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— Novel Ideas and Pilot Projects —

COLUMBINE POLLINATION SUCCESS NOT DETERMINED BY A PROTEINACEOUS REWARD TO HUMMINGBIRD POLLINATORS

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Abstract—Plants provision pollinators with a variety of nutritious or otherwise beneficial rewards. Hummingbirds (primarily *Calypte anna*) pollinate the columbine *Aquilegia eximia*. In addition to drinking nectar, they glean entrapped insects from its sticky surfaces. To test the hypothesis that this insect carrion, an abundant and easily-collected protein source, serves as a provision to the pollinator and increases pollination I experimentally manipulated this reward and measured pollination success. I set up three treatments - an insect carrion addition, carrion removal, and an unmanipulated control - on small patches of the plant in each of five populations of *A. eximia*. Pollination success, measured by seed set in emasculated flowers, was unaffected by carrion level. Pollination success positively correlated with average floral display in each patch; this suggests that local nectar reward in an area is more important than this proteinaceous reward in determining pollination success. Stickiness in this system may function as an effective exclusion mechanism for smaller-bodied pollinators. While this study did not demonstrate that captured insects increased reproductive success of this columbine, this interaction (and pollinator exclusion) may play a role in other hummingbird-plant interactions, as hummingbird pollination and insect-entrapment occur together in at least nine species of six plant families.

Keywords: *hummingbird pollination, sticky plants, pollinator rewards, Aquilegia*

INTRODUCTION

Plants provision pollinators with a wide diversity of rewards, including nutritious substances (nectar, pollen), substances with unique chemical properties (resins, waxes), and possibly even shelter and heat (Kevan 1975; Simpson & Neff 1981). A great many plants – species in at least 110 genera of 49 families – entrap insects on their sticky surfaces (LoPresti et al. 2015). These entrapped insects serve as a food source for mutualistic true bugs and spiders, which further reduce herbivory in some systems (Romero et al. 2008, Krimmel and Pearse 2013; LoPresti et al. 2015, LoPresti and Toll 2017). In the "protocarnivorous" *Roridula,* mutualistic true bugs feed on entrapped insects, fertilizing the plant with their feces (Anderson 2005). In addition, these mutualistic bugs move pollen within a plant (geitogamously), acting as the primary pollinator, though only as self-pollinators (Anderson et al. 2003).

Insect-entrapment has been thought to reduce pollination (Eisner et al. 1998); this hypothesis has been most frequently investigated in carnivorous plants with sticky traps. The risk of entrapping insects has been suggested to drive the physical separation of traps from flowers, as well as color and scent differences, in carnivorous sundews (Droseraceae: *Drosera* spp.) and most butterworts (Lentibulariaceae: *Pinguicula* spp.) (Zamora 1999; El-Sayed et al. 2015). However, having flowers on a tall stalk, for which a large flower-trap separation is merely a byproduct, also increases pollinator visitation more broadly and both comparative, observational, and experimental evidence suggests that the risk of pollinator entrapment is not responsible for trap-flower separation in *Drosera* species (Anderson & Midgely 2001; Anderson 2010; Jürgens et al. 2015).

While trapping of insects is sometimes thought to reduce the effectiveness of pollinators, the trapped insects have never before been considered as a reward which may increase plant reproductive success. Further, stickiness has not been investigated as an exclusion mechanism for small-bodied pollinators, another potential function.

Hummingbird diets consist of floral nectar and a variety of small arthropods, the proportion of each varies by species and season. Hummingbirds catch insects on the wing, glean arthropods from vegetation (Wagner 1946) and pick out entrapped insects from spiderwebs (Young 1971). The Anna's Hummingbird (*Calypte anna*), common in Northern California, is no exception. Pearson (1954) observed a single male of this species for two entire days and found that it foraged for insects an average of 85 times per day. In 2014 and 2016, while conducting insect censuses, I witnessed Anna's Hummingbirds gleaning insects off of the sticky pedicels of the hummingbird-pollinated serpentine columbine (*Aquilegia eximia*) on several occasions.

A. eximia entraps hundreds of insects, nearly all < 5 mm in total length, on the densely glandular aboveground parts

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TABLE 1: Known and possible hummingbird-pollinated plants which also entrap arthropod carrion on sticky surfaces. List from LoPresti et al. 2015 and personal observations.

Family	Genus	Species
Hummingbird pollinated & insect entrapping		
Bromiliaceae	*Vriesea*	*bituminosa*
Caryophyllaceae	*Silene*	*laciniata*
Lamiaceae	*Salvia*	*spathacea*
Orobanchaceae	*Castilleja*	spp.
Phyrmaceae	*Mimulus*	*cardinalis*
Ranunculaceae	*Aquilegia*	*eximia*
		*formosa**
		shockleyi
Solanaceae	*Nicotiana*	spp.
	Petunia	*exserta*
Hummingbird-pollinated & possibly insect-entrapping		
Caryophyllaceae	*Silene*	*virginica*
		regia
Nyctaginaceae	*Mirabilis*	*triflora*
Scrophulariaceae	*Scrophularia*	*macrantha*

*some populations entirely glabrous, others glandular-sticky and insect-entrapping

of the plant and provisions mutualistic true bugs and other predators (LoPresti et al. 2015). Hummingbird gleaning from sticky plants was thus far unrecorded, yet might occur widely, as sticky plants are common and geographically widespread (LoPresti et al. 2015). Additionally, a number of insect-entrapping plants, in several families, are primarily hummingbird-pollinated (Tab. 1). Given that this is a reliable and easily exploited resource during the flowering season, I hypothesized that this proteinaceous reward might serve as a pollinator reward and increase pollination success. To test this hypothesis, I performed removals and additions of insect carrion to patches of columbines at the UC-Davis McLaughlin Reserve.

MATERIALS AND METHODS

In early July, I selected five study populations (Bear Meadow [BM], Correa House [CH], High County Line Seep [HCLS], OHV Seep [OHV], and Upper Quarry Valley Seep [UQV]). Within each population I selected three patches of columbine which were roughly equal in size (no more than 1 m^2, with < 5 individual plants), between one and five meters from other patches, and separated from the other selected patches by at least one nonexperimental patch. I randomly assigned these to three treatments: a control, a carrion removal (all entrapped insects carefully removed with forceps), and a carrion addition (15-20 fruit flies added to any near-flowering pedicel). Fruit flies are comparable in size to the insects normally found entrapped at the study site, most of which are flies (LoPresti et al. 2015). Fruit flies are a standard hummingbird food used in maintaining Anna's hummingbirds in captivity (Brice 1982). As serpentine columbine readily self-pollinates (pers. obs.), to get an estimate of pollination services to the patch, I emasculated all flowers in the patch prior to flower opening. I also added bands of tanglefoot (Scott's LLC), to prevent caterpillar herbivory of buds, flowers and fruit.

Every 3-5 days for the flowering season (early July to early October), I visited each patch, refreshing treatments, emasculating all buds, recording number of open flowers at that time, individually marking all open flowers, collecting any fruit with partially developed seeds, and removing any herbivores and herbivore eggs. Seeds were then counted on a per carpel basis, as some had herbivory on one or more carpel.

I analyzed all data and created figures in R vers. 3.1.3. The response variable for all analyses was average seeds per carpel (thus each fruit provided one observation). I used a linear mixed-model (package lme4), with carrion level as a continuous predictor variable (0, 6, 17 for the treatments) and floral display (# of flowers open at same time as the collected fruit was a flower) as fixed effects and site as a random effect.

RESULTS

I collected 398 fruit from fourteen experimental patches over the course of this experiment, 371 were included in analyses; the remaining 27 flowered between checks, so I did not have reliable estimates of floral display for them. One patch treatment, the carrion-removal treatment at Bear Meadow, dried before any fruit developed.

The primary hummingbird visitor to columbines was *Calypte anna*, followed by *Selasophorous rufus*, though the latter represented less than < 5% of visits during the late summer (unpublished data) and were absent earlier in the flowering season. Few other visitors to columbine occur here. On a few occasions, I witnessed syrphid flies (Diptera: Syrphidae spp.), solitary bees and honeybees (*Apis mellifera*) collecting pollen; however, these observations were rare relative to hummingbird visits. The carpenter bee *Xylocopa californica*, was a common nectar robber in 2014 and 2015, though after a series of intense wildfires in late summer 2015, this large-bodied, wood-nesting species and its conspicuous holes in nectar spurs were entirely absent during the 2016 season.

Carrion treatments had no significant effect on pollination success (Fig. 1). A mixed-effects model with site as a random effect and floral display and carrion level fit no better than one without carrion level (Likelihood ratio test, X^2 = 0.39, df = 1, P = 0.53). A model with just floral display as a fixed effect fit significantly better than one without (X^2 = 6.78, df = 1, P = 0.01). The coefficient of floral display in that model was 0.33 (+ 0.12; t = 2.77, P < 0.01), with an intercept of 20.27. Floral display ranged from 1-13, meaning that a flower which occurred in a patch of with nine other flowers produced, on average, ~4 seeds/carpel more than a flower alone in its patch (an 18% increase), but this relationship depended on site (Fig. 2).

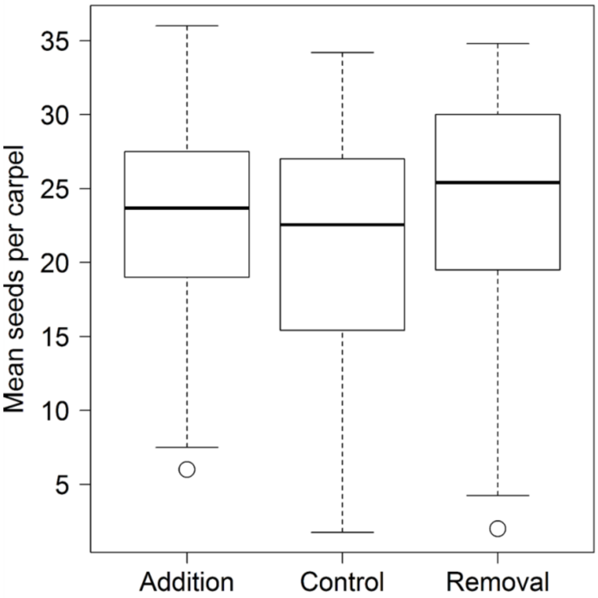

FIGURE 1: Relationship between treatment carrion levels and pollination success (seeds per carpel of emasculated fruit). No significant treatment effect was found. Treatments are carrion addition (A), control (C), and carrion removal (R). Sample sizes (A, C, R order) are: OHV (18, 21, 15); UQV (25, 15, 23); BM (7, 17, 0); CH (29, 39, 49); HCLS (55, 18, 40).

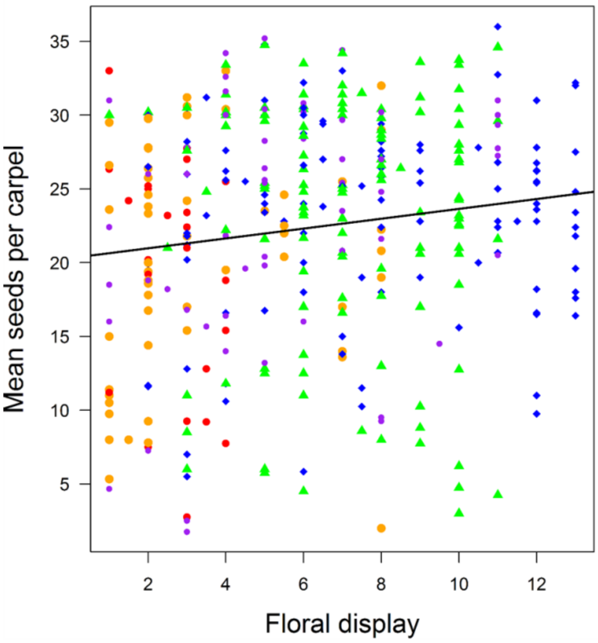

FIGURE 2: Relationship between floral display (# of flowers open at the time the fruit was flowering) and seeds per carpel for each fruit. Line of best-fit is model slope and intercept coefficients (not plotted with a specific site's random effect slope). Site points are: blue diamond (HCLS), purple small circle (UQV), red large circle (BM), green triangle (CH), and yellow medium circle (OHV). Sample sizes are as in Fig. 1.

DISCUSSION

Pollination success did not depend on experimental carrion level, however it did correlate positively with the number of open flowers within the patch at the time of flowering (though with considerable unexplained variation). This suggests that nectar resources at the patch level play a greater role in female reproductive success for these columbines than a proteinaceous insect resource does. Similarly, Brody and Mitchell (1997) found that floral number correlated with pollination success in another hummingbird-pollinated plant, *Ipomopsis aggregata*. The peduncles and pedicels of plants in patches of *A. eximia* overlap extensively, likely making individual plants in a patch appear indistinguishable to a pollinator, an analogous situation to the distinct single flowering spikes of *I. aggregata*. *A. eximia* grows in small patches along seasonally-flowing streams and many individuals do not flower in a given year. The greater pollination success of plants in patches with more flowers may indicate a benefit of growing with greater numbers of conspecifics or co-flowering with neighbours, though it also could be due to an underlying factor correlated with floral number, i.e. plant vigour. While significant, and pronounced, this floral display effect may even be underestimated; emasculation prevented within-plant and within-patch pollen movement, which I would expect to be higher in larger patches. (The study design necessarily precluded any estimates of the male components of fitness, an important caveat).

Visitation rates to the different patches were not quantified and this was a major shortcoming of the study. Patch visitation rate is quite low, probably less than ten visits/day/patch, and logistically, it was impossible for me to conduct many-hour observations on enough patches to get good data. I also tried motion sensitive game cameras (Bushnell Natureview), which were somewhat usable for single flower observations, but did not work for patch-level observations, despite many attempts. Future studies of this sort should endeavour to quantify visitation, especially for studies including male components of fitness.

While carrion does not increase pollination success, it is possible that this resource is still important to Anna's hummingbirds. While this species can survive short periods solely on nectar, they need protein in a longer-term diet (Brice 1992). Additionally, protein level determined degree of feather iridescence, an assumed sexually-selected trait (Meadows et al. 2012). Even if carrion on sticky plants is necessary and commonly exploited, an effect of carrion on pollination might not be expected. Anna's Hummingbirds spend an average of less than five minutes foraging for insects per day compared to almost two hours foraging for nectar (Pearson 1954). Given that time budget, it is possible that far more insect foraging was done on those carrion-addition and control patches, but the slight increased attention was insufficient to boost pollination success. Further, these activities may be separated in time or space. Pearson (1954) separates the foraging flights for insects and flowers; my own observations of insect-gleaning by hummingbirds on columbines did not involve nectaring, and it is possible that foraging bouts are always strictly separated.

(It is also possible that they glean insects entrapped on the sticky flowers, which would certainly escape my observation [see Wagner 1946]).

With these caveats, the diversity of plants which are insect-entrapping and hummingbird-pollinated suggest other systems where this phenomenon could occur. Several of these are visited by Anna's hummingbirds, but others are pollinated primarily by different genera of hummingbirds, which may forage differently (see Wagner 1946 for detailed observations in foraging across several hummingbird genera). If found in another system, it is almost certainly not an adaptation; having examined dozens of sticky, insect-entrapping plant species in California, I strongly believe that indirect defence and physiological protection drive the repeated evolution of this trait and that the observed feeding of hummingbirds is a solely facultative interaction (e.g. Meehan 1879). Another characteristic of hummingbirds as pollinators may be more important in relation to stickiness of the plant: body size.

The relationship between insect-entrapping plants and pollinators may be complicated. Eisner et al. (1998) noted a bee pollinator of *Mentzelia pumila* (Loasaceae) entrapped by the plant's "grappling hook" trichomes and hypothesized a cost of stickiness from pollinator entrapment. While it has been suggested that carnivorous plants separate their flowers from insect-trapping apparatuses or use colours and odours in order to not catch pollinators, evidence is slim (Anderson and Midgely 2001; Anderson 2010; Jurgens et al. 2015; El-Sayed et al. 2016). Many small flies and bees – pollinators of other plants – get caught on *A. eximia* (e.g. Fig. 3). It is quite likely that stickiness deters or prevents small-bodied pollinators from utilizing *A. eximia*.

FIGURE 3: A honeybee (*Apis mellifera*) caught on the sticky inflorescence of *A. eximia*. At time of entrapment, it likely was nectar robbing; robbing holes of *Xylocopa californica* are visible on spurs in the photos and honeybees use them secondarily. (Photo taken 23-June-2015; in 2016, *X. californica* was absent at this location).

Excluding less- or ineffective pollinators may be as important as attracting better ones. In *Penstemon*, adaptations favouring hummingbird pollination evolved in concert with adaptations against bees (Castellanos et al. 2004). In *A. eximia*, there are a suite of traits which probably exclude small-bodied pollinators: the nectaries are located atop long spurs, with a constriction below the nectary (making access to the nectar difficult for anything without a long tongue), the flower is pendulant (making it more difficult for bee or lepidopteran visitors) and the plant, including flowers, is extremely sticky. Stickiness of flowers themselves (e.g. *A. eximia*) or calyxes (e.g. *Silene* spp.) are common traits in sticky plants (see supplementary information in LoPresti et al. 2015). Stickiness of calyces or flowers has been hypothesized to prevent ants and other ambulatory non-pollinators from interfering with pollination (Thomas 1988, Villagra et al. 2014); however, it may also prevent small-bodied flying insects from pollinating. Concluding that stickiness, a pleiotropic trait with physiological, herbivore resistance, and possible pollination benefits, is an adaptation for any of these functions would be impossible with a single species (though an ecological effect could certainly be demonstrated). Stronger evidence could be found with comparative studies of clades with pollinator shifts and insect-entrapment could examine whether stickiness is evolutionary linked with large-bodied pollinators. Specifically, the genera *Aquilegia*, *Mimulus*, and *Nicotiana* are well-studied both phylogenetically and ecologically and have sticky and nonsticky species as well as multiple pollinator shifts and would be ideally suited for these studies.

Conclusion

Despite a bevy of papers on hummingbird consumption of arthropods, none have tested whether pollination services are affected by this protein resource. We found that hummingbirds exploited the carrion on a sticky plant's surface, though manipulating the level of this proteinaceous reward did not affect pollination success. This study supported previous results showing that seed set of emasculated flowers is positively affected by floral display. Finally, I present a hypothesis that stickiness may effectively exclude small-bodied pollinators.

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