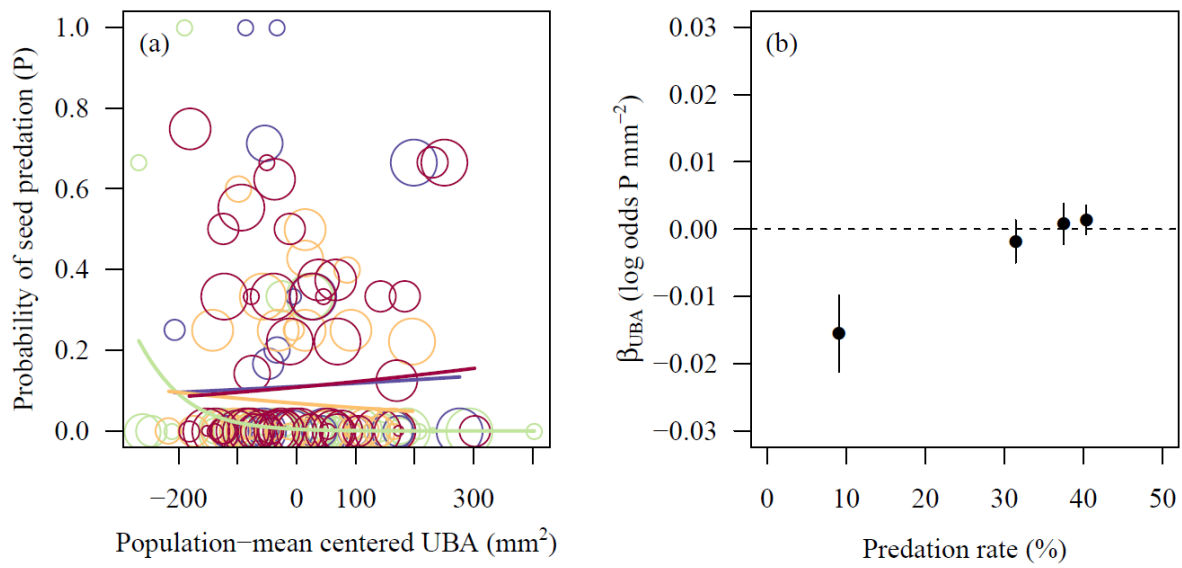


Fig. 1.

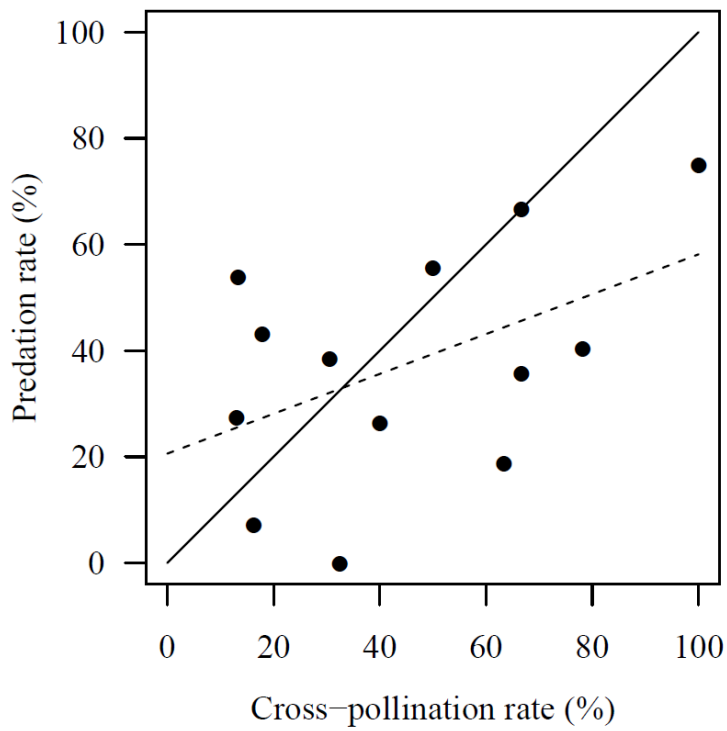
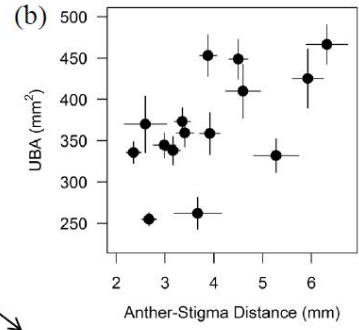
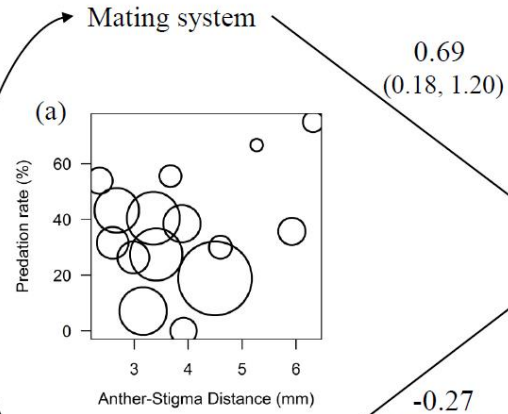


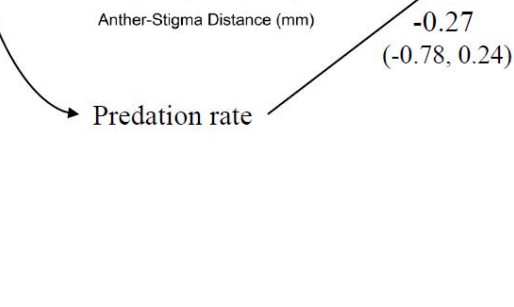
Fig. 2.



0.34
(-0.21, 0.73)



UBA ← U
0.76



590

591 Fig. 3.