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 Ehrлен, 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).
For example, hawthorn trees in northern Spain occurring in more fragmented habitats experienced less reliable pollination by bees and flies, and less frugivory by birds, but more intense seed predation by mice (Garcia and Chacoff, 2007). Similarly, both the bee pollinators and lepidopteran and dipteran seed predators of Centaurea jacea in Germany were less abundant in experimental populations located at sites containing less semi-natural habitat, but the effect of landscape structure on interactions were species-specific and depended on the spatial scale analyzed (Steffan-Dewenter et al., 2001). We may therefore expect both the absolute and relative intensities of mutualistic and antagonistic interactions to vary across heterogeneous landscapes, but whether this leads to systematic differences in selection remains an empirical question.

The neotropical vine Dalechampia scandens L. (Euphorbiaceae) is pollinated by female apid and megachilid bees (Armbruster and Webster, 1982; Armbruster, 1985). A recent study in Costa Rica showed that the floral traits and mating systems of populations varied predictably along a gradient of pollination reliability (Opedal et al., 2016a): populations at pollinator-poor sites had evolved blossom traits associated with greater rates of autonomous selfing, including smaller involucral bracts (a floral-advertisement trait; Armbruster et al., 2005) and reduced herkogamy. While these results suggest that population-mean floral trait values have tracked variation in the reliability of pollination, pollinators may not be the only biotic interactors affecting the evolution of D. scandens blossom traits. This was demonstrated by a study in a Mexican population, where the net selection on blossom traits was determined by interactions with both pollinators and predispersal seed predators (Pérez-Barrales et al., 2013). While pollinators selected for larger floral bracts, seed predators apparently responded to the same cues, generating conflicting selection. If seed predators consistently select for reduced floral advertisement, seed-predator-mediated selection might shift trait values away from the mating-system-related optimum determined by the local reliability of pollination. Specifically, if the strength of selection increases with the intensity of predation (Vanhoenacker et al., 2013), more intensively predated populations would be expected to have smaller floral bracts than expected from their historical pollination environment.

To assess whether predispersal seed predators modify pollinator-mediated floral trait evolution in D. scandens, we first studied spatio-temporal variation in seed-predator-mediated selection, i.e. the relationships between floral advertisement and the probability of seed predation within populations. Second, we assessed the joint influence of mating system and
seed predation rate on among-population divergence in floral advertisement. Specifically, we asked (1) whether seed predators exert selection on floral advertisement, and (2) whether selection varies in time and space depending on the rate of seed predation. Using data from a larger number of populations, we tested (3) whether predation rates covary with pollination reliability among populations, and (4) whether the rate of attack by seed predators predicts population-mean floral trait values after controlling for the effect of mating systems.

MATERIALS AND METHODS

Study system

Dalechampia scandens L. (s.l.) (Euphorbiaceae) is a species complex of perennial woody vines native to the lowland Neotropics (Armbruster, 1985). It occurs in naturally open areas and anthropogenic disturbances, including limestone outcrops, open shrublands, light gaps, and roadsides. Male and female flowers are aggregated into bisexual blossom inflorescences (pseudanthia – ‘false flowers’), which function as pollination units. Blossoms are protogynous, with a female phase of c. 2-3 days followed by a bisexual phase. The blossoms are visited and pollinated by female apid and megachilid bees, which collect floral resin from a gland-like structure associated with the male flowers (Armbruster, 1985). Outcrossing rates in four natural populations in Costa Rica ranged from 0.16 to 0.49, indicating a mixed mating system with a tendency towards selfing (Opedal et al., 2016a). The male and female subinflorescences are together subtended by a pair of involucral bracts that open during the day to allow pollination, and close at night to protect the floral tissues (Armbruster, 1985; Armbruster et al., 1997). The bracts are normally creamy white during anthesis, when they function as an advertisement towards pollinators. During fruit maturation, the bracts change color to dark green and increase their rates of photosynthesis, becoming more cryptic and providing carbon for the developing seeds (Pélabon et al., 2015b). A maximum of nine seeds per blossom is dispersed by explosive dehiscence of capsules (Armbruster, 1982). Developing seeds are subject to predation by seed weevils (Curculionidae: Baridinae). The weevils oviposit on blossoms prior to seed development, and may thus use the bracts to find receptive inflorescences and/or the bract size as a cue to determine the future availability of resources for their larvae (Pérez-Barrales et al., 2013).

During the peak blooming seasons (Oct.-Dec.) of 2014 and 2015, we studied 20 populations in north-western Costa Rica (see Appendix S1 in the Supplementary Data with this article for exact locations), belonging to the ‘large-glanded’ taxon of the D. scandens complex (Bolstad et al., 2014). Neighboring populations are separated by 1.8 – 36.5 km, and
contemporary gene flow between populations is uncommon or absent (Opedal et al., 2017b).

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

Effects of floral advertisement on seed predation within populations
We studied the effect of a floral-advertisement trait on the probability of seed predation in each of three populations, one of which was studied in two consecutive years, as part of a long-term study of spatio-temporal variation in selection on Dalechampia scandens blossom traits (see also Pérez-Barrales et al., 2013). During each study, we marked distinct patches comprising one or sometimes several intertwined individuals. In each patch, we recorded daily the number of pollen grains deposited onto the stigmas of individually-marked blossoms. On the first day of the bisexual phase (the day the first male flower opened), we measured a set of blossom traits involved in interactions with pollinators and predispersal seed predators. We also measured the height of each blossom above ground. In this study, we focused on the area of the upper floral bract, computed as the product of bract length and width. Bract area is positively correlated with the size of the resin gland and thus represents an honest signal of the quantity of reward offered to pollinators (Armbruster et al., 2005; Pélabon et al., 2012). Previous work suggests that bees preferentially visit blossoms with larger bracts (Armbruster et al., 2005; Pérez-Barrales et al., 2013). However, bract area may also be shaped by interactions with seed predators (Pérez-Barrales et al., 2013). We collected developing infructescences approximately four weeks after measurements were made and recorded the number of viable and predated seeds. Predated seeds are easily identified as empty seed coats, often with exit holes of adult weevils. Undehisced capsules were dissected to ascertain whether the seeds had been eaten.

Population-level comparative study: relationships among mating system, seed-predation rate, and upper bract area
Our previous work has shown that blossom traits vary predictably along a gradient of pollination reliability and mating systems. Here, we focus on the potential of seed predators to modify the outcome of pollinator-mediated floral-trait evolution. To assess among-population covariation of predation rate, pollination reliability, and blossom traits, we recorded all or a subset of these variables in 20 populations (Appendix S1). To quantify realized pollination reliability, we recorded allogamous pollen loads on the stigmas of female-phase blossoms (n = 14 – 101 blossoms, mean = 43.2, median = 36), when autogamous selfing is not yet possible. We measured upper bract area and anther-stigma distance (herkogamy), a key floral trait mediating mating-system variation among populations and species (Opedal et al., 2017a;
Opedal, 2018), on randomly selected blossoms in early bisexual condition \((n = 4 – 55\) blossoms, mean = 23.9, median = 24). Trait differences among populations are largely genetically determined, as demonstrated by the correlation of phenotypic traits among populations measured in the wild and under common-environment greenhouse conditions (Opedal et al., 2016a). We collected developing infructescences in 20 populations \((n = 3 – 101\) infructescences, mean = 22.9, median = 13). Following explosive dehiscence of capsules, we recorded the number of seeds produced, and whether each seed had been eaten. Adult weevils emerging from predated infructescences were identified as Nanobaris plumbata (Curculionidae: Baridinae), a species distributed apparently from southern Mexico to Panama (Champion, 1909; J. Prena, pers. com.).

### Analyses

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

We modelled the effect of upper bract area on the probability of seed predation at the blossom level by fitting a generalized linear mixed-effect model with binomial error distribution and logit link function (glmmADMB; Fournier et al., 2012). We included only those blossoms that set seeds in the analysis \((n = 155)\). The response variable included the number of seeds eaten and the number of seeds surviving for each blossom, thus weighing the probability of predation by the total number of seeds produced. We also included blossom height above ground as a covariate, and patch as a random effect. This approach treats blossoms nested within patches as the unit of study, which is justified by the fact that seed predators are unlikely to differentiate between blossoms on individual vines when these grow intertwined in a patch (Bolstad et al., 2010; Pérez-Barrales et al., 2013). Thus, we interpret any significant relationship between floral advertisement and the probability of seed predation at the blossom level as evidence for seed-predator-mediated phenotypic selection. To test for differences in seed-predator oviposition patterns among populations, we compared a full model including interactions between population and population mean-centered upper bract area and blossom height to a simpler model excluding the bract area \(\times\) population interaction using AICc (Burnham and Anderson, 2002). Mean-centering was done by subtracting the population mean from individual trait values. Because environmental factors vary between years and the insect seed predators in different years are different individuals, we treated the observations of the Palo Verde population in two consecutive years as different populations.

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

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

**RESULTS**

*Effects of floral advertisement on seed predation within populations*

Both the absolute and relative intensities of mutualistic and antagonistic interactions differed among the three focal populations, and between years at Palo Verde (Table 1). At Horizontes in 2015 and at Palo Verde in 2014, most blossoms were visited by pollinators, and seed
predators attacked a substantial proportion of infructescences. At Puente la Amistad in 2014 predation was of comparable magnitude, but pollination was unreliable. Conversely, at Palo Verde in 2015, pollination was reliable but seed predation was rare.

The effect of upper bract area on the probability of seed predation (Fig. 1), after controlling for effects of blossom height, was population specific (the full model was supported over the simpler model with no trait × population interaction, ΔAICc = 4.67, and over an intercept-only model, ΔAICc = 3.90). At Palo Verde in 2015, blossoms with smaller bracts were more likely to suffer seed predation. At Palo Verde and Puente la Amistad in 2014, and at Horizontes in 2015, the probability of seed predation was independent of upper bract area. Thus, seed-predator-mediated selection was detected only in the least heavily predated population (Fig. 1b).

Population-level comparative study: relationships among mating system, seed-predation rate, and upper bract area

Across 20 populations, 409 (13.9%) of the 2933 seeds scored were eaten by seed weevils. The percentage of predated infructescences at the population level ranged from 0% to 75% (mean = 34.5%, median = 32.5%, n = 20 populations, Appendix S1). Populations suffering greater predation rates produced fewer viable seeds (r = -0.71, 95% CI = -0.88, -0.39). Predation rates were similar between years across populations (30.6% of infructescences were predated in 2014, and 27.9% in 2015, respectively).

Predation rates covaried positively yet non-significantly with rates of allogamous pollination across populations (r = 0.48, 95% CI = -0.10, 0.81, Fig. 2). The regression slope of predation rate on allogamous pollination rate (β = 0.38 ± 0.21, Fig. 2) was less than one, corresponding to a tendency for seed predation to be relatively more intense in populations experiencing less reliable pollination.

Herkogamy and upper bract area covaried positively across populations, placing populations along an axis of increasing trait values (Fig. 3b). After controlling for assumed mating system (herkogamy), the relationship between predation rate and population-mean upper bract area was negative yet non-significant (Fig. 3). Hence, there was a weakly supported tendency for more intensively predated populations to have smaller floral bracts than expected from their mating-system history.

DISCUSSION
Variation in interactor communities across heterogeneous, fragmented landscapes may lead to variation in the selective pressures acting on plant populations, i.e. creating geographic selection mosaics (Thompson, 2005; Gómez et al., 2009; Sun et al., 2016). Two important parameters needed for predicting the long-term consequences of anthropogenic habitat destruction and fragmentation for plant populations are therefore i) whether different interactors (e.g. pollinators vs. antagonists) differ in their response to environmental change and ii) the degree to which spatial and temporal variation in interaction intensities lead to differences in selection (Benkman, 2013; Vanhoenacker et al., 2013). Across a fragmented landscape in north-western Costa Rica, *D. scandens* populations experience contrasting levels of pollination reliability, and have apparently adapted to the resulting reproductive environment (Opedal et al., 2016a). Here, we showed that those populations also experience contrasting intensities of seed predation, with a tendency for greater predation intensities in populations with more reliable pollination. We also observed a tendency for the intensity of seed predation relative to pollination to be higher at sites experiencing unreliable pollination (Fig. 2). For example, the pollinator-poor Puente la Amistad population experienced seed predation at a rate comparable to or even greater than that observed in the more pollinator-rich Palo Verde and Horizontes populations (Table 1). However, variation in the intensity of seed predation has not detectably influenced the evolution of floral advertisement (involucral-bract size) in *D. scandens* populations.

Predispersal seed predation by *Nanobaris* seed weevils occurred independently of variation in floral advertisement in all populations except Palo Verde in 2015, where the probability of predation decreased with increasing bract area (Fig. 1). These result are inconsistent with the expectations that seed predators use increasing floral advertisement as a cue indicating greater availability of resources for their offspring in the future (Brody, 1992; Strauss and Irwin, 2004; Strauss and Whittall, 2006; Parachnowitsch and Caruso, 2008; Pérez-Barrales et al., 2013; Sun et al., 2016), and that the strength of antagonist-mediated selection increases with increasing interaction intensity (Benkman, 2013; Vanhoenacker et al., 2013). Although the negative effect detected in the Palo Verde 2015 study was statistically significant, the low number of infructescenses attacked in that study suggests that this effect could be a false positive (Type I error). We can think of no obvious direct mechanism explaining weevil preference for smaller floral bracts, although one possibility would be that bract area correlates negatively with some other trait attractive to weevils, such as color (Carlson and Holsinger, 2010) or fragrance (Theis and Adler, 2012). These results also
contrast with a study conducted in a Mexican *D. scandens* population, where 30% of infructescences were affected by seed predation, and seed weevils were more likely to oviposit on blossoms with larger bracts (Pérez-Barrales et al., 2013). This pattern was not detected in the same population in the following year, however, despite a predation rate of 18% (R. Pérez-Barrales, unpublished data). Overall, in six studies conducted in four different *D. scandens* populations in two different regions, relationships between floral advertisement and the probability of seed predation have been detected only twice (including the Palo Verde 2015 study), and in opposite directions. These observations lead us to conclude that, while seed predators may occasionally mediate phenotypic selection on *D. scandens* blossom traits, it occurs infrequently and is not predictable from population-mean seed predation rates within the range observed in the *D. scandens* study populations.

The hypothesis that joint attraction of pollinators and seed predators generate conflicting selection on floral advertisement has received reasonably strong empirical support (Brody, 1992; Brody and Waser, 1995; Brody and Mitchell, 1997; Cariveau et al., 2004; Pérez-Barrales et al., 2013; Sun et al., 2016), yet the likelihood of detecting seed-predator-mediated selection may depend on several factors. Working in a large set of *Primula farinosa* populations, Vanhoenacker et al. (2013) demonstrated stronger seed-predator-mediated selection at greater intensities of predation, yet the relationship tended to be non-linear and accelerating at greater predation intensities. Furthermore, the strength of seed-predator-mediated selection may depend not only on the intensity of seed predation, but also on the reliability of pollination (Brody, 1992; Vanhoenacker et al., 2013). This effect arises in part because the reliability of pollination may affect the degree to which seed set differs between attractive vs. non-attractive phenotypes, and hence the reliability of floral advertisements as a cue indicating seed production. While pollen limitation on seed set has been demonstrated in at least one study detecting predator-mediated selection (Brody, 1992), others have detected selection in the absence of apparent pollen limitation (Cariveau et al., 2004; Parachnowitsch and Caruso, 2008). Furthermore, Bartkowska and Johnston (2012) found that pollinators, but not seed predators, mediate selection in a pollen-limited population of *Lobelia cardinalis*. In the current study, we failed to detect seed-predator-mediated selection across focal populations that differed both in the rate of predation and in the reliability of pollination. Thus, while further work is needed to resolve these contrasting results, one possible explanation for the lack of weevil choosiness within *D. scandens* populations is that variation in seed set is too limited to generate strong preferences for floral phenotypes associated with
larger seed sets (see Brody, 1992). Such effects could perhaps be expected when pollination is reliable, or when a self-compatible species is capable of effective autonomous self-pollination.

The general lack of detectable effects of upper bract area on seed predation within populations was mirrored in the patterns observed among populations. If seed predators preferentially oviposit on large-bracted blossoms, we would expect a negative relationship between predation rate and the size of advertisement traits among populations. After controlling for mating-system-related variation in upper bract area by including herkogamy in the path analysis, the relationship between predation rate and upper bract area was indeed negative, but statistically non-significant (Fig. 3). This result argues against our causal hypothesis, that the intensity of seed predation determines the strength of predator-mediated selection and thus contributes to the evolutionary divergence of floral traits. Weak relationships between current predation intensity, strength of selection, and floral traits also argue against the alternative hypothesis that seed predators are differentially attracted to populations with different mean trait values (Dart and Eckert, 2015). There are at least two non-mutually-exclusive explanations for this finding. First, the weak relationships between the phenotypic traits and current predation intensity may be due to recent changes in predation intensity. Indeed, while variation in herkogamy and upper bract area represents the outcome of long-term interactions with pollinators, antagonists, and other selective factors, our data on predation intensity were collected over only two years. Second, the current and/or long-term relative abundance of seed weevils across populations may depend on other factors not included in our analysis.

Dart and Eckert (2015) have suggested that florivores are attracted to large-flowered populations of *Camissoniopsis cheiranthifolia* due to the greater quantity of resources available. Similarly, seed weevils benefit from laying eggs on blossoms producing many seeds, and seed predation rates might therefore depend on the average seed set in each population. While the average number of outcrossed seeds produced in *D. scandens* populations is likely to increase with the rate of allogamous pollination, and indirectly with blossom size, the opposite may be true for the number of selfed seeds. In *D. scandens*, seeds resulting from selfing are the same size as those resulting from outcrossing (Opedal et al., 2015; Pélabon et al., 2015a), suggesting they are equally valuable as resources for seed predators. Indeed, mating-system-related local adaptation in mixed-mating plant species may tend to reduce among-population variation in seed set, and hence the amount of resources
available for seed predators. If seed predators respond to mean resource availability at the population level, seed predation rates would then be expected to vary independently from pollination environments and floral traits, as observed in *D. scandens*. We lack data on average open-pollinated seed set for most of our study populations, precluding a strong test of this hypothesis. However, the range observed across our focal populations was indeed limited (Table 1), and across all populations the range of seed sets of blossoms developing fruits (and hence collected for the purpose of this study) was also relatively limited (mean = 6.45 seeds, s.d. = 0.82, range = 4.23 - 8.25, Appendix S1).

Judging from their rates of interaction with *D. scandens* populations, the relative abundances of bee pollinators and coleopteran seed predators are largely decoupled across our study area, suggesting that these species groups respond differently to landscape-scale environmental heterogeneity. While pollination tended to be more reliable in populations occurring in less-disturbed habitats (along gravel roads through forested areas), predation tended to be more intense in heavily disturbed habitats along highways (Appendix S1). The primary pollinators of *D. scandens* in the study area are female euglossine bees (Table 1, Fig. 3). These forest-associated bees appear largely to avoid highway roadsides and other heavily disturbed habitats (Brosi, 2009; Briggs et al., 2013; Opedal et al., 2017b), suggesting that habitat destruction may be an indirect driver of mating-system and floral-trait evolution in this system. In contrast, *Nanobaris* seed weevils (Fig. 3) seem less affected by habitat type in our study area and were relatively abundant at several pollinator-poor sites. If low population densities of euglossine bees at heavily disturbed sites is indeed caused by habitat destruction, this finding adds to previous studies suggesting stronger effects of habitat destruction and fragmentation on mutualistic than antagonistic interactions (Magrach et al., 2014; Brudvig et al., 2015).

The main conclusion of this study is that, although predispersal seed predation may reduce the average fitness of *D. scandens* populations and thus be ecologically important (Kolb et al., 2007), seed-predator-mediated selection is unlikely to have been an important driver of floral evolution, at least for the traits we measured. By excluding a possible confounding effect, this observation strengthens our previous conclusion that pollination-related selection drives the evolutionary divergence of blossom traits among *D. scandens* populations (Opedal et al., 2016a). Specifically, the study populations appear to have tracked variation in adaptive landscapes generated by pollinator communities, but seed predators had 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**

Population-level predation and pollination data are available in Appendix S1 in the Supplementary Data with this article. Trait measurements are available in the Dryad Digital Repository: [https://doi.org/10.5061/dryad.8ph35](https://doi.org/10.5061/dryad.8ph35) (Opedal et al., 2016b).
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.

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

**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 $\beta_{UBA}$, 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.
Fig. 1.

Fig. 2.
Fig. 3.