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Can Conflicting Selection from Pollinators and Nectar-Robbing Antagonists Drive Adaptive Pollen Limitation? A Conceptual Model and Empirical Test

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ABSTRACT: Pollen limitation is widespread, despite predictions that it should not be. We propose a novel mechanism generating pollen limitation: conflicting selection by pollinators and antagonists on pollinator attraction traits. We introduce a heuristic model demonstrating antagonist-induced adaptive pollen limitation and present a field study illustrating its occurrence in a wild population. For antagonist-induced adaptive pollen limitation to occur, four criteria must be met: (1) correlated attraction of pollinators and antagonists; (2) greater response by antagonists than pollinators to altered investment in attraction traits; (3) reduced investment in pollinator attraction, leading to pollen limitation; and (4) higher fitness for plants with reduced investment in pollinator attraction. We surveyed nectar robbery and reproductive output for 109 *Odontonema cuspidatum* (Acanthaceae) plants in a pollen-limited population over 2 years and used experimental floral arrays to evaluate how flower number affects pollination and nectar robbery. Both pollinators and nectar robbers preferred larger floral displays and nectar robbery reduced reproductive output, suggesting conflicting selection. Survey and experimental data agreed closely on the optimum flower number under antagonist-induced pollen limitation; this number was substantially overrepresented in the population. While criteria for antagonist-induced adaptive pollen limitation are restrictive, the necessary conditions may often be realized. Considering interactions beyond the plant-pollinator dyad illuminates previously overlooked mechanisms generating pollen limitation.

Keywords: conflicting selection, costs of reproduction, nectar robbery, *Odontonema cuspidatum*, pollen limitation, pollination.

Introduction

Antagonists are commonly attracted by the same traits that plants use to draw pollinators (Adler and Bronstein 2004; Theis and Adler 2012; Ågren et al. 2013; Sletvold et al. 2015; Sun et al. 2016; Knauer and Schiestl 2017). Where both antagonists and pollinators favor particular trait values, this can lead to conflicting selection on these attraction traits (Sletvold et al. 2015; Sun et al. 2016; Ramos and Schiestl 2019). Depending on the relative strength of selection exerted by the antagonist and mutualist (Sletvold et al. 2015) as well as the degree of spatial and temporal variability in these pressures (Siepielski and Benkman 2010; Siepielski et al. 2013; Ågren et al. 2013), such conflicting selection can favor intermediate phenotypes, maintain within-population phenotypic diversity (Siepielski and Benkman 2010; Shumate et al. 2011), or lead to a spatial mosaic of populations with distinct dominant phenotypes (Ågren et al. 2013). Despite known examples of conflicting selection via correlated attraction of pollinators and antagonists affecting floral trait evolution, the potential role of such selective pressures in generating pollen limitation of plant reproduction (i.e., when seed production is limited by a lack of pollen receipt rather than a lack of resources) has received little attention. Here, we outline a mechanism by which conflicting selection by pollinators and antagonists may give rise to adaptive pollen limitation. We first present a conceptual model explaining the mechanism, then offer data from field surveys and experimental manipulation that are consistent with the model.

Pollen limitation is ubiquitous in animal-pollinated plants (Burd 1994; Ashman et al. 2004; Knight et al. 2006). Haig and Westoby (1988) proposed a foundational framework for understanding pollen limitation. Their framework assumes that there is a trade-off between investment of resources in pollinator attraction and investment in ovule provisioning, and that the number of ovules fertilized is an increasing function of investment in pollinator attraction. Given these assumptions, optimum investment in pollinator attraction should occur at the intersection of the functions relating investment in pollinator attraction to (1) the number or

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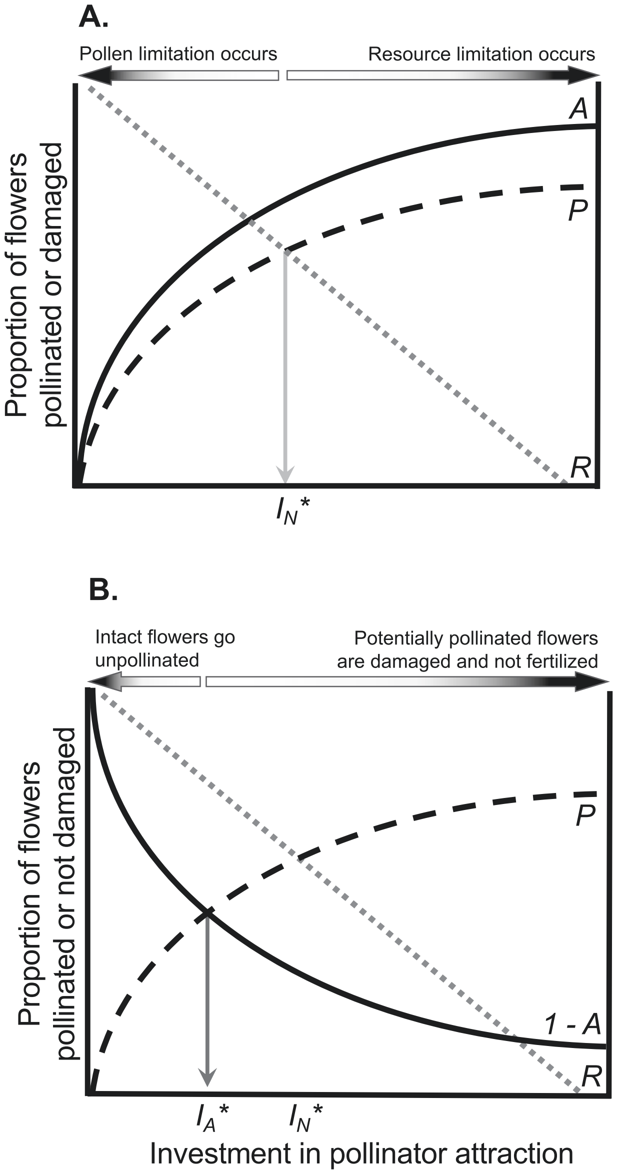

Figure 1: Conceptual diagram illustrating how investment in pollinator attraction is affected by the presence of a floral antagonist and may lead to pollen limitation. *A*, According to the Haig-Westoby framework, optimum investment in pollinator attraction (I^*_{N}) is determined by the intersection of the pollination function (*P*, representing effective pollination at a given level of investment; dashed line) and the resource availability function (*R*, representing resources available for ovule provisioning; gray dotted line). Pollen limitation occurs when investment in pollinator attraction is less than I^*_{N}; where investment is more than I^*_{N}, reproduction is resource limited. The shapes of the functions illustrated here assume (1) a strict trade-off between allocation to pollinator attraction and allocation to ovule provisioning and (2) diminishing returns on increasing investment in pollinator attraction, but model predictions are not sensitive to the precise shape of these functions. The antagonist function (*A*; solid line) indicates that attraction of pollinators and antagonists is correlated. *B*, Replotting panel *A* with the antagonist function inverted (*1-A*), showing the proportion of flowers not damaged by the antagonist. Note that if the strength of the antagonist response to investment in pollinator attraction is greater than the pollinator response, $I^*_{\mathrm{A}} < I^*_{\mathrm{N}}$ and pollen limitation will occur. In the text, I^*_{N} is referred to as the Haig-Westoby optimum, while I^*_{A} is referred to as the antagonist-induced optimum.

proportion of ovules fertilized (*P* in fig. 1) and (2) the number or proportion of ovules that could be provisioned (*R* in fig. 1). At this intersection (designated I^*_{N} in fig. 1), plant reproductive output is simultaneously limited by resource availability and pollen receipt. This qualitative result holds for a wide range of functions specifying the pollination and resource curves, but the pollination function is generally modeled with an asymptotic response to increased allocation to pollinator attraction while the resource function is modeled with a decreasing linear response (Haig and Westoby 1988; Ashman et al. 2004; Burd 2008). Within the Haig-Westoby framework, pollen limitation occurs when investment in pollinator attraction is less than the optimum (i.e., to the left of I^*_{N}; fig. 1), leaving potentially provisionable ovules unfertilized.

Haig and Westoby's (1988) framework predicts that pollen limitation should be rare, since it represents a suboptimal investment in pollinator attraction. Yet pollen limitation is common. Most explanations invoke variability in pollen receipt, which subsumes two dominant theories. The first suggests that pollen limitation arises from the failure of a plant population to respond to an altered pollination regime that results in decreased fertilization (Thomson 2001; Ashman et al. 2004; Vamosi et al. 2006). In this scenario, ecological change results in decreased pollen receipt compared with the conditions in which the plant evolved. The plant, adapted to a prior equilibrium level of pollen receipt, is "locked in" to producing more ovules than will be pollinated. The second theory posits that pollen limitation represents an adaptive response to stochastic variability across space or time in pollen receipt (Burd 1995*a*; Ashman et al. 2004). Burd (1995*a*) showed theoretically that in many cases—particularly when variance in pollen receipt across flowers within an individual is high—producing more ovules than are pollinated on average is adaptive, as it allows the plant to take advantage of rare instances of high levels of pollination.

While the two mechanisms outlined above likely generate many instances of pollen limitation, we believe the constrained focus on plant-pollinator interactions in existing theory on pollen limitation overlooks potential additional mechanisms that necessarily involve other types of biotic interactions. One such mechanism, which we call antagonist-induced adaptive pollen limitation, is an adaptive response by a plant to conflicting selection from pollinators and floral antagonists (e.g., florivores, nectar robbers) on pollinator attraction traits. Figure 1 provides a conceptual diagram, adapted from the Haig-Westoby framework, that depicts how antagonist-driven adaptive pollen limitation arises; below we outline this mechanism in greater detail.

The Conceptual Model

For antagonist-induced adaptive pollen limitation to occur, four criteria must be met.

Criterion 1. Both pollinators and antagonists must be attracted by the same trait(s). This is illustrated in figure 1*A* by the shape of the curves *P* and *A*, where *A* (hereafter referred to as the antagonist function) represents the relationship between investment in pollinator attraction and proportional antagonist damage (ranging from 0, no damage, to 1, all flowers damaged); both *P* and *A* are increasing functions of investment in pollinator attraction. This establishes the potential for conflicting pressures on plant allocation to pollinator attraction.

Criterion 2. The response of the antagonist to a change in investment in pollinator attraction must be equal to or greater than the response of the pollinator (for an evaluation of how widespread we expect this to be, see "Discussion"). When this is the case, the maximum slope (and possibly the asymptotic value) of *A* will be greater than that of *P*. Therefore, the intersection of *1-A* (the inverse of the antagonist function; i.e., the proportion of undamaged flowers) will intersect with *P* to the left of I_{N}^{*} (fig. 1*B*).

Criterion 3. The resulting conflicting pressures should lead to reduced investment in the relevant attraction trait(s) and therefore a level of pollen receipt lower than that which would maximize viable seed production (i.e., pollen limitation).

Criterion 4. Reduced investment in pollinator attraction should nevertheless result in higher fitness for plants compared with those that allocate more to pollinator attraction.

There are two ways in which criteria 3 and 4 may be met. First, antagonist damage may have such a strong negative impact on the plant's ability to reproduce that, in the presence of the antagonist, maximum reproductive success during that reproductive event is achieved at a level of investment in pollinator attraction below the Haig-Westoby optimum. Alternatively, for an iteroparous species (i.e., a species that reproduces multiple times), reduced allocation toward pollinator attraction may be advantageous if this increases the likelihood of surviving to reproduce again. In this case, what would be the optimum level of investment in pollinator attraction? While the answer to this question will depend in part on the precise nature of the trade-off between present and future reproduction, a first approximation of this optimum is indicated by the intersection point of curves *P* and *1-A* (I_{A}^{*}; fig. 1*B*). At this intersection, attraction of pollinators and antagonists is such that all flowers could, at least in theory, interact with either a pollinator or an antagonist but not both. Assuming that antagonist-damaged flowers are rarely successfully pollinated, this intersection point describes optimum investment in pollinator attraction. Why? On the one hand, investing more in pollinator attraction would result in losing flowers to antagonists that would otherwise attract pollinators (representing lost investment both of the additional resources allocated to pollinator attraction and of the resources spent on initial ovule formation for those flowers lost to antagonists). On the other hand, investing less in pollinator attraction would result in flowers that were neither damaged nor pollinated, representing wasted allocation of resources to the formation of ovules that will not be fertilized. The degree to which this partitioning of flowers into exclusive "for-pollinator" or "for-antagonist" categories actually occurs is likely to depend on the mechanism by which antagonists affect seed production (i.e., by reducing pollinator attraction vs. reducing ovule viability; for details, see "Discussion").

The optimum investment in pollinator attraction in the presence of the antagonist (the antagonist-driven optimum) will be lower than the Haig-Westoby optimum predicted by the intersection of pollen limitation and resource limitation functions, so long as criterion 2 is satisfied. This reduced investment in pollinator attraction will result in pollen limitation. Uniquely, in comparison to the other explanations of pollen limitation outlined above, this mechanism suggests that pollen limitation may be adaptive even in an environment of relatively predictable levels of pollen receipt (although it can also operate when pollen receipt varies unpredictably).

Empirical Test of the Conceptual Model

We evaluated evidence for the idea that conflicting pressures from antagonists and pollinators lead to pollen limitation using nectar robbery and pollination of the iteroparous shrub *Odontonema cuspidatum* (Acanthaceae) as a model system. *Odontonema cuspidatum* is likely pollinated primarily by hummingbirds (G. Fitch, unpublished data; Meyer and Lavergne 2004) and, in the study region in Chiapas, Mexico (where it is native), is also heavily nectar robbed, primarily by stingless bees of the genus *Trigona* (*T. fulviventris* and *T. nigerrima*; Meliponini: Apidae; Fitch and Vandermeer 2021*a*). Nectar robbery occurs when a flower visitor extracts nectar from the flower via an animal-made hole rather than the corolla opening.

In the study population, robbed flowers of *O. cuspidatum* are 41% less likely to set fruit than unrobbed flowers (Fitch and Vandermeer 2021*a*). Moreover, at the plant level, nectar-robbing intensity (i.e., the proportion of flowers that are robbed) increases as flower number increases, at least for floral displays of moderate size (Fitch and Vandermeer 2021*a*). In other species, hummingbird visitation is likewise positively related to flower number (Schemske 1980; Rodriguez-Robles et al. 1992; but see Brody and Mitchell 1997). We therefore suggest that flower number is a key trait governing the attraction of both pollinators and antagonists (i.e., nectar robbers) to *O. cuspidatum* (satisfying criterion 1). We evaluated whether the antagonist response to flower number is stronger than the pollinator response (satisfying criterion 2) and determined the Haig-Westoby and antagonist-induced optima using a combination of field surveys and a manipulative experiment. Assuming a stronger response of antagonists to flower number, we predicted that naturally occurring plants would have fewer flowers than predicted by the Haig-Westoby optimum (I^*_{N}; fig. 1) and would be pollen limited (satisfying criterion 3). Finally, we predicted that floral display sizes close to the antagonist-induced optimum I^*_{A}; fig. 1*B*), as predicted by data on pollinator and antagonist response to flower number, would be overrepresented among naturally occurring plants (consistent with criterion 4). However, we hypothesized that the variable intensity of nectar robbery experienced by plants (Fitch and Vandermeer 2021*a*) would maintain high within-population variability in floral display size (Siepielski and Benkman 2010).

Material and Methods

Study System

This study took place at Finca Irlanda, a large (~300 ha), shaded, certified-organic coffee farm in southeastern Chiapas, Mexico. In this area, *Odontonema cuspidatum* occurs commonly as an understory plant. *Odontonema cuspidatum* is a sprawling shrub, 1.5–2.5 m in height. The plant flowers primarily from May to August, in the early part of the rainy season. Plants produce indeterminate, branching terminal racemes holding slender red flowers, 1.5–3.0 cm long. Individual inflorescences hold from ~10 to several hundred flowers, and plants produce from one to several dozen inflorescences per flowering period. Plants can live for at least 5 years and generally produce flowers every year beginning in their second or third year (G. Fitch, unpublished data).

Flowers of *O. cuspidatum* are visited by a wide range of nectar- and pollen-feeding animals. Legitimate visitors are primarily hummingbirds, with less frequent visits from butterflies and small solitary bees (G. Fitch, unpublished data). While *Trigona* bees act as primary nectar robbers, a host of hymenopterans are secondary robbers of *O. cuspidatum* flowers (i.e., they rob using previously made holes); these include several species of ants and wasps, as well as other species of stingless bees.

Evaluation of Pollen Limitation

We evaluated the strength of pollen limitation in the studied population of *O. cuspidatum* in 2016–2018, using a total of six *O. cuspidatum* individuals with four or more inflorescences. Four of these plants were growing within areas of coffee cultivation under relatively high-light conditions, while two were growing in a forest fragment with reduced light availability. Total flower number for the manipulated plants ranged from 131 to 1,286. Six plants is a small sample size from which to estimate pollen limitation, but logistical constraints (both the time involved in hand-pollination of large numbers of flowers and the fact that there was only a limited number of plants available, as plants could not be both involved in this experiment and included in the monitoring study) made it difficult to include more plants. Despite this small sample size, we detected strong pollen limitation, indicating that this level of replication was sufficient for this system (see “Results”).

On each plant, one inflorescence was randomly assigned to each of the following treatments: (1) ambient pollination and nectar robbery (unmanipulated) and (2) hand cross-pollination (bagged to exclude nectar robbers; stigmas were saturated with mixed pollen from two other *O. cuspidatum* individuals using a wire filament loop; for details, see Fitch and Vandermeer 2020). Inflorescences were bagged during bud development, before any flowers were large enough to be nectar robbed. Hand-pollination occurred between 0600 and 0800 hours each day during the flowering period. All hand-pollinated flowers were marked on the pedicel with nail polish so that, in case we did not pollinate all flowers, we could distinguish between pollinated and unpollinated flowers. To control for the effects of handling and nail polish application, open flowers were similarly marked on inflorescences that were not hand-pollinated. On fruit maturation, we harvested inflorescences and determined fruit set (fruits per flower) and seeds per inflorescence for each inflorescence, including only marked ovaries in our calculations. While we did not quantify nectar robbery on the plants in the hand-pollination trials, nectar robbers were observed on all open inflorescences.

Inflorescence-level treatments like those we used have been shown to overestimate pollen limitation because of resource reallocation by plants (Zimmerman and Pyke 1988; Knight et al. 2006). However, there are several reasons to suspect that reallocation effects are small in this case. First, inflorescences of *O. cuspidatum* are terminal, and we selected inflorescences for manipulation that were spatially separated within the plant; terminal inflorescences and spatial separation among inflorescences both reduce the possibility of reallocation (Wesselingh 2007). Moreover, stems of *O. cuspidatum*, if separated from the parent plant, commonly root to become new individuals, indicating that individual stems draw from independent resource pools, which makes reallocation unlikely (Wesselingh 2007).

Nevertheless, to control for potential effects of inflorescence-level treatment, in 2019 we conducted full-plant hand-pollination on five individual *O. cuspidatum* that had been propagated from stem cuttings in August 2017 and grown in plastic nursery sleeves filled with soil obtained on the farm. These propagated plants were grown in the farm nursery, which has light exposure similar to that experienced by the naturally occurring plants growing in the area of coffee cultivation. In all cases, 2019 was the first year these plants had flowered; all plants produced either one or two inflorescences. Hand-pollination was carried out using cross pollen as described for the inflorescence-level treatments above, and fruit set and seeds per plant were compared with data from five individuals of similar condition (i.e., propagated in August 2017 and first flowering in 2019, in a high-light environment) that received ambient pollination.

To determine whether plants were pollen limited, we examined whether hand cross-pollination had an effect on fruit set (i.e., fruits per flower), seeds per fruit, and seeds per inflorescence in naturally occurring plants. We compared each measure for inflorescences that experienced hand cross-pollination and those receiving ambient pollination using paired *t*-tests. Since we found evidence for pollen limitation (see "Results"), we took the mean fruit set of hand-pollinated inflorescences to represent the proportion of flowers pollinated at the Haig-Westoby optimum for investment in pollinator attraction.

We assessed the effects of whole-plant hand cross-pollination on potted plants using a Mann-Whitney *U*-test comparing fruit set in plants receiving hand-pollination to those receiving ambient pollination.

Field Surveys and Estimation of Optimal Investment in Pollinator Attraction (I^*_{N} and I^*_{A})

Survey methods are described in greater detail in Fitch and Vandermeer (2021*a*). In brief, in May–July 2017–2018, we surveyed 109 individual *O. cuspidatum* plants (33 of these 109 were surveyed in 2017 only, 15 in 2018 only, and 61 in both years) for nectar robbery every 5 days for the duration of the flowering period. At each survey, we checked all flowers more than 1.5 cm in length, whether open or not, for perforations at the corolla base, which is evidence of nectar robbery. Flowers longer than 1.5 cm are generally either open or within 1 or 2 days of opening; flowers shorter then 1.5 cm are unopened and are rarely robbed. Results for open and unopened flowers were tabulated separately, as were results for each inflorescence. At the conclusion of the flowering period, fruit set, seeds per fruit, and seeds per plant were assessed as described in Fitch and Vandermeer (2021*a*).

To determine the theoretical fruit set that a plant would achieve in the absence of nectar robbery, we developed the following formula:

$$s_{\mathrm{T}} = s_{\mathrm{O}} + (r \times d), \tag{1}$$

where s_{T} represents fruit set in the absence of nectar robbery, s_{O} represents observed fruit set, r represents the proportion of flowers that were robbed, and d represents the population-level mean effect of nectar robbery on fruit set, derived from a subset of plants in which we compared the fates of robbed and unrobbed flowers (Fitch and Vandermeer 2021*a*):

$$d = \frac{\text{fruit set for unrobbed flowers} - \text{fruit set for robbed flowers}}{\text{fruit set for robbed flowers}}. \tag{2}$$

In the population of *O. cuspidatum* under study, $d = 0.70$ in 2017 and 0.68 in 2018 (Fitch and Vandermeer 2021*a*).

To test for a relationship between plant size (as measured by the number of stems) and flower number, we used a generalized linear model (GLM) with a Poisson error distribution; number of flowers was the response variable, and number of stems the predictor.

We evaluated the relationship between total flower number and (1) nectar robbery and (2) corrected fruit set. For each relationship, we fit four different functions (linear, quadratic, logistic, and Michaelis-Menten) to the data using the nls function. We used small sample size–corrected Akaike information criterion scores to determine the best model. Once the best model was selected, we used the predicted best-fit curves in two ways. First, we used the fit of theoretical fruit set to floral display size to determine I^*_{N}, the Haig-Westoby optimum flower number, based on the mean fruit set achieved by hand-pollinated inflorescences. Second, we plotted together the curves for theoretical fruit set and for the proportion of flowers that were not nectar robbed (as in fig. 1*B*) to determine I^*_{A}, the antagonist-induced optimum flower number. In both cases, confidence intervals (CIs) for I^*_{N} and I^*_{A} were generated by bootstrapping.

We tested whether reproductive output was related to total flower number using the following metrics of reproductive output: fruit set, seeds per fruit, and seeds per plant. In all cases we used GLMs with negative-binomial error distribution. For fruit set, number of fruits was the response variable, with log(total number of flowers) included as an offset. The β value of the offset is fixed at 1, allowing us to effectively model proportional data while avoiding the pitfalls of using proportions in regression analysis (Reitan and Nielsen 2016). For both seeds per fruit and seeds per plant, number of seeds was the response variable; for seeds per fruit we additionally included log(number of fruits) as an offset. Because we counted the number of seeds from all fruit only in 2018, we have data on seeds per plant only for that year, but models for fruit set and seeds per fruit incorporated data from 2017 and 2018. For these models, year was included as a covariate.

Experimental Floral Arrays: Is Flower Number the Key Attraction Trait?

To test whether nectar robbers and pollinators responded specifically to flower number rather than another correlated trait, we conducted an experiment using replicated experimental floral arrays. Each of the seven replicates consisted of one array each of 1, 3, 6, and 10 *O. cuspidatum* inflorescences. The inflorescences included in each replicate were cut from a single plant or adjacent plants to control for the effects of variation in floral traits that might influence flower visitor attraction. Additionally, we harvested inflorescences of approximately the same size, although flower number nevertheless differed among inflorescences. Inflorescences, along with a 0.25–0.5-m length of stem, were cut from the plant the day before they were to be deployed and kept in water. The following morning, between 0600 and 0700 hours, each array was placed in a 1-L clear plastic container filled with water and was set out in the field. Arrays within a replicate were placed ~10 m apart; replicates were distributed within a 2-ha section of the farm, in areas under coffee production. Replicates were placed within 100 m of blooming *O. cuspidatum* plants and ≥100 m from one another. Each day, we deployed one to three replicates, depending on the availability of inflorescences.

Between 1300 and 1400 hours on the same day, we surveyed each array for nectar robbery and pollen removal. Nectar robbery was surveyed with the same methods as the field surveys, which are described above. We used pollen removal as a proxy for pollinator visitation, having previously determined that a single visit from a hummingbird was usually sufficient to remove the bulk of pollen from a flower's stamens. To check for pollen removal, a thin wire-filament loop was run over the surface of the stamen and checked for pollen. If the loop came away with more than trace amounts of pollen (i.e., >10 visible grains), we recorded the flower as unvisited by a pollinator. After checking for nectar robbery and pollen removal, we placed all inflorescences in a screened, pollinator- and nectar-robber-free room, grouped by replicate, until the following morning.

Individual inflorescences continued to bloom, and newly opened flowers continued to contain nectar, for at least 3 days after cutting, so we continued to use inflorescences on three consecutive days as long as the quality of the floral display did not visibly deteriorate. Deteriorated inflorescences were replaced with fresh-cut inflorescences from the same plant as necessary. After 3 days of deployment, all inflorescences from a replicate were discarded. On consecutive days, inflorescences were used for the same replicate but randomly assigned to array. Data collection for this experiment lasted from June 27 to July 18, 2018.

To investigate how inflorescence number affected (1) flower number, (2) nectar robbery, and (3) pollen removal in experimental arrays, we used nonlinear mixed models, implemented with the nlme function in the R package nlme (Pinheiro et al. 2019). For all response variables, we compared the same four functions as for naturally occurring plants and selected the best model as described above. For nectar robbery our response variable was number of flowers robbed, and for pollen removal it was number of flowers with pollen removed. For nectar robbery and pollen removal we constructed two models each, one with flower number included as an offset in order to evaluate how the proportion of flowers robbed or visited by pollinators was influenced by inflorescence number, and one without the offset to evaluate how the number of flowers robbed or visited was influenced by inflorescence number. For all models, the number of inflorescences (i.e., array size) was included as a fixed effect; replicate was included as a random effect, to account for potential differences in floral attractiveness across plants from which inflorescences were harvested.

All underlying data and code associated with data analysis are freely available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.sn02v6x4c; Fitch and Vandermeer 2021*b*).

Results

Evaluation of Pollen Limitation

We hand cross-pollinated 382 flowers on six inflorescences on six different naturally occurring plants. For inflorescence-level treatments, hand-pollination resulted in fruit set nearly five times higher than that seen in inflorescences experiencing ambient pollination (ambient mean ± SE: 0.12 ± 0.02; hand-pollinated: 0.57 ± 0.04; $t = 5.22$, $\mathrm{df} = 5$, $P = .003$). Similarly, hand-pollination resulted in a doubling of seeds per fruit (ambient: 1.5 ± 0.5; hand-pollinated: 3.4 ± 0.1; $t = 4.41$, $\mathrm{df} = 3$, $P = .02$) and a fivefold increase in seeds per inflorescence (ambient: 40.9 ± 20.6; hand-pollinated: 206.0 ± 53.3; $t = 4.35$, $\mathrm{df} = 3$, $P = .02$). These results indicate that the population of *O. cuspidatum* under study experiences strong pollen limitation and that fruit set at the Haig-Westoby optimum (I^*_{N}) should be approximately 0.6 (i.e., the fruit set achieved in hand-pollinated inflorescences). Plant-level results from propagated plants were qualitatively similar, with fruit set again five times higher for hand-pollinated plants, but with substantially reduced fruit set overall compared with naturally occurring plants (ambient: 0.04 ± 0.01; hand-pollinated: 0.22 ± 0.01; $W = 0$, $P = .01$), likely due to the severe resource limitation experienced by these plants. Consumption of developing fruit by *Chlosyne janais* (Nymphalidae) larvae on several plants precluded comparison of seed production for whole-plant pollen manipulation.

Field Surveys and Estimation of Optimal Investment in Pollinator Attraction (I^*_{N} and I^*_{A})

Total flower number on monitored plants ranged from 18 to 1,402 flowers. Median flower number was 100 flowers,

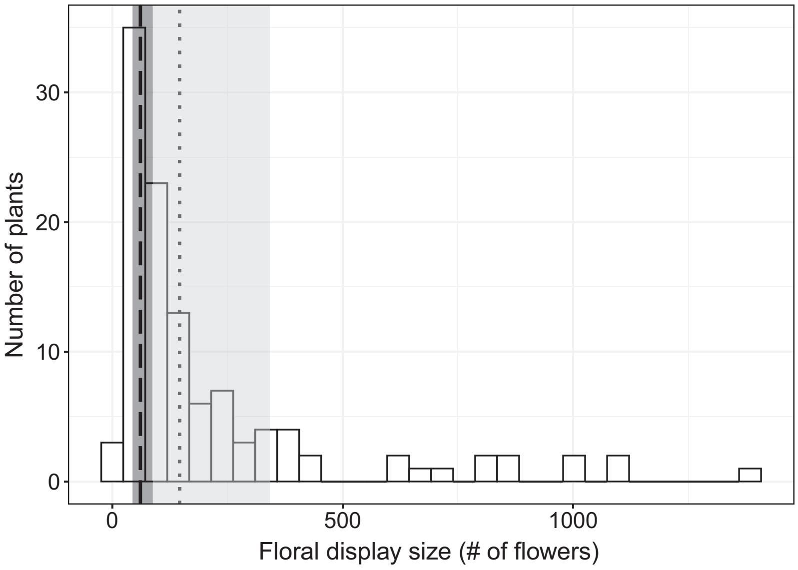

Figure 2: Frequency distribution of total flower number for surveyed plants. The dashed line represents optimum flower number under antagonist-induced adaptive pollen limitation; the dark gray area represents the 95% confidence interval (CI) for the antagonist-induced optimum (corresponding to the shaded region in fig. 3*C*). The dotted line and light gray area represent the optimum and 95% CI, respectively, predicted from the Haig-Westoby framework (the two shaded regions touch but do not overlap).

while the mode was 41 flowers (fig. 2). Flower number was not significantly correlated with plant size, as estimated by the number of discrete stems emerging from the soil ($\beta = 0.03 \pm 0.02$, $z = 1.45$, $P = .15$).

Nectar robbery showed a positive response to *O. cuspidatum* flower number (fig. 3*A*); fruit set showed no correlation with *O. cuspidatum* flower number ($\beta = 0.00$, $z = -1.57$, $\mathrm{df} = 126$, $P = .1$). However, when fruit set data were corrected for the negative effect of nectar robbery following equation (1), expected fruit set in the absence of nectar robbery (hereafter referred to as "theoretical pollination") showed a positive saturating response to flower number (fig. 3*B*). Thus, both nectar robbers and pollinators appear to respond to floral display size such that per-flower nectar robbery and pollination are higher for larger floral displays, satisfying criterion 1 of the conceptual model.

For both proportional nectar robbery and proportional theoretical pollination, the relationship with flower number was best represented by a Michaelis-Menten function (table 1), although in the case of theoretical pollination the Michaelis-Menten was barely distinguishable from the logistic. Both the half-saturation constant (K) and the asymptote (V_m) were lower for theoretical pollination than nectar robbery ($K = 26 \pm 7$ vs. 41 ± 11, $V_\mathrm{m} = 0.69 \pm 0.04$ vs. 0.86 ± 0.07; fig. 3*B*), indicating a stronger response by nectar robbers than pollinators to floral display size and satisfying criterion 2.

When the Michaelis-Menten fits for (1) theoretical antagonist-free pollination and (2) the inverse of nectar-robbing intensity (i.e., the proportion of flowers not robbed) as a function of flower number are plotted together, their curves intersect at a flower number of 62 flowers (95% CI: 44–88 flowers; fig. 3*C*). According to the Michaelis-Menten fit for pollination in the absence of nectar robbery, this flower number results in only 48% (95% CI: 42%–54%) of flowers setting fruit, 30% less than the maximum fruit set predicted by V_m (fig. 3*C*) and >15% less than the mean fruit set for hand cross-pollinated inflorescences (57% ± 4%; see above). Twenty-seven percent of surveyed plants had a flower number that fell within the bounds of the 95% CI for the predicted optimum flower number, despite this range representing only 3% of the total observed range in flower number (fig. 2, dark gray region). By contrast, the 95% CI for the flower number needed to achieve fruit set matching that seen in hand-pollinated plants (i.e., the optimum according to the Haig-Westoby framework [Haig and Westoby 1988; Burd 2008]; 0.57 ± 0.04) was 88–342 flowers (predicted optimum: 146 flowers). Despite the 95% CI for the Haig-Westoby optimum encompassing fully 18% of the observed range in total flower number, only 20% of plants had a total flower number within this range (fig. 2, light gray region). For plants within the 95% CI for the antagonist-driven optimal total flower number (44–88 total flowers), the mean ± SE per-observation flower number was 13 ± 1 flowers.

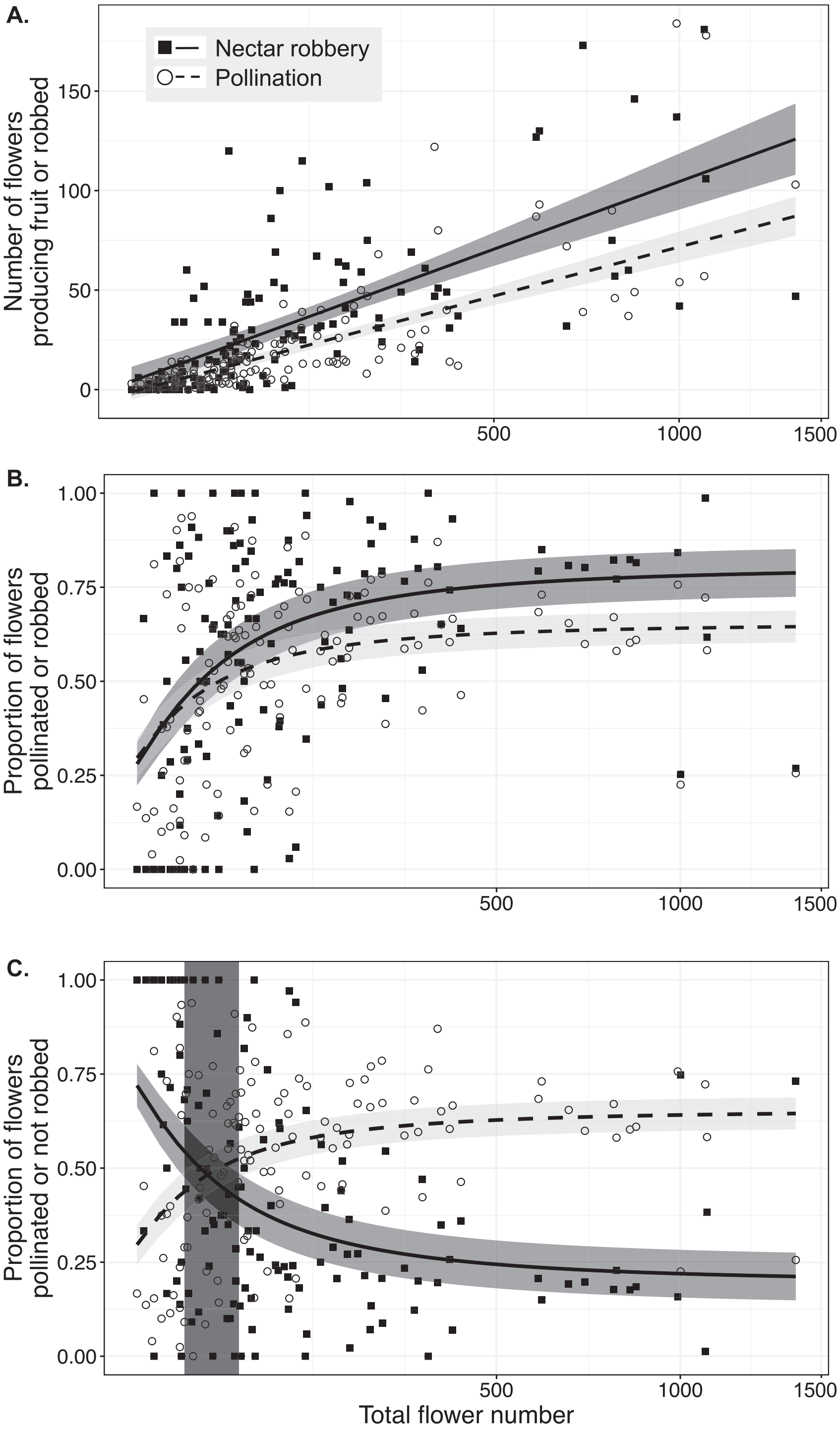

Figure 3: Relationship between flower number and pollination and nectar robbery in naturally occurring plants. In *A*, open circles represent the total number of fruits produced, and filled squares represent the total number of flowers robbed per plant. In both *B* and *C*, open circles represent theoretical pollination in the absence of nectar robbery (see text). In *B*, filled squares represent the proportion of flowers experiencing nectar robbery; in *C*, filled squares represent the proportion of flowers that were not robbed. In *A*, lines represent the linear best fit between flower number and fruit production (dashed line) or nectar robbery (solid line). In *B* and *C*, lines represent the Michaelis-Menten function fit to each data set; shaded areas represent 95% confidence intervals. In *C*, the vertical shaded region indicates the predicted optimum range of flower number, assuming conflicting selection on flower number from pollinators and nectar robbers (corresponding to the dark gray region of fig. 2). Note that the proportion of flowers robbed in *B* and *C* uses the total number of flowers surveyed as the denominator, rather than the flower number as determined at the end of the flowering season; the former is generally a smaller number, since we did not necessarily survey every flower produced by each plant.

As with fruit set (see above), seeds per fruit was not significantly affected by flower number ($\beta = -0.03 \pm 0.02$, $z = -1.37$, df $= 87$, $P = .17$). Per-plant seed production, however, was positively correlated with flower number ($\beta = 0.74 \pm 0.10$, $z = 7.37$, df $= 77$, $P < .001$).

Experimental Arrays: Is Flower Number the Key Attraction Trait?

Arrays of different sizes differed significantly in the number of open flowers, with flower number increasing linearly with inflorescence number (table 1; fig. 4*A*). Both nectar robbery and pollen removal increased with inflorescence number, providing further indication that criterion 1 is satisfied by this system. As with naturally occurring plants, this response was better represented by a saturating function than linear, logistic, or quadratic functions (table 1). And as in naturally occurring plants, both the half-saturation constant (K) and asymptote (V_m) were higher for nectar robbery than pollen removal (nectar robbery: $K = 0.67 \pm 0.39$, $V_m = 0.76 \pm 0.10$; pollen removal: $K = 0.44 \pm 0.29$, $V_m = 0.51 \pm 0.08$; fig. 4*B*), again satisfying criterion 2. When the function for nectar robbery is inverted, the intersection point for the inverse nectar robbery function and pollen removal function is at 2.4 inflorescences, although the 95% CI is quite wide (fig. 4*C*). According to the linear function relating the number of open flowers to array size, 2.4 inflorescences corresponds to 10 open flowers. This closely matches the mean per-observation flower number for naturally occurring plants within the 95% CI for the antagonist-driven optimal total flower number (13 ± 1 flowers).

When the number (rather than proportion) of flowers experiencing nectar robbery is considered, the relationship between inflorescence number and nectar robbery is best described by a linear function (table 1; fig. 4*A*). For the number of flowers experiencing pollen removal, on the other hand, a logistic function provides the best fit (table 1; fig. 4*A*). This indicates that increasing flower number beyond approximately six inflorescences results in minimal increase in the number of flowers pollinated while continuing to attract more nectar robbers.

Discussion

Results from both our field surveys and our experimental manipulations suggest that pollen limitation in *Odontonema cuspidatum* is the result of adaptive response to conflicting pressures on flower number exerted by hummingbird pollinators and bee nectar robbers. While researchers have previously noted conflicting selection pressures on floral traits exerted by pollinators and nectar robbers (Gélvez-Zúñiga et al. 2018), this study is among the first to point out the potential causal link with pollen limitation.

Table 1: Model comparison for the relationships between inflorescence number and flower number, nectar robbery, and pollen removal in naturally occurring plants and experimental arrays of *Odontonema cuspidatum*

Model	Pearson's r	AICc	ΔAICc
	Naturally occurring plants		
Nectar robbery—proportion:			
Michaelis-Menten	**.43**	**39.26**	**.00**
Logistic	.43	41.54	2.28
Quadratic	.41	116.32	77.06
Linear	.22	198.86	159.60
Theoretical pollination—proportion:			
Michaelis-Menten	**.39**	**−33.71**	**.00**
Logistic	.40	−32.54	1.17
Quadratic	.36	80.28	114.00
Linear	.16	163.06	196.78
	Experimental arrays		
Flower number:			
Michaelis-Menten	.80	413.50	2.28
Logistic	.80	425.34	4.12
Quadratic	.80	413.49	2.27
Linear	**.80**	**411.22**	**.00**
Nectar robbery—proportion:			
Michaelis-Menten	**.55**	**31.99**	**.00**
Logistic	.56	34.02	2.03
Quadratic	.31	45.83	13.84
Linear	.45	59.77	27.78
Pollen removal—proportion:			
Michaelis-Menten	**.68**	**−3.06**	**.00**
Logistic	.33	7.98	11.04
Quadratic	.36	10.69	13.74
Linear	.48	28.85	31.90
Nectar robbery—number:			
Michaelis-Menten	.85	386.42	3.20
Logistic	.85	385.60	2.39
Quadratic	.85	383.40	.19
Linear	**.85**	**383.22**	**.00**
Pollen removal—number:			
Michaelis-Menten	.73	364.93	8.05
Logistic	**.83**	**356.89**	**.00**
Quadratic	.83	358.00	1.11
Linear	.76	361.02	4.13

Note: Theoretical pollination refers to observed fruit set corrected for the negative effect of nectar robbery on pollination, following equation (1). Boldface type indicates the best model for that variable, determined by small sample size–corrected Akaike information criterion (AICc) score. ΔAICc indicates the difference in AICc score from the best model for that variable.

For pollen limitation to arise as an adaptive response to conflicting pressures from pollinators and antagonists, four criteria must be met. The first criterion requires that the same trait(s) mediate attraction of both pollinators and antagonists. This criterion was met in both naturally occurring

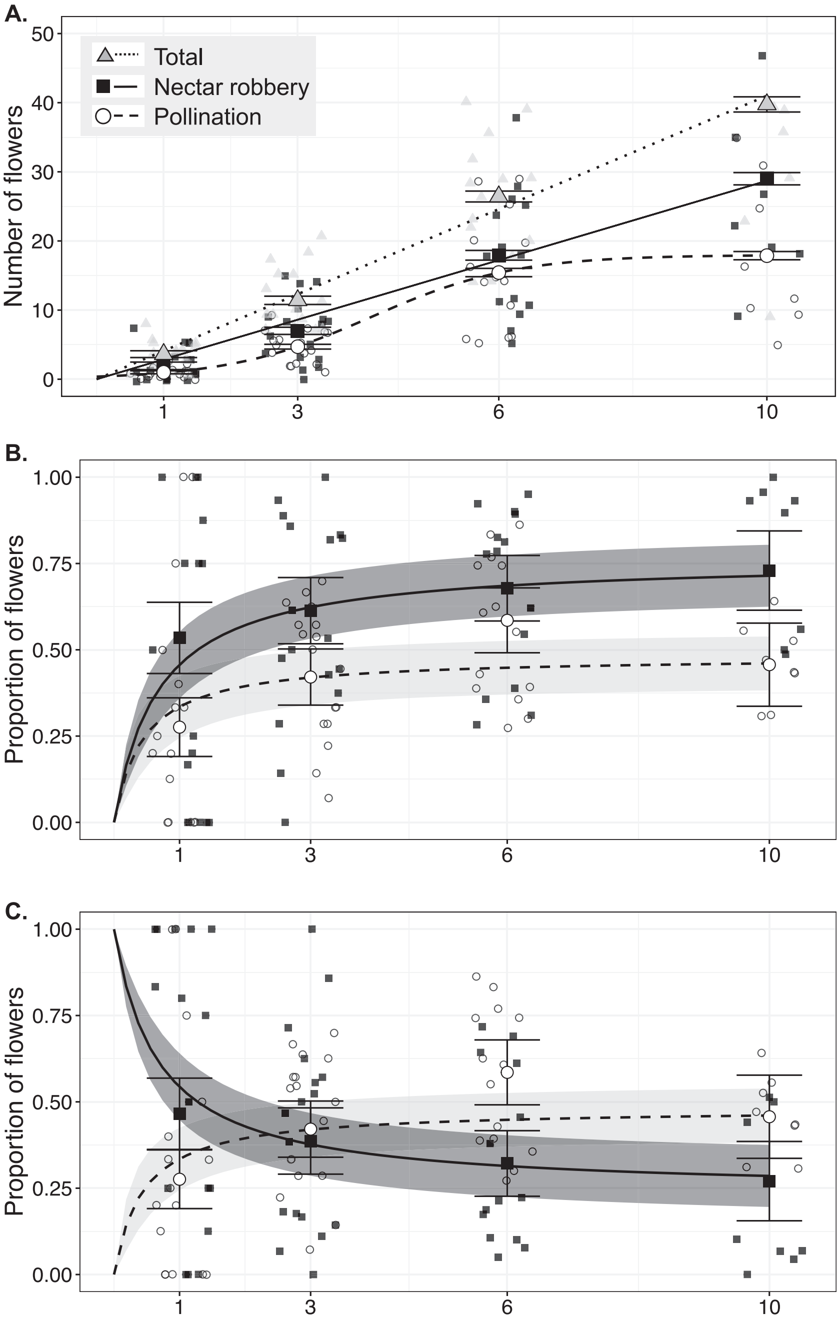

Figure 4: Number (*A*) and proportion (*B*, *C*) of flowers in experimental arrays experiencing pollen removal and nectar robbery. Large points with error bars represent means ± SE for each array size; small points show individual observations, jittered on the *X*-axis for clarity. Lines represent the fit of the best model from among four candidates (linear, quadratic, logistic, Michaelis-Menten). In *B* and *C*, shaded area indicates 95% confidence intervals.

plant and experimental arrays. In naturally occurring plants, *O. cuspidatum* attractiveness to both pollinators and nectar robbers was mediated by flower number, with both the proportion of flowers robbed and the theoretical proportion of flowers that would be pollinated in the absence of nectar robbery showing positive asymptotic responses to flower number. Moreover, a similar pattern of positive asymptotic response to flower number of both nectar robbery and pollen removal emerges from the data from our experimental arrays. This confirms that flower number—rather than another, correlated trait—mediated both pollinator and nectar robber attraction.

The second criterion is that the antagonists' response to an increase in allocation to pollinator attraction must be stronger than the pollinators' response. Data from both surveys and experimental arrays indicate that this occurs in our study system: levels of nectar robbery are consistently higher than levels of pollination for a given floral display size.

The third criterion is that conflicting pressures lead to reduced investment in the relevant trait(s), lowering pollinator attraction and leading to pollen limitation. Again, our data confirm that this occurs in *O. cuspidatum*. The antagonist-induced optimum flower number for this population of *O. cuspidatum*, as predicted by field survey data, is less than half the predicted Haig-Westoby optimum (fig. 3*C*). At the antagonist-induced optimum, fruit set is >15% lower than the levels seen in hand cross-pollinated *O. cuspidatum* plants, indicating that plants at the antagonist-induced optimum experience pollen limitation. These results are closely matched by those from our experimental arrays; estimates of the antagonist-driven optimum flower number from the two data sets are remarkably similar (i.e., 10 vs. 13 open flowers at a time).

The fourth and final criterion to be satisfied is that plants reducing investment in pollinator attraction must nevertheless experience higher fitness than those investing at the Haig-Westoby optimum. We cannot conclusively determine whether this is occurring in the study population of *O. cuspidatum* given that we do not have information on lifetime fitness; the evidence we do have is mixed in its support for this criterion. The distribution of total flower number in the population supports the idea that a flower number close to the antagonist-induced optimum is selected for. Total flower numbers within the range of the antagonist-driven optimum were dramatically overrepresented in naturally occurring plants (fig. 2). This does not appear to be solely the result of resource availability dictating allocation to flower production, as flower number was not significantly correlated with plant size (although light availability does influence flower production, presumably via its influence on the availability of photosynthate; Fitch and Vandermeer 2020).

Despite apparent convergence on the antagonist-driven optimum flower number, the population exhibits high variability in flower number. This maintenance of trait variation is consistent with our hypothesis that high levels of variability in nectar-robbing intensity—both across individuals and between years within individuals—should maintain trait variation. Indeed, the overrepresentation of plants with a very large number of flowers (fig. 2) suggests the possible existence of an alternative strategy. Very large floral displays may represent a form of antagonist satiation; there is some indication that levels of nectar-robbing intensity decline for plants with very large floral displays (in fig. 3*B*, note two points in lower right), although these same plants also experienced relatively low levels of pollination, drawing into question the utility of antagonist satiation in this case.

We found a positive linear correlation between flower number and seed production, suggesting that despite higher levels of nectar robbery, short-term fitness is maximized by maximizing flower number. But in this population, a large flower number in one year is strongly correlated with reduced flower production the following year (G. Fitch and J. Vandermeer, unpublished data). Costs of reproduction such as this are common in plants (Obeso 2002). Such costs suggest a potential trade-off between short-term and lifetime fitness maximization, increasing the likelihood that a smaller number of flowers, minimizing the loss of flowers to nectar robbers, may maximize lifetime fitness. Moreover, in the experimental arrays, the number of flowers experiencing pollen removal showed a saturating response to inflorescence number, indicating minimal benefit of floral displays larger than approximately six inflorescences (26 ± 1 open flowers at a time). Thus, the linear relationship between total flower number and seed production may reflect benefits of a longer flowering period rather than of producing more total flowers. This explanation does not contradict the idea that the antagonist-induced optimum flower number maximizes fitness.

This study suggests that conflicting selection between antagonists and pollinators is an important driver of pollen limitation in *O. cuspidatum*. But how widespread is antagonist-induced adaptive pollen limitation likely to be? According to our conceptual model, the conditions under which antagonists would drive pollen limitation are rather restrictive. Below, we discuss three primary restrictions. Despite these restrictions, we suspect that the necessary conditions for antagonist-induced adaptive pollen limitation occur widely and that antagonist-induced adaptive pollen limitation may be common.

One restrictive requirement of the model is that antagonists must respond at least as strongly as pollinators to an incremental increase to investment in pollinator attraction. At first glance, greater sensitivity of the antagonist than the pollinator to pollinator attraction traits seems unlikely. Yet such an outsized response from antagonists might be expected when the antagonist occurs at substantially higher densities than the pollinator. This is fairly likely to occur if the pollinator is a vertebrate and the antagonist is an insect or even if the pollinator is a solitary insect while the antagonist is a social insect with the potential for rapid recruitment of large numbers of foragers to a food resource. At least for nectar robbery, these are common (although certainly not universal) scenarios: social Hymenoptera comprise a plurality of documented nectar robbers (Irwin et al. 2010), while the pollinators of flowers that experience heavy nectar robbery are often birds (e.g., Irwin 2006; Rojas-Nossa et al. 2016; Gélvez-Zúñiga et al. 2018). At somewhat longer timescales, if insects whose larvae consume floral tissues use pollinator-attracting floral traits to locate oviposition sites (as is the case for, e.g., some Lepidoptera; Irwin et al. 2003; Adler and Bronstein 2004), the effect of antagonist attraction is likely to outweigh that of pollinator attraction, since a single visit by an ovipositing adult will generally result in multiple larvae that will then consume the plant.

A second restriction of the model is that most individual flowers should be either "for pollinators" or "for antagonists"—that is, flowers that are visited by a pollinator should produce seeds, flowers attacked by an antagonist should not, and there should be little overlap in flowers visited by both pollinators and antagonists. The degree to which this occurs will likely depend on the mechanism by which antagonist activity reduces plant reproduction. If the antagonist reduces the attractiveness of flowers to pollinators but does not directly damage reproductive organs, then the fate of a flower (pollinated or damaged) is essentially determined by the identity of its first visitor (assuming relatively high single-visit pollination efficiency). If the first visitor pollinates the flower, future interactions with an antagonist will not impact seed production. Alternatively, if an antagonist visits first, it is unlikely that the flower will be subsequently visited by pollinators, leaving it unpollinated. It is common for floral antagonists—including nectar robbers and florivores—to affect reproduction by reducing pollinator attraction (Lohman et al. 1996; Irwin and Brody 2000; Castro et al. 2008; Sõber et al. 2010; Varma et al. 2020), including in our study system (Fitch and Vandermeer 2020).

On the other hand, if antagonists directly damage reproductive organs, the important question is not who visits the flower first but whether the flower interacts with an antagonist at any point. In this case, there is likely to be substantial overlap in for-pollinator and for-antagonist flowers, unless antagonists avoid flowers that have been previously visited by pollinators. While antagonist avoidance of pollinated flowers is possible (particularly for antagonists seeking nectar rewards), its occurrence has not been widely documented. We therefore suggest that antagonist-induced adaptive pollen limitation is more likely to occur where the antagonist reduces fitness by deterring pollinators rather than damaging reproductive organs.

We note, however, that the partitioning of flowers as either for pollinators or for antagonists need not be complete for antagonist-induced pollen limitation to operate. In our study system, nectar robbery significantly reduces pollination, but approximately 18% of robbed flowers still set fruit (vs. 32% of unrobbed flowers; Fitch and Vandermeer 2021*a*). This indicates that while pollinators prefer unrobbed flowers, they do pollinate robbed flowers, leading to some overlap in the flowers that are "for" pollinators and those that are "for" antagonists. Nevertheless, antagonist-induced adaptive pollen limitation appears to occur in this system. Further study, both empirical and theoretical, will be necessary to determine how much nonoverlap in for-pollinator and for-antagonist categories is needed for antagonist-induced adaptive pollen limitation to arise.

Finally, antagonist-induced adaptive pollen limitation is likely to be more common in iteroparous than semelparous plant species. Reducing investment in traits that attract both pollinators and antagonists will be adaptive for semelparous species only if the negative effect of attracting additional antagonists outweighs the positive effect of attracting additional pollinators. In iteroparous species, on the other hand, reducing short-term investment in reproduction increases the likelihood of survival and future reproduction. Therefore, the negative effect of attracting antagonists need not fully outweigh the positive effect of attracting pollinators for reduced investment in pollinator attraction to be adaptive. Much of the existing theoretical work on pollen limitation has focused, at least implicitly, on semelparous species. This is due, in part, to challenges associated with estimating pollen limitation for iteroparous species, since experimental manipulation of pollen receipt in iteroparous species can overestimate pollen limitation if reproductive output is not measured for all reproductive events (Zimmerman and Pyke 1988; Knight et al. 2006). However, that pollen limitation is difficult to experimentally assess in iteroparous species does not mean that it does not occur. The current study suggests that a more explicit focus on iteroparous species may illuminate additional mechanisms that give rise to pollen limitation.

Existing explanations of pollen limitation have focused exclusively on the plant-pollinator interaction. This work highlights how expanding our field of view to consider other impinging interaction types can illuminate heretofore-overlooked mechanisms. The present study should be considered a proof of concept: we show data consistent with the hypothesis that antagonist-induced pollen limitation may occur and be adaptive. Yet we are not able to conclusively rule out a role for other mechanisms—particularly plant response to stochastic variability in pollen receipt—that may be leading to pollen limitation in *O. cuspidatum*.

More generally, we do not know how widely antagonist-induced adaptive pollen limitation occurs. In the present scenario, the attractiveness trait under apparent conflicting selection was flower number. We know that pollinators and antagonists can exert conflicting selection on other traits, including flower morphology (Irwin et al. 2003; Ågren et al. 2013; Sletvold et al. 2015; Gélvez-Zúñiga et al. 2018), nectar rewards (Adler and Bronstein 2004), flower scents (Theis and Adler 2012), and flower phenology (Sletvold et al. 2015); can antagonist-induced adaptive pollen limitation also arise in these cases? Must the antagonist strictly attack floral tissues, or could a more generalized herbivore, still cueing to pollinator attraction traits (e.g., Irwin et al. 2003), exert the same pressures? Might a qualitatively similar pattern emerge in a system with two pollinators, where a high-quality pollinator competes for access to flowers with a poorer pollinator (e.g., Burd 1995*b*)? The answers to all of these questions await further study; we hope that their pursuit can further motivate discussion regarding the ecological and evolutionary drivers of pollen limitation.

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Statement of Authorship

G.F. and J.V. conceived of the study and developed methods and experimental design. G.F. conducted data collection and data analysis. G.F. wrote the first draft of the manuscript, with substantial input from J.V.; both authors contributed to editing the manuscript.

Data and Code Availability

All data and code associated with this study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.sn02v6x4c; Fitch and Vandermeer 2021*b*).

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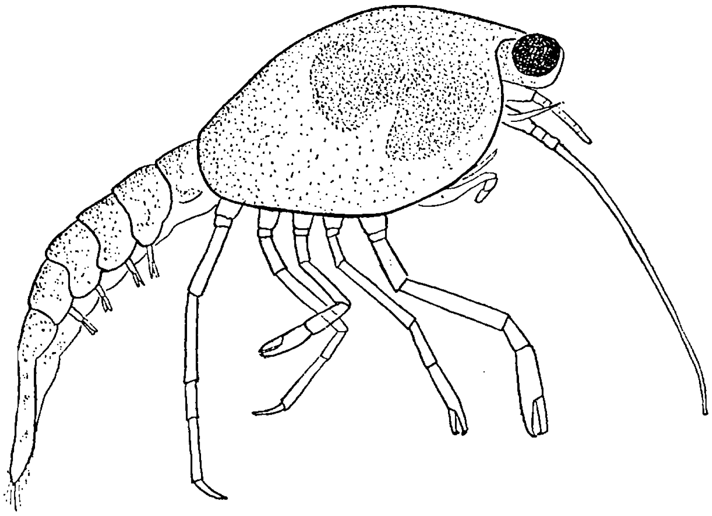

"A remarkable fact in the life history of the crayfish is that the young associate with the mother for many days after leaving the egg, being at first fastened to her and later going back to her for protection until finally quite independent. . . . The young of *C. clarkii* were obtained from eggs laid in confinement by adults shipped from New Orleans, November 18, 1904; some 18 out of 61 surviving the journey." Figured: "Right side of living larva, in second stage." From "The Attached Young of the Crayfish *Cambarus clarkii* and *Cambarus diogenes*" by E. A. Andrews (*The American Naturalist*, 1907, 41:253–274).