

Light availability influences the intensity of nectar robbery and its effects on reproduction in a tropical shrub via multiple pathways

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PREMISE: The multiple exogenous pathways by which light availability affects plant reproduction (e.g., via influence on attraction of mutualists and antagonists) remain surprisingly understudied. The light environment experienced by a parent can also have transgenerational effects on offspring via these same pathways.

METHODS: We evaluated (a) the influence of light availability on floral traits in *Odontonema cuspidatum*, (b) the relative importance of the pathways by which light influences nectar robbery and reproductive output, and (c) the role of parental light environment in mediating these relationships. We conducted a reciprocal translocation experiment using clonally propagated ramets and field surveys of naturally occurring plants.

RESULTS: Light availability influenced multiple floral traits, including flower number and nectar volume, which in turn influenced nectar robbery. But nectar robbery was also directly influenced by light availability, due to light effects on nectar robber foraging behavior or neighborhood floral context. Parental light environment mediated the link between light availability and nectar robber attraction, suggesting local adaptation to low-light environments in floral visitor attraction. However, we found no transgenerational effect on reproduction.

CONCLUSIONS: Our findings demonstrate that exogenous pathways by which light influences plants (particularly through effects on floral antagonists) can complicate the positive relationship between light availability and plant reproduction. Our results are among the first to document effects of light on floral antagonists and clonal transgenerational effects on flower visitor attraction traits.

KEY WORDS Acanthaceae; clonal transgenerational effects; floral antagonists; indirect effects; maternal effects; *Odontonema cuspidatum*; pollination; reciprocal translocation.

Light is a key resource for plants, providing the energy that is the basis for carbon assimilation; light availability therefore has strong effects on plant growth and reproduction. In low-light conditions, plant growth rates are commonly reduced (Coleman et al., 1994; Kilkenny and Galloway, 2008; Galloway and Etterson, 2009). Moreover, plants growing in low light may allocate a greater proportion of resources toward tissues that aid in light capture, rather than reproduction (McConaughay and Coleman, 1999; Delerue et al., 2013). These two factors, operating independently or in tandem, can result in reduced reproductive output in low- as compared to high-light conditions (Fig. 1F), whether through the production of fewer ovules (Mattila and Salonen, 1995; Kilkenny and Galloway, 2008;

Cao et al., 2017) or through reduced per-ovule provisioning levels (Niesenbaum, 1993; Kilkenny and Galloway, 2008).

Yet, beyond the endogenous pathways (that is, pathways involving only the effects of light availability on the plant in question) described above, there are multiple exogenous pathways (mediated by other organisms) by which light availability can influence reproductive output (Fig. 1). For example, light may influence patterns of herbivory via effects on herbivore behavior (Suárez-Vidal et al., 2017) or on plant chemistry and palatability (Dudt and Shure, 1994; McDonald et al., 1999). Similarly, light availability can influence pollination by affecting either pollinator behavior (Fig. 1B, C; Herrera, 1995; Kilkenny and Galloway, 2008)

or floral traits that mediate pollinator attractiveness, including flower number (Cunningham, 1997; Kilkenny and Galloway, 2008; Cao et al., 2017) and flower size (Kilkenny and Galloway, 2008).

Importantly, an individual plant's response to light availability may be mediated or constrained by the light environment in which its parent(s) grew (Galloway and Etter, 2007, 2009; Heger, 2016). Such conditioning of offspring response to environmental stimuli by parental environment is known as a transgenerational effect. Transgenerational effects—also known as parental effects, and including maternal effects—are common in plants and can reflect aspects of both the abiotic and biotic environments of parents (Roach and Wulff, 1987; Rossiter, 1996). Transgenerational effects have been studied primarily in sexually reproducing plants, but there is mounting evidence for their importance in clonal plants as well (Latzel and Klimešová, 2010; Dong et al., 2017; Münzbergová and Hadincová, 2017; Dewan et al., 2018). However, the study of transgenerational effects in plants—whether in clonal or sexually reproducing populations—has focused largely on growth or defense traits, with very little attention paid to transgenerational effects on traits mediating floral attractiveness.

In addition to the effects of light environment on plant traits, light availability may also affect pollination via the influence of light on other plant–animal interactions. One interaction type that may be an important mediator of plant–pollinator interactions is nectar robbery (NR; Fig. 1D, E)—that is, the extraction of nectar from a flower via an opening other than the corolla mouth (Irwin et al., 2010). The effect of this interaction on the plant can be negative, neutral, or positive (Maloof and Inouye, 2000; Burkle et al., 2007; Irwin et al., 2010). Since nectar robbers are frequently also pollinators of other plant species—and even of other flowers of the same species—they may respond to similar traits as pollinators (Irwin et al., 2010). Indeed, nectar robbers have been shown to prefer plants with more flowers, much like pollinators (Irwin, 2006; Gélvez-Zúñiga et al., 2018). Even if nectar robbers and pollinators use different cues to locate food sources—particularly likely when robbers and pollinators have different sensory biases (e.g., arthropod robbers and vertebrate pollinators; Schiestl and Johnson, 2013; Gegear et al., 2017)—light may simultaneously influence multiple plant traits, thereby affecting pollinator and nectar robber attraction in potentially complex ways. But the extent to which NR is influenced—whether directly or indirectly—by abiotic conditions has scarcely been evaluated. Aiming to fill this knowledge gap, the study reported here combined field surveys and a reciprocal translocation experiment

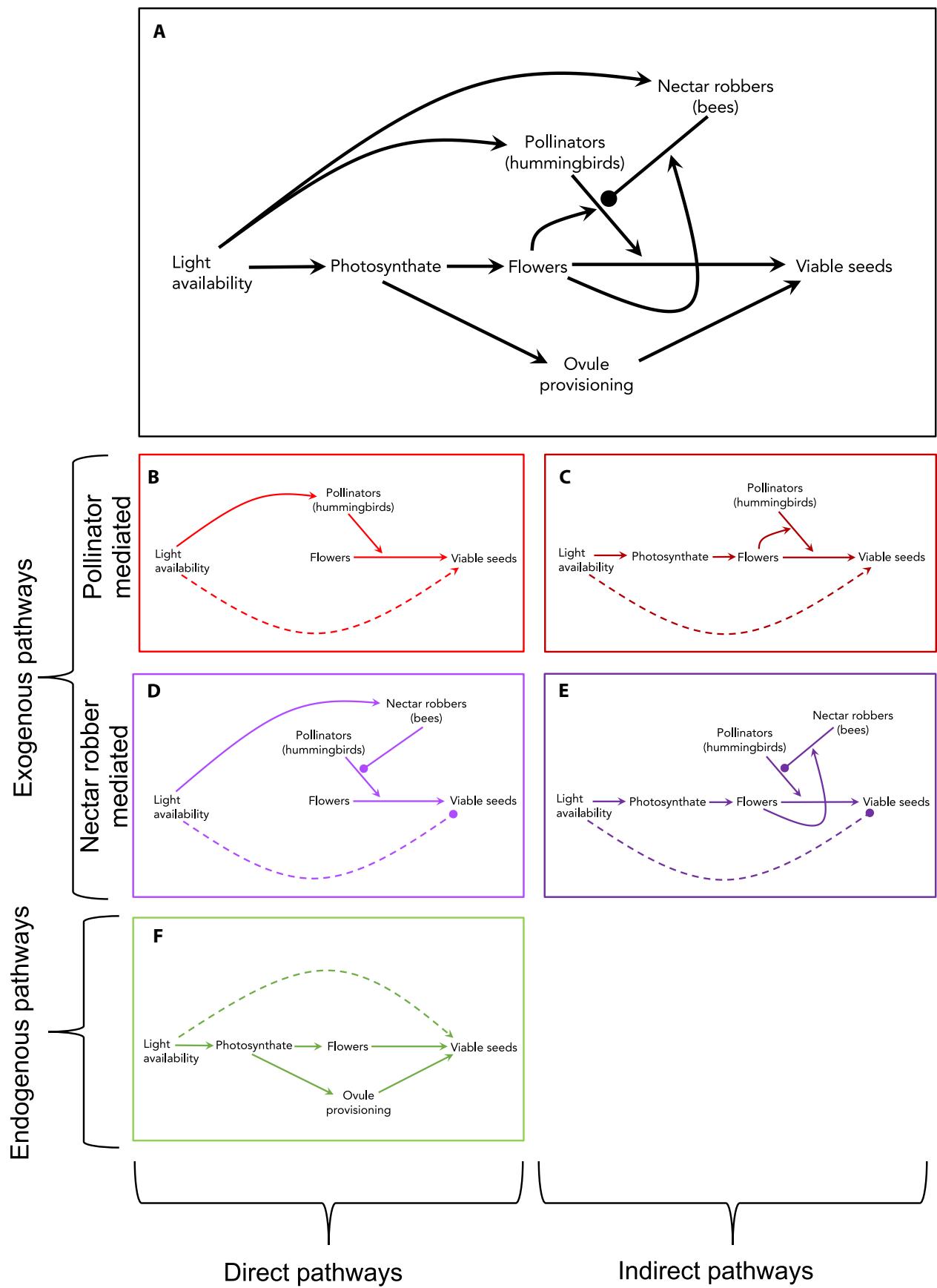
using the polycarpic understory shrub *Odontonema cuspidatum* (Nees) Kuntze (Acanthaceae). In the study area (southeastern Chiapas, Mexico), *O. cuspidatum* experiences high levels of NR from stingless bees (Hymenoptera: Apidae: Meliponini); robbed flowers are significantly less likely than unrobbed flowers to produce fruit (Fitch and Vandermeer, 2020a [Preprint]). The study, which took place in a coffee agroecosystem comprising areas of coffee cultivation with a canopy tree cover of varying density and small forest fragments, addressed the following questions:

1. Does light availability affect flower number, flower morphology, or nectar rewards in *O. cuspidatum*?
2. Does light availability influence the intensity of NR by stingless bees, and, if so, is this due to direct effects of light on bee foraging behavior or mediated by floral traits?
3. What is the relative importance of endogenous effects, pollinator-mediated effects, and nectar-robbing-mediated effects of light availability on *O. cuspidatum* reproductive output?
4. Does parental light environment mediate the effect of offspring light environment during growth or flowering on pollination and NR (i.e., are there transgenerational effects of parental light environment on the link between light availability and pollination and/or NR)?

We expected that (1) light availability would influence multiple aspects of floral attraction traits, with higher light availability leading to the production of more flowers, larger flowers, and more floral nectar. We further predicted that (2) plants in high-light conditions would experience higher levels of NR, primarily due to the predicted effects of light availability on floral traits. Direct effects of light availability on flower visitor behavior are often due to increased activity levels associated with higher temperatures (Herrera, 1995; Kilkenny and Galloway, 2008). We therefore hypothesized that light availability would have little effect on bee foraging behavior, given that temperature may be relatively unimportant in regulating bee foraging in warm tropical environments (Willmer, 1991; but see Figueiredo-Mecca et al., 2013; Aleixo et al., 2017).

Given that NR has a negative impact on *O. cuspidatum* fruit set (Fitch and Vandermeer, 2020a [Preprint]) and that pollen supplementation results in dramatic increases in fruit set and seed production (G. Fitch and J. H. Vandermeer, unpublished manuscript), indicating that reproduction is pollen limited, we expected that (3) the exogenous effects of light on *O. cuspidatum* reproductive output, mediated both by pollinators and nectar robbers, would be

FIGURE 1. Conceptual diagram illustrating the multiple pathways by which light availability can influence seed production, pollination, and nectar robbery in *Odontonema cuspidatum*. Arrows indicate positive effects and filled circles indicate negative effects. In direct pathways, light availability modifies an interaction partner; in indirect pathways, light availability modifies an interaction link. Dotted links indicate the predicted net effect of light availability on seed production for the illustrated pathway. Note that multiple pathways may operate in tandem. (A) Complete path diagram. (B–E) Exogenous pathways (i.e., pathways that involve nectar robbers and/or pollinators). (B) Direct pollinator pathway: light directly affects pollinator behavior; pollinator behavior affects seed production. (C) Indirect pollinator pathway: light affects pollinator behavior by affecting floral traits; pollinator behavior affects seed production. (D) Direct nectar robber pathway: light directly affects nectar robber behavior; nectar robbery decreases pollination. (E) Indirect nectar robber pathway: light affects nectar robber behavior by affecting floral traits; nectar robbery decreases pollination. (F) Endogenous pathways (i.e., pathways that involve only light effects on the plant). In C and E, the flow diagrams imply that light affects floral traits via changes to photosynthate availability, but in this study we did not investigate the physiological mechanisms underlying correlation between light availability and floral traits. Note that some possible pathways (e.g., nectar robbery directly affects seed production) are omitted because they were eliminated as potential causal pathways in this study system (see text).



stronger than the endogenous effects. Finally, (4) we expected that strong direct effects of light in the growth environment would overwhelm any effects of the parental environment, and thus that transgenerational effects would be absent.

MATERIALS AND METHODS

Study system

Odontonema cuspidatum (Acanthaceae) is a polycarpic shrub endemic to southern Mesoamerica, where it occurs in the forest understory, particularly in light gaps and along watercourses; it is also commonly planted as an ornamental and for erosion control (Daniel, 1995; G. Fitch, personal observation). Broken stems of *O. cuspidatum* readily root to become independent ramets (G. Fitch, personal observation).

In the study area, *O. cuspidatum* blooms primarily during the rainy season, from June to August, bearing indeterminate branching racemes of tubular red flowers. Plants are self-fertile but not capable of autogamy (Appendix S1). Flowers are pollinated primarily by hummingbirds (G. Fitch, unpublished data) but are also attractive to a wide range of nectar-feeding insects. Many of these insects engage in nectar robbery, extracting nectar from perforations in the base of the corolla tube. Primary nectar robbers (i.e., those that make the perforation themselves) include two species of stingless bee in the genus *Trigona* (*T. fulviventris* and *T. nigerrima*, Hymenoptera: Apidae; Meliponini; Appendix S2). Other Hymenoptera, as well as several species of Lepidoptera, secondarily rob, using previously made perforations. Flowers are commonly robbed before opening, generally once they are <1.5 cm long and <2 d before opening; NR prior to opening generally does not impact blooming. Nectar robbery leads to a $\sim 40\%$ reduction in probability of setting fruit (Fitch and Vandermeer, 2020a [Preprint]).

Fertilized flowers produce explosively dehiscent capsules containing up to four seeds. In the population under study, fruit set is quite low: on average, $<20\%$ of flowers produce fruit (Fitch and Vandermeer, 2020a [Preprint]).

Research was conducted at Finca Irlanda (15.17358, -92.33827), a shaded organic coffee farm in southeastern Chiapas, Mexico. The farm, approximately 300 ha in extent and 900–1150 m a.s.l., consists of a coffee plantation with a diverse tree canopy, along with several forest fragments. On the farm, *O. cuspidatum* occurs both within areas of coffee cultivation and in forest fragments.

Field surveys

We randomly selected 109 *O. cuspidatum* individuals within the study area for inclusion in field surveys (hereafter, we refer to these plants as “naturally occurring”). Each selected plant was individually marked with flagging tape at its base and was monitored during the flowering period in 2017 and 2018. Among the 109 surveyed plants, 33 individuals surveyed in 2017 either died or did not flower in 2018; in 2018 we included an additional 15 plants that did not flower in 2017. We recorded the GPS coordinates of each plant and determined the degree of canopy cover directly above the crown of the plant—our measure of light availability—using CanopyApp version 1.0.3 (University of New Hampshire, Durham, New Hampshire, USA). Canopy cover ranged from 22% to 98%. Distances between surveyed plants ranged from 10 to 2200 m. The

hummingbirds that serve as primary pollinators of *O. cuspidatum* have foraging ranges that span this distance and move readily between forest fragments and areas of coffee production (S. Barney et al., University of Michigan, unpublished manuscript), so all surveyed individuals represent a single population.

In 2017–2018, plants were surveyed for NR weekly during flowering. NR leaves visible perforations at the base of the corolla tube. At each survey, all flowers ≥ 1.5 cm in length on inflorescences that contained at least one open flower were checked for evidence of robbery, and we recorded the number of robbed and unrobbed flowers per inflorescence. We tallied robbery for open and unopened flowers separately.

Beginning ~ 2 wk after flowering ended on the earliest-flowering inflorescence, and continuing weekly until all inflorescences had matured, we assessed fruit set by counting the number of fruit and number of persistent ovaries (i.e., flowers that had not set fruit) on mature inflorescences. Inflorescences that had been damaged by insect herbivores (primarily *Chrysone* sp. [Nymphalidae: Lepidoptera] larvae; representing $<5\%$ of inflorescences) were not included in measures of fruit set, though we included counts from these inflorescences in plant-level flower number. To measure seed set, up to five fruits (in 2017) or all undamaged fruits (in 2018) were collected from each inflorescence. Collected fruits were placed in a drying oven until all had dehisced (~ 24 h), and then seeds were counted.

Floral traits

On a subset of 18 of the plants that were surveyed for nectar robbery, chosen to represent the overall gradient of canopy cover, we measured the following aspects of floral morphology: corolla tube length, corolla flare, corolla mouth width, and corolla base width. These traits were chosen because they are readily measurable in the field and have been shown to influence flower visitor attraction in other species (e.g., Galen, 1999; Rojas-Nossa et al., 2016; Gélvez-Zúñiga et al., 2018). On each plant, five open flowers were randomly selected for measurement. Measurements occurred during 21–29 June 2018 and were made using digital calipers (Thomas Scientific, Swedesboro, New Jersey, USA).

On another subset of monitored plants (49 in 2017, 19 in 2018, with 7 included in both years; for details of sampling scheme, see Appendix S3)—again chosen to represent the range of canopy cover seen across monitored plants—we assessed nectar volume and sugar content. Nectar sampling in 2018 was primarily to fill gaps in the range of canopy cover experienced by plants sampled in 2017, with repeat sampling of a subset of individuals to determine the degree of interannual variability in nectar traits within individuals, which was found to be low and showed no consistent temporal trend (G. Fitch, unpublished data). Unbagged flowers consistently had no standing nectar crop, so we measured nectar production on flowers from which pollinators were excluded with mesh bags. We bagged two inflorescences per plant and checked bagged inflorescences for open flowers twice per week. Nectar volume was measured by removing the nectar from a flower with a 75 μL microcapillary tube (Drummond Scientific, Broomall, Pennsylvania, USA) and measuring the height of the nectar in the tube using digital calipers. To measure nectar sugar content, we used a pocket refractometer (Eclipse 45-81; Bellingham & Stanley, Tunbridge Wells, UK). Only plants for which we had measures of both nectar volume and nectar sugar content for at least four flowers were included in data analysis.

We used correlation between floral traits and light availability to assess the endogenous response of *O. cuspidatum* floral traits to

light (Fig. 1B). We did not investigate the physiology underlying these correlations, and only infer that these correlations are due to light impacts on photosynthate availability.

Reciprocal translocation experiment

See Figure 2 for a schematic diagram of the reciprocal transplant experiment design. In August 2017, we cut 12 stems (hereafter “ramets”) each from 12 plants, six growing in high light (canopy cover <50%; high-light parental environment [PE]) and six in low light (canopy cover >80%; low-light PE). Cut ramets were potted in 500 cm³ nursery sleeves filled with potting soil from the Finca Irlanda nursery. Half the ramets from each plant were placed in the Finca Irlanda nursery, where light availability was high (23% canopy cover; high-light growth environment [GE]); the other half were placed together in a nearby forest fragment with dense canopy (95% canopy cover) and low light availability (low-light GE). Canopy cover at these sites fell within the range of canopy cover experienced by naturally occurring plants. Ramets were left to grow for 10 mo, until the onset of flowering. During the dry season, all ramets were given a soaking watering once per week but were otherwise unattended.

In June 2018, 38 of the potted ramets flowered and were placed in the field in arrays of two or three ramets prior to the onset of flowering (for number of ramets in each PE-GE-FE combination,

see Fig. 2). Arrays were located >10 m and <100 m from existing *O. cuspidatum* plants in bloom, and >10 m from other arrays. Eighteen ramets were placed in low-light conditions (canopy cover >85%; low-light flowering environment [FE]) in a forest fragment to bloom, and 20 were placed in high-light conditions (canopy cover <35%; high-light FE) in an area of coffee cultivation. These ramets were monitored for NR and assessed for fruit and seed set, as outlined above for naturally occurring plants, with the difference that monitoring of potted ramets for NR occurred every other day rather than weekly. Several ramets were heavily damaged by *Chlosyne* sp. larvae during the course of the experiment and were excluded from analyses of season-long flower production and reproductive output. Five ramets from four different treatments were heavily damaged by *Chlosyne* sp. larvae during the course of the experiment and were excluded from analyses of season-long flower production and reproductive output. In all cases, damage occurred only after flowering was under way, so we included data on NR and per-observation flower number for all plants. We could identify no ecological correlate with *Chlosyne* sp. herbivory. Because of the small number of ramets that flowered in 2018, we were not able to assess floral traits, other than flower number, on ramets in the reciprocal translocation experiment. While 38 ramets spread over six treatments results in a small number of individuals per treatment, the fully factorial design maximized statistical power by enabling us to group

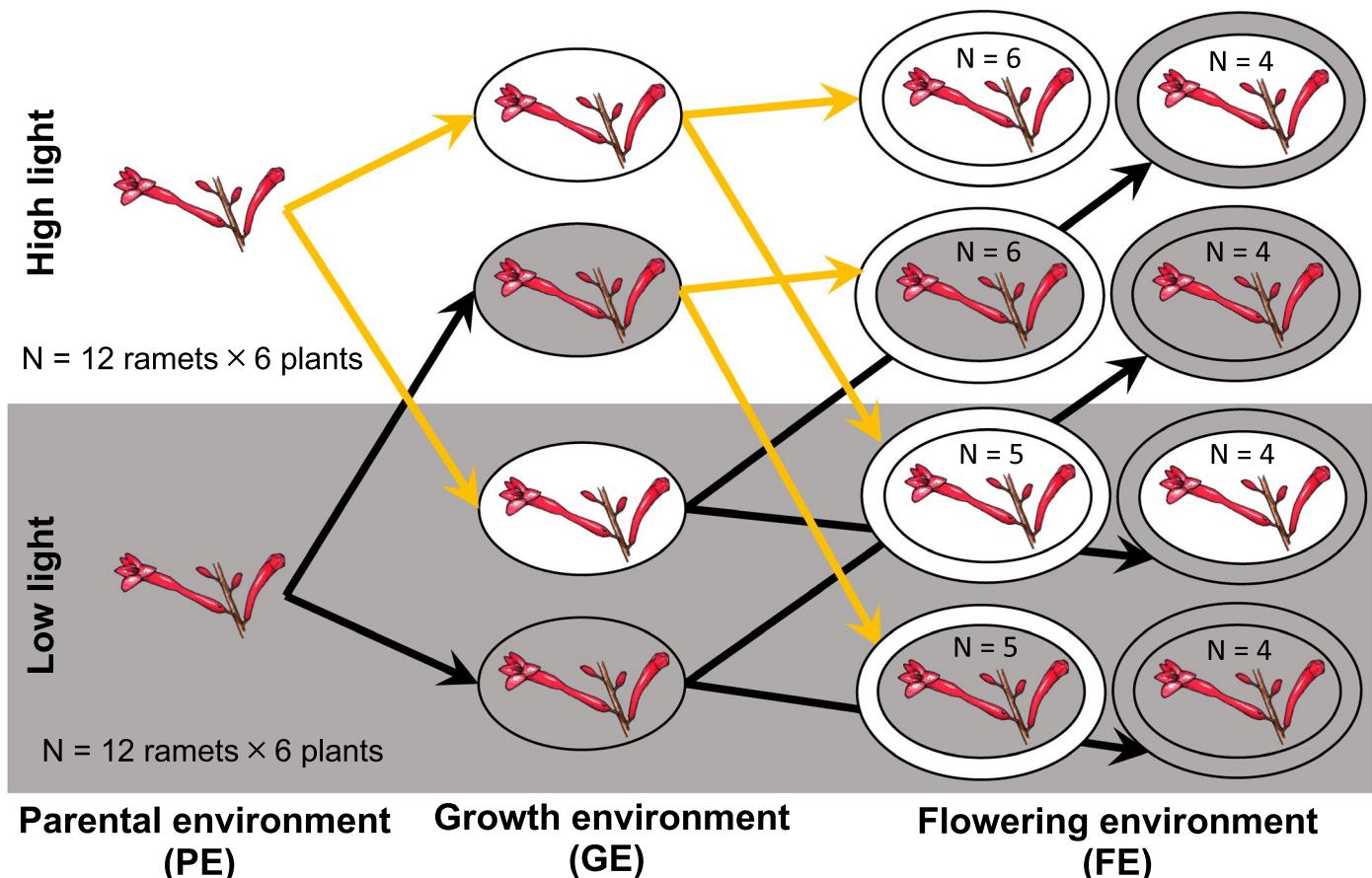


FIGURE 2. Schematic diagram of the experimental design for the reciprocal translocation experiment. Arrows indicate translocation; *N* denotes the number of plants included in the treatment.

individuals across multiple treatments when considering the effect of any one environment.

Data analysis

All analyses were conducted in R version 3.6.1 (R Core Team, 2018). All models were checked for conformity to assumptions: linear models were checked for normality and heteroskedasticity; Poisson generalized linear models (GLMs) and generalized linear mixed models (GLMMs) were checked for overdispersion.

To assess the effect of light availability on floral traits, we modeled each trait as a function of canopy cover using mixed-effects models, with plant as a random effect. Continuous traits were modeled using linear mixed models (LMMs), while discrete traits (i.e., flower number) were modeled with GLMMs with Poisson error distribution, as implemented in the package “lme4” (Bates et al., 2015). To check for correlation among the measured floral traits, we determined Pearson’s correlation coefficient for each trait pair; traits were largely uncorrelated (the highest correlation, between basal width and corolla mouth width, was 0.41; Appendix S4).

To test for effects of light availability, flower number, and floral traits on nectar robbing intensity (NRI), we used the number of robbed flowers as the response variable, offset by log(total number of flowers assessed for NR) in order to effectively model the proportion of flowers robbed. To assess the effects of per-observation flower number, we used a Poisson GLMM with plant identity as a random effect; the response variable was per-observation measures of robbed and total flowers. For all other models, we used season-long mean NRI as the response variable in negative binomial GLMs. For season-long total flower number, we included year as an additional predictor to account for the fact that data came from two years. For flower morphology and nectar traits, we used plant-level mean trait values as the predictor variables.

We used three metrics of reproductive output to test for effects of light availability and NRI on reproductive output in naturally occurring plants: fruit set, seed set, and seeds per plant. To model fruit set, we used a negative binomial GLM with number of fruit as the response variable, offset by log(total number of flowers produced), which was determined as described above. Canopy cover, season-long proportion of flowers robbed, and year were the predictors. Models for seed set and seeds per plant were similar to those for fruit set, except that year was not included as a predictor, since we had data from only 2018. For both models, the predictor variable was the number of seeds collected; the number of fruits collected was additionally included as an offset in the model of seed set.

For the reciprocal translocation experiment, we evaluated the effects of PE, GE, and FE on both flower number and NR. We evaluated the effect of each environment on two aspects of flower number: (1) the number of open flowers at each observation and (2) the season-long total number of flowers produced. In both cases, we used a GLMM with the three environments as fixed effects and ramet nested within replicate as a random effect; for the model assessing the effect of environment on number of flowers open at a time, date of observation was included as an additional random effect.

To assess the effects of PE, GE, and FE on NR of ramets in the reciprocal translocation experiment, we used per-observation measures of NRI, rather than a season-long measure. Because ramets in the reciprocal translocation experiment were monitored more frequently—such that we observed most of the flowers each ramet produced while they were open—our response variable was number of

open robbed flowers, rather than all (open and unopened) robbed flowers. Our model for NRI of these ramets included log(total number of open flowers) as an offset and date and ramet nested within replicate as random effects. We began with a maximal model that included PE, GE, and FE, and all two- and three-way interactions between environments, as well as total flower number (including open flowers and closed flowers ≥ 1.5 cm long). We then conducted stepwise simplification of the model, eliminating interaction terms in order of *P*-value and comparing model fits using the Akaike information criterion corrected for small sample size (AIC_c). AIC_c values for all models differed by >2 , so we used the best model for inference.

RESULTS

Field surveys

Effects of light availability on floral traits—Among the floral morphology traits measured (corolla length, corolla flare, corolla mouth width, and corolla base width), only corolla flare was affected by light availability, with plants growing in low light having significantly wider petals than those growing in high light (Table 1). Flower number was also significantly impacted by light availability: plants growing in low light produced fewer flowers overall—and fewer flowers at a time—than plants receiving more sunlight (Table 1). Per-flower nectar volume was also significantly lower in low-light plants, but nectar sweetness was not affected by light availability (Table 1).

Effects of light availability and floral traits on nectar robbery—NRI was not significantly related to light availability in naturally occurring plants (GLM: $\beta = 0.05 \pm 0.04$, $z = 1.31$, $df = 166$, $P = 0.19$). Among the measured floral traits, only flower number—measured as season-long total or as number of open flowers per observation—had an effect on NRI (Table 2). In both cases, flower number correlated positively with NRI.

TABLE 1. Effect of light availability on floral traits of naturally occurring plants. Estimates were derived from a GLMM with Poisson error distribution (for the three measures of flower number) or LMM (for all other traits). Light availability (measured as the inverse of canopy cover) and all traits except flower number were scaled to the mean to allow for comparison of effect sizes. Significant ($P < 0.05$) effects of light availability on a trait are in bold.

Trait	$\beta \pm SE$	<i>t</i> or <i>z</i>	df	<i>P</i>
Total flower number	0.30 ± 0.08	3.51	124	<0.001
Open flowers per observation	0.31 ± 0.05	6.03	643	<0.001
Flowers ≥ 1.5 cm long per observation	0.31 ± 0.05	6.18	643	<0.001
Corolla flare	-0.27 ± 0.12	-2.35	82	0.03
Corolla mouth width	0.16 ± 0.16	1.02	82	0.3
Corolla base width	0.07 ± 0.14	0.50	82	0.6
Corolla length	-0.07 ± 0.12	-0.60	82	0.5
Nectar volume	0.18 ± 0.08	2.16	475	0.03
Nectar sweetness	0.04 ± 0.13	0.29	336	0.8

Boldface indicates a significant effect ($P < 0.05$).

TABLE 2. Effect of floral traits on nectar robbery in naturally occurring plants. Model output from GLMMs with Poisson error distribution and plant as a random effect. In all models, the response variable was season-long total number of robbed flowers, with $\log(\text{total number of flowers})$ included as an offset in order to assess the effect of floral traits on the proportion of flowers experiencing nectar robbery.

Trait	$\beta \pm \text{SE}$	<i>z</i>	df	<i>P</i>
Total flower number	0.14 ± 0.02	5.48	125	<0.001
Open flowers per observation	0.08 ± 0.03	2.47	644	0.01
Flowers ≥ 1.5 cm long per observation	0.02 ± 0.03	0.47	645	0.6
Corolla flare	0.005 ± 0.07	0.07	29	0.9
Corolla mouth width	0.10 ± 0.07	1.45	29	0.2
Corolla base width	0.08 ± 0.07	1.14	29	0.3
Corolla length	0.03 ± 0.06	0.57	29	0.6
Nectar volume	0.03 ± 0.05	0.49	65	0.6
Nectar sweetness	-0.06 ± 0.06	-0.10	63	0.3

Boldface indicates a significant effect ($P < 0.05$).

Effects of light availability and nectar robbery on reproductive output

output—In naturally occurring plants, neither fruit set nor seeds produced per plant was correlated with either light availability or NRI (Table 3). Seed set was not correlated with NRI but was marginally negatively correlated with light availability (Table 3).

Reciprocal translocation experiment

Effect of light availability on floral traits—Of the three light environments considered (PE, GE, and FE), only GE had an effect on flower number (Table 4). Ramets in low-light GE produced fewer total flowers and also had significantly fewer flowers ≥ 1.5 cm on a per-observation basis, though the number of open flowers per observation was not affected by GE. The magnitude of the effect of shading on flower number is comparable to that seen in naturally occurring plants (Fitch and Vandermeer, 2020a [Preprint]).

Effects of light availability and floral traits on nectar robbing intensity—Flowering environment was the most important predictor of NRI, with ramets in the high-light FE experiencing higher NRI (Table 5). In addition to FE, the best model for NR of experimental ramets included flower number, PE, GE, and a PE \times FE interaction (Table 5). Removing any single predictor resulted in a model with significantly poorer fit ($\Delta AIC_c > 2$ in all cases), though there was no significant main effect of PE.

As in naturally occurring plants, nectar-robbing intensity was positively correlated with total flower number (Table 5). Ramets grown in low light experienced higher NRI regardless of where they flowered, though this effect was relatively small and disappeared when flower number was removed from the model. Finally, ramets

from low-light PE and low-light FE experienced significantly more NR than ramets from low-light PE and high-light FE, generating the significant PE \times FE interaction included in the best model of NRI (Fig. 3). There was no parallel relationship for experimental ramets from high-light PE.

Reproductive output—Fruit set was significantly correlated with both GE and FE, though in contrasting manners. Fruit set was nearly three times higher in ramets from high-light GE compared to low-light GE (0.21 vs. 0.07 fruits/flower; Table 4); the effect of FE was modest by comparison, but ramets in high-light FE had a significantly lower fruit set than those in low-light FE (0.16 vs. 0.18 fruits/flower; Table 4). Parental environment had no effect on fruit set. Because of high levels of pre-dispersal seed predation on experimental ramets, we were unable to measure seed production on a sufficient number of ramets to draw conclusions about the effect of light availability on seed production.

DISCUSSION

While light availability is generally thought to positively influence plant reproduction by increasing the availability of resources to allocate to reproduction, the presence of multiple exogenous pathways linking light to plant reproduction (Fig. 1) has the potential to complicate this direct link. In this study, in addition to increasing plant resources, light availability also influenced both pollination and NR, via both direct and indirect pathways.

Light availability influenced multiple floral traits associated with pollinator attraction, in somewhat contrasting ways. Greater light availability was associated with higher flower number and increased nectar volume, but with smaller corolla flare. Increases in flower number and nectar volume in plants receiving more light are consistent with the idea that light availability influences photosynthate production and, therefore, resource allocation to pollinator attraction and reproduction. It may be that plants growing in low-light conditions compensate for reduced flower number and reward volume by increasing corolla flare to increase attractiveness to pollinators. However, although larger corolla flare increases pollinator attraction in many species (e.g., Conner and Rush, 1996; Galen, 1999; Mothershead and Marquis, 2000), we do not know whether this is true for *O. cuspidatum*.

Light availability likewise influenced NRI. We hypothesized that this link would be mediated primarily by light availability's effects on floral traits. However, in the reciprocal translocation experiment, GE had only a small effect on NRI. Moreover, NRI was higher in plants from low-light GE, contrary to our expectation. Thus, although flower number—which is influenced by light availability—has a modest effect on NRI, variation in NRI cannot be explained

TABLE 3. Effects of nectar robbery and light availability on reproductive output in naturally occurring plants, estimated using negative binomial GLMs. Estimates for fruit set use two years of data; estimates for seed set and seeds per plant use a single year of data. Significant ($P < 0.05$) effects are in bold.

Measure of reproductive output	Nectar robbery			Light availability			Year (2018)			df
	$\beta \pm \text{SE}$	<i>z</i>	<i>P</i>	$\beta \pm \text{SE}$	<i>z</i>	<i>P</i>	$\beta \pm \text{SE}$	<i>z</i>	<i>P</i>	
Fruit set—all plants	-0.07 ± 0.19	-0.37	0.7	0.002 ± 0.05	0.03	0.9	-0.34 ± 0.11	-3.06	0.002	124
Seed set—all plants	-0.06 ± 0.10	-0.54	0.6	-0.19 ± 0.10	-1.91	0.06	—	—	—	55
Seeds per plant—all plants	0.23 ± 0.15	1.54	0.1	0.14 ± 0.15	0.93	0.4	—	—	—	55

Boldface indicates a significant effect ($P < 0.05$).

TABLE 4. Effect of light availability on floral traits and fruit set of experimental ramets. In all cases, β estimates represent the effect of the high-light environment in comparison to the low-light environment. Significant ($P < 0.05$) effects are in bold.

Response variable	Parental environment			Growth environment			Flowering environment			df
	$\beta \pm SE$	<i>z</i>	<i>P</i>	$\beta \pm SE$	<i>z</i>	<i>P</i>	$\beta \pm SE$	<i>z</i>	<i>P</i>	
Total flowers	0.42 \pm 0.30	1.41	0.2	1.01 \pm 0.27	3.71	<0.001	0.26 \pm 0.31	0.86	0.4	32
Flowers ≥ 1.5 cm, per observation	0.16 \pm 0.15	1.06	0.3	0.34 \pm 0.16	2.18	0.03	0.23 \pm 0.16	1.48	0.1	198
Open flowers, per observation	0.15 \pm 0.16	0.97	0.3	0.26 \pm 0.18	1.46	0.1	0.14 \pm 0.17	0.79	0.4	198
Fruit set	0.01 \pm 0.18	0.06	0.9	1.44 \pm 0.35	4.33	<0.001	0.36 \pm 0.18	2.0	0.046	31

Boldface indicates a significant effect ($P < 0.05$).

primarily by variation in floral traits. Instead, FE was the most important predictor of NRI, with ramets flowering in high light experiencing more NR. Analogous patterns in insect pollinator visitation have, elsewhere, been invoked as evidence that higher light availability directly affects insect activity by increasing local temperature (Herrera, 1995; Kilkenny and Galloway, 2008). However, these studies were conducted in temperate regions, whereas the present study occurred in a warm tropical climate where low temperature is less likely to limit flower visitor behavior (Willmer, 1991). Another possibility is that low-light conditions affect foraging behavior not via temperature but by reducing visual acuity and sensitivity of foraging bees (Streinzer et al., 2016). In that case, plants growing in low-light conditions would experience reduced NR, both because foraging efficiency would be lower than in high-light conditions and because the diurnal time frame in which foraging could occur would be narrowed.

Alternatively, the link between FE light conditions and NRI may be mediated by the community composition of co-flowering plants, which we have shown elsewhere to be an important driver of NRI (Fitch and Vandermeer, 2020a [Preprint]). In this scenario, the low density of co-flowering plants in low-light environments reduces local density of foraging nectar robbers and, therefore, reduces NR. Because our study design utilized preexisting light availability regimes, with their concomitant floral communities, we are unable to disentangle the relative impact of light availability versus (light-availability-influenced) co-flowering community composition on NRI. Nor did we directly measure pollinator or nectar robber visitation rates to either experimental or naturally occurring

plants. A more general concern with the reciprocal translocation experiment is that the relatively small number of experimental plants that flowered in 2018 limited our sample size. Thus, while the results from the reciprocal translocation study are consistent, in many respects, with findings from naturally occurring plants, they should nevertheless be interpreted with caution, particularly where they conflict with results from surveys.

One unexpected finding from the reciprocal translocation study was that, once we accounted for the effect of flower number, NRI was actually higher for ramets from low-light GE, regardless of FE. It may be that high light availability reduces the attractiveness of other traits, unmeasured in this study, that mediate nectar robber attraction (e.g., floral volatiles). In such a scenario, the positive effects of light availability on flower number and directly on nectar-robbing behavior would generally cancel out this hypothesized reduction in attractiveness. This is consistent with our findings from both naturally occurring and experimental plants that GE light availability per se did not influence NRI.

Data from the reciprocal translocation experiment and field surveys support conflicting inferences regarding the importance of light availability for *O. cuspidatum* reproductive output. In the reciprocal translocation experiment, GE was the most important determinant of fruit set, with higher fruit set in ramets grown in high-light conditions. This suggests that reproductive output is limited primarily by photosynthate availability—in other words, that endogenous pathways linking light availability and reproductive output (Fig. 1F) are more important than exogenous pathways (Fig. 1B–E). Since *O. cuspidatum* occurs primarily in high-light microhabitats (e.g., light gaps, streambeds), a strong direct response to light availability is perhaps to be expected. But data from field surveys indicate that neither NRI nor light availability significantly influences reproductive output. In naturally occurring plants, the effect of light availability on reproduction (via either endogenous or exogenous pathways) may be obscured by other factors (e.g., soil properties, biotic interactions, plant age or size) that were controlled in the reciprocal transplant experiment.

The modest negative relationship between FE light availability and fruit set in the reciprocal translocation experiment suggests that the solely pollinator-mediated pathways linking light availability and reproductive output (Fig. 1B, C) are relatively unimportant in determining *O. cuspidatum* reproduction. In this population of *O. cuspidatum*, robbed flowers receive less pollination than unrobbed flowers, and as a result robbed flowers are significantly less likely to set fruit (Fitch and Vandermeer, 2020a [Preprint]). Since NRI was positively correlated with light availability in the FE, pollinators avoiding robbed flowers will, all else being equal, pollinate more flowers on shaded plants. Interestingly, this suggests that

TABLE 5. Best model for predicting robbery of ramets from reciprocal translocation experiment. For the different environments, β estimates indicate the effect of the low-light environment. Significant ($P < 0.05$) effects are in bold.

Predictor	$\beta \pm SE$	<i>z</i>	<i>P</i>	ΔAIC_c for omitting variable
Total flower number	0.04 \pm 0.02	2.43	0.02	3.7
Parental environment	0.19 \pm 0.23	0.84	0.40	14.4
Growth environment	0.67 \pm 0.29	2.35	0.02	2.9
Flowering environment	-3.02 ± 0.58	-5.24	<0.001	21.3
Parental environment \times flowering environment	2.23 ± 0.62	3.61	<0.001	9.8

Boldface indicates a significant effect ($P < 0.05$).

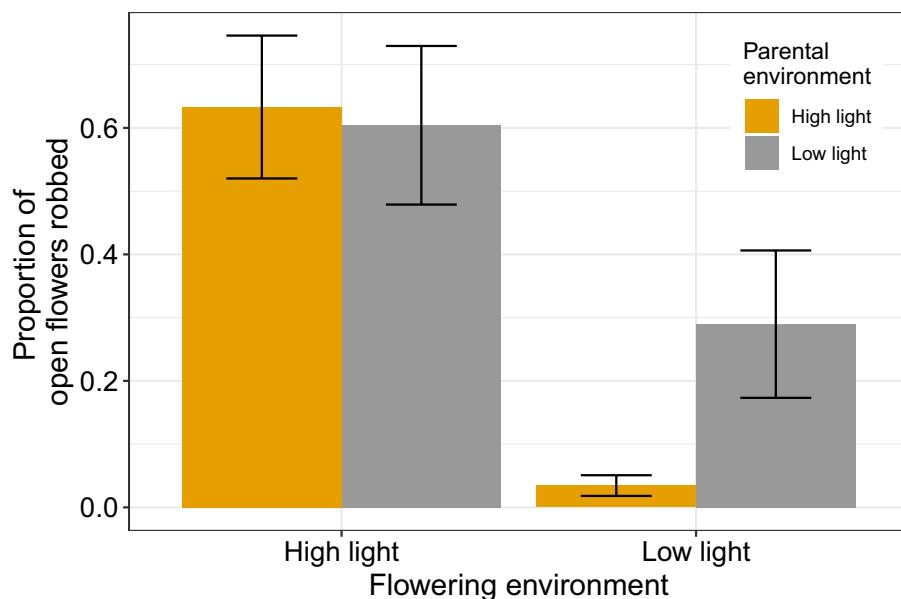


FIGURE 3. Effect of light availability at flowering time on nectar robbery, as mediated by the light environment experienced by the parent plant. Error bars represent standard error; letters indicate significantly different levels of nectar robbery; N denotes the number of plants included in the treatment.

light availability (and/or its impacts on the co-flowering community; see above) influences nectar robber preference for *O. cuspidatum* more than pollinator preference. This could also explain the lack of correlation between light availability and fruit set in naturally occurring plants, because the contrasting impacts of (1) reductions in photosynthate availability and (2) increases in pollination as light availability decreases would negate one another.

Our finding of a significant effect of PE \times FE interaction on NRI in experimental ramets suggests that clonal transgenerational effects (Latzel and Klimešová, 2010) influence plants' attractiveness to nectar robbers. Ramets sourced from parents growing in low-light conditions experienced significantly higher NRI when they flowered in low- versus high-light conditions, regardless of ramet GE. This suggests local adaptation of traits mediating nectar robber attraction to low light availability, conditioned by PE. Even when we controlled for the effects of NRI on fruit set—thereby recovering the effect of FE light availability on pollination—we found no evidence for a PE \times FE interactive effect on fruit set. This is further evidence that nectar robbers are, surprisingly, more sensitive to *O. cuspidatum* floral traits than pollinators—at least to those traits that are affected by PE. Moreover, given the negative effect of NR on reproductive success in *O. cuspidatum*, this suggests that clonal transgenerational plasticity—at least in relation to pollination—is not adaptive in this population. Further work is needed to elucidate the specific traits influencing NRI that exhibit transgenerational effects. In addition, while we suspect that light influences floral traits primarily by increasing photosynthate availability, in the absence of physiological studies we cannot be certain of the causal pathway linking light availability and floral traits.

Our results highlight how complex, interacting effects of light on interactions between plants and mutualist and antagonist partners can complicate the simple assumption that increases in light availability should lead to increased plant reproductive success. Indeed, despite strong positive effects of light availability on plant growth and ovule

production in *O. cuspidatum*, we found no effect of light availability on seed production. This was apparently due to strong effects of light availability on the nectar-robbing behavior of stingless bees, which in turn influenced pollination and seed production.

The effects of light availability on floral antagonists have received little attention to date; this study suggests that this oversight has limited our understanding of the often complex relationship between light and plant reproduction. While we suspect that light availability commonly influences plant-floral antagonist interactions, further work in other plant-pollinator-floral antagonist systems is needed to evaluate the generalizability of our findings. In particular, future research that more precisely identifies the causal mechanism(s) by which light influences floral antagonists—focusing on a taxonomically diverse set of antagonists—will greatly advance our ability to predict the net effects of light on plant reproduction in such complex systems.

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AUTHOR CONTRIBUTIONS

G.F. and J.H.V. conceived of the study and designed the methodology. G.F. collected and analyzed the data. G.F. led the writing of the manuscript, with substantial feedback from J.H.V. Both authors gave final approval.

DATA AVAILABILITY

All data and code associated with this manuscript are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.kkwh70s2z> (Fitch and Vandermeer, 2020b).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. *Odontonema cuspidatum* mating system.

APPENDIX S2. Photograph showing *Odontonema cuspidatum* inflorescence being nectar-robbed by the stingless bee *Trigona fulviventris* (black circle), and with perforation from previous robbing (white circle).

APPENDIX S3. Flow diagram of sampling scheme for naturally occurring *Odontonema cuspidatum* plants.

APPENDIX S4. Correlation among *Odontonema cuspidatum* floral trait values (Pearson's r).

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