

Breeding system of the critically endangered Lakela's Mint and influence of plant height on pollinators and seed output

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Abstract Understanding reproductive systems of rare plants is critical for conservation efforts. Lakela's Mint, *Dicerandra immaculata* Lakela var. *immaculata*, is an endangered plant endemic to an approximately 4.8-km long area in Florida, USA. We used an experimental garden and three populations of Lakela's Mint to determine: (1) what is the breeding system (autonomous, asexual, self-fertile, cross-fertile) and are insects necessary for reproduction; (2) which native and nonnative insect species visit flowers and is the frequency of visits to a plant influenced by its height; (3) does the number of flowers visited within a plant by individual insects differ among native and nonnative insect species and due to plant height; and (4) is seed output influenced by plant height? Our results indicate that the breeding system of Lakela's Mint was facultative outcrossing. Insect-pollinated flowers produced more seeds than flowers that reproduced autonomously or asexually. The honey bee *Apis mellifera* L., a nonnative species, was the most frequent visitor to plants and visited more flowers within plants than native pollinators, but its behavior was not influenced by plant height. Native pollinators such as *Bombus impatiens* Cresson were attracted more frequently to shorter plants, but visited fewer flowers than on taller plants. Despite having fewer total and pollinated flowers,

shorter plants had a higher output of intact seeds than taller plants, which could be due to differences in efficiency between native and nonnative pollinators or other factors. Our results add insight into factors influencing seed output and interactions between pollinators and rare plants.

Keywords Agamospermy · *Apis mellifera* · *Bombus impatiens* · Cross-pollination · *Dicerandra immaculata* · Self-pollination

Introduction

Understanding the reproductive systems of rare plants is critical for promoting their preservation and management (Bond 1994; Wilcock and Neiland 2002). This is especially true for short-lived species that regenerate only through seed (Bond 1994; Schenck et al. 1994) because population dynamics are closely linked to seed dynamics (Pavlik et al. 1993). For such species, reproduction may be asexual when no donation of pollen occurs or through a breeding system that involves self-pollination (geitonogamy), which may occur within the same flower (autogamy) or between different flowers within the same plant (allogamy), or through cross-pollination between genetically distinct plants (xenogamy) (Richards 1986; Barrett 1998; Neal and Anderson 2005; Eckert et al. 2009). Plants may use a mixture of geitonogamy and xenogamy (Cruden 2000; Eckert 2000). Most plants that require pollen for reproduction sometimes fail to achieve their full pollination potential (Burd 1994; Larson and Barrett 2000).

Failure in pollination can occur because of pollen or pollination limitation (Wilcock and Neiland 2002) and rare plants in fragmented habitats are particularly susceptible to pollination failure (Cunningham 2000). Pollen

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limitation occurs when the quality or quantity of pollen is insufficient (Campbell and Halama 1993; Aizen and Harder 2007), when a lack of outcrossing promotes inbreeding depression and expression of harmful recessive alleles (Brown and Kephart 1999; Keller and Waller 2002), and when there is self-incompatibility within a population (DeMauro 1993). Pollination limitation occurs when there is no transfer of pollen to stigmas of flowers (Wilcock and Neiland 2002), which can be associated with the abundance and behavior of pollinators, particularly in relation to floral or other plant traits within a population (Ehrlén 1992; Campbell and Halama 1993; Larson and Barrett 2000; Knight et al. 2005). The effective pollination hypothesis suggests that tall plants attract pollinators away from neighboring plants and across greater distances (Aarssen 1995). Variation in the behavior of pollinators often influences fitness of individual plants. For example, bumblebees avoid short plants of *Polemonium viscosum* Nutt. (Polemoniaceae) in favor of tall ones, yielding greater annual fecundity (Galen 1989). Taller *Verbascum thapsus* L. (Scrophulariaceae) plants are visited more frequently by pollinators compared with shorter unvisited neighboring individuals, and pollinators are required for maximum seed yield (Donnelly et al. 1998). The size of a floral display, which is the number of open flowers on an individual plant, and the flowering plant density, which is the number of local plants in bloom, are also positively correlated with increased abundance of pollinators and increased likelihood that a single pollinator will choose a plant for the center of its foraging path (Mitchell et al. 2004; Nattero et al. 2011).

European honey bees (*Apis mellifera* L.) and other exotic pollinators have been introduced to many parts of the world where they sometimes disrupt pollination of native plants (Goulson 2003) due to interference competition when native pollinators are physically excluded from a floral resource or through exploitative competition when floral resources are lost (Huryñ 1997; Thompson 2004). Assemblages of native pollinators can be more effective at pollination than introduced species because of their capability in transferring pollen or because of differences in behavior (Kevan and Baker 1983; Garibaldi et al. 2013). Nonnative pollinators may collect resources without contributing equivalent pollination services compared with native species, or they may forage in such a way that successful outcrossing events are reduced, for example through more numerous visitation of flowers within the same plant, with subsequent reduction in plant fitness (Gross and Mackay 1998). A reduction in plant fitness due to the behavior of pollinators could be harmful to rare plants that are already faced with other environmental challenges such as global climate change and habitat loss or fragmentation.

Lakela's Mint, *Dicerandra immaculata* Lakela var. *immaculata* (Lamiaceae), is a critically endangered short-lived perennial plant that persists in fragmented Florida scrub habitat on the Atlantic Coastal Ridge in Indian River and St. Lucie Counties, FL, USA (Nelson 1996). Populations of Lakela's Mint persist only at eight sites: five historical sites and three new sites where populations were introduced by Bok Tower Gardens, a non-profit public garden (BTG; Lake Wales, FL, USA). If Lakela's Mint is similar to some other species in its genus, then it likely requires pollen and insect visitation to produce seeds (Evans et al. 2004). Using an experimental garden and three populations of Lakela's Mint, we sought to answer the following questions: (1) what is the breeding system (autonomous, asexual, self-fertile, cross-fertile) and are insects necessary for reproduction; (2) which native and nonnative insect species visit flowers and is the frequency of visits to a plant influenced by its height; (3) does the number of flowers visited within a plant by individual insects differ among native and nonnative insect species and due to plant height; and (4) is seed output influenced by plant height?

Materials and methods

Study species

Lakela's Mint has multiple inflorescences, each of which is between 15–25 cm long and has overlapping cymes that bear up to five flowers in leaf axils (Kral 1982). Flowering typically begins in early October and concludes in November. Flower petals are a rose-lavender color and lack spots on the petals, unlike other *Dicerandra* species that have spotted petals. The four stamens per flower extend beyond the corolla and the anthers are spurred, which means that insects are likely necessary to prompt the release of pollen (Nelson 1996). The stigma extends farther than the stamens. The stigma and anthers are laterally spread out within the corolla, which is predicted to reduce nectar robbing compared to the flower morphology in other species of *Dicerandra* and deposit pollen on the sides of pollinators instead of the venter (illustrated and discussed in Deyrup and Menges 1997).

Breeding system

We determined the breeding system by using plants within an experimental garden at BTG. The Lakela's Mint population was established in the experimental garden in 2002 and was then augmented yearly starting in 2009. There were 273 plants within the experimental garden representing four of the five historical populations of Lakela's

Mint at the time of our study. No genetic work has been done to determine the genotypic diversity within the garden, but plants were propagated primarily from cuttings from over 100 parent plants. For our work we selected plants that varied in source population, size, and flowering phenology.

We used five treatments to determine the breeding system of Lakela's Mint (after Evans et al. 2004). The first four treatments were all applied to separate inflorescences on the same 12 plants, whereas the fifth treatment (the control) was applied to one inflorescence each of a second set of 12 plants. (1) Autonomous self-pollination: We tested for spontaneous self-pollination by enclosing a mean of 22 flower buds (minimum of 8 and maximum of 65) on one stem of each plant with mesh organza bags (Papermart, Orange, CA, USA) to exclude insects. Any pollination that occurs is likely the result of autogamy, but we cannot rule out allogamy. (2) Asexual reproduction (agamospermy): Flowers were enclosed with bags and anthers were removed from eight flowers on one stem of each plant the morning the flowers opened to test for asexual reproduction. Prior observations indicated that pollen was not released until 10:00–14:00 h on the first day that flowers opened, so we removed anthers before this time while they still appeared to be covered in a sheath. (3) Geitonogamy: We tested for self-compatibility by bagging eight flowers on one stem of each plant and transferring pollen by hand from an anther of one flower to the stigma of another flower within the same plant. Flowers were visited daily between mid-morning to mid-afternoon and pollen was transferred when stigmas appeared receptive. (4) Cross-pollination: We transferred pollen by hand from anthers on donor plants to the recipient stigmas of eight flowers on one inflorescence of a separate plant primarily as a control to ensure that our results for the geitonogamy treatment were due to self-compatibility/incompatibility and not our technique. Flower buds on the donor and recipient plants were bagged to prevent visits by insects once they opened. Donor plants were selected haphazardly throughout the experimental garden in order to represent different source populations. (5) Insect-pollination (control): Control plants were not enclosed during flowering in order to determine the reproductive output of a plant when pollinated by insects. A focal inflorescence was chosen on each plant and all but eight flowers were removed from the inflorescence.

These five treatments were applied to plants between 3–18 October 2013. Bags were left in place on all plants, and added on control plants once seed capsules formed (which should indicate that flowers were pollinated), until seeds ripened and dehisced in January 2014. We then collected the bags and counted the number of seed capsules and seeds produced per stem and treatment combination. Each flower has four ovules, and can therefore produce up

to four seeds per seed capsule. Our sample size was reduced to 11 control plants because one bag became dislodged before we could collect seeds.

Differences among the five treatments in the number of seeds produced per seed capsule were tested by a general linear model (PROC GLM; SAS Institute 2011). We used a square-root transformation on the data to meet assumptions of normality prior to analysis. We present the mean and standard error of the mean (SEM) of non-transformed data in the “Results”.

Pollinators and plant height

Floral visitors of Lakela's Mint were studied at three sites in St. Lucie and Indian River Counties (specific locations of the sites are not given to protect the populations). The three sites had sandy, well-drained soil that was near neutral in pH (6.7–6.9) and supported Florida scrub, which is characterized by a canopy of *Pinus clausa* (Chapm. Ex Engelm.) Vasey ex Sarg., *Quercus* spp., and *Carya floridana*, a shrubby midstory, and open, sandy gaps in the understory (Richardson et al. 2013). Site 1 supported a population of 475 individuals, which are primarily offspring of 1500 plants introduced by BTG from 2002–2009. Site 2 supported a wild population of 1000–5000 Lakela's Mint. Site 3 supported a wild population and introduced plants and their offspring for a total population size of 600 individuals. BTG augmented the population at Site 3 in 2007–2008. We visited each site four times between 12–24 October 2012 and twice between 10–30 October 2014 during daylight hours. During each visit in 2012 we haphazardly selected 20 flowering plants per site and observed each plant for 5 min. Total observation time in 2012 was 1200 min. We counted and identified all pollinators that visited a plant during an observation and counted the number of flowers visited within the plant by a subset of individual pollinators. We also measured, in centimeters, the maximum height of each plant.

We repeated our observations in 2014 to detect annual variation in the composition of the pollinator community. We observed pollinators on a total of 36 plants for 180 min combined at Sites 2 and 3. Site 1 was closed to extended access in 2014 due to vandalism of the plant population. The composition of the pollinator community was unchanged from 2012 and we detected only minor variation in the frequencies the primary pollinators visited plants (see “Results”), which is why we reduced sampling effort in 2014.

Few pollinator species were abundant (see “Results”), so we calculated the percentage of visits to plants within a site and year by the honey bee *A. mellifera* (Hymenoptera: Apidae), bumble bee *Bombus impatiens* Cresson (Hymenoptera: Apidae), and then all other pollinators as a

single group. Separate χ^2 contingency tests were used to determine whether the frequency of these three categories of pollinators were different between years and between sites within a year. We also used separate general linear models, blocked by site and date, to determine whether the frequency of visitation by *A. mellifera*, *B. impatiens*, and other pollinators was influenced by the height of plants.

The number of flowers visited within a plant by *A. mellifera* was compared to native pollinators using a paired *t* test (PROC TTEST; SAS Institute 2011), which included 33 paired observations. To obtain these paired observations, we first followed a native pollinator until it settled on a plant and then we counted the number of flowers the pollinator probed during a single visit. Observing the same plant, we then counted the number of flowers probed by *A. mellifera* during a single visit. We next used separate general linear models (PROC GLM; SAS Institute 2011), blocked by site and date, to determine whether the number of flowers visited within a plant by *A. mellifera* and native pollinators was influenced by the height of the plant. Data for *A. mellifera* were log10 transformed to meet assumptions of normality, but non-transformed data are presented in the “Results”. *Bombus impatiens* was combined with all other native pollinators for these analyses in order to have an adequate sample size.

Reproductive output

At each of the three sites in 2014 we haphazardly selected 20 plants. On each plant we counted the number of reproductive stems, counted the number of flowers on each of three stems (stems are subsamples), and we measured the height of the plant. We later enclosed the three stems in mesh bags after seed capsules formed. Bags were left in place until seeds ripened and dehisced and then we collected the bags and counted the number of seed capsules, total seeds, and number of intact (i.e., likely viable) seeds. All seeds were cut open under a dissecting microscope to determine whether they contained intact embryos, unformed embryos, or were empty.

We used separate general linear models (PROC GLM; SAS Institute 2011), blocked by site, to determine whether plants of different heights varied in their ratio of seed capsules to flowers, ratio of seeds to seed capsules, and ratio of intact seeds to total seeds. When data were collected from subsamples within a plant, stems were nested within plants in the statistical models. Data were square-root transformed, when necessary, to improve normality. Non-transformed data are presented in the “Results”. We also used separate negative binomial models (PROC GENMOD; SAS Institute 2011), blocked by site, to determine whether plants of different heights varied in their mean abundance of flowers, total seeds, and intact seeds.

Stems were nested within plants in the statistical models when data were collected from subsamples.

Results

Breeding system

Seeds were produced from flowers in each treatment in the experimental garden, but the mean number of seeds per seed capsule varied across treatments ($F = 9.66$, $df = 4, 54$, $P < 0.001$). Flowers that were insect-pollinated (control) or were cross-pollinated by hand averaged nearly 3.5–10 times more seeds per seed capsule than those that reproduced autonomously or asexually (Fig. 1). The number of seeds per seed capsule produced by geitonogamous self-pollination did not differ from any of the other treatments (Fig. 1).

Pollinators and plant height

Apis mellifera was the most frequent pollinator to visit plants at all sites in both years, comprising 68–94 % of pollinator visits, and *B. impatiens* was the next most frequent visitor, comprising between 2–16 % of visits (Table 1). Insect species that were infrequently seen on Lakela’s Mint included other species from the hymenopteran families Halictidae, Vespidae, and Apidae, such as *B. pennsylvanicus* (De Geer) and *Xylocopa* spp. We also observed lepidopterans from the families Hesperidae, Nymphalidae, and Noctuidae, dipterans from the family Syrphidae, and a few species that were not identified to

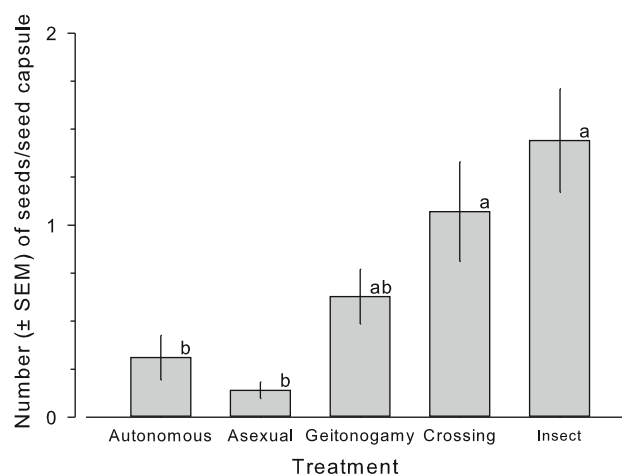


Fig. 1 Mean number (SEM) of seeds per seed capsule produced by Lakela’s Mint flowers subjected to five treatments in a common garden: Autonomous = spontaneous self-pollination; Asexual = agamospermy; Geitonogamy = self-pollination; Crossing = cross-pollination; and Insect = insect-pollinated control. Means with different letters are significantly different (Tukey’s means separation test, $P < 0.05$)

family. Whereas *A. mellifera* was introduced to North America, the other identified species are native.

Despite the fact that *A. mellifera* was consistently the most frequent visitor, the relative frequency of *A. mellifera*, *B. impatiens*, and other pollinators differed among years ($\chi^2 = 15.4$, $df = 2$, $P < 0.001$) and sites in 2012 (Table 1; $\chi^2 = 15.6$, $df = 4$, $P = 0.004$). In 2014 the percentage of *A. mellifera* was lower and the percentage of *B. impatiens* and other pollinators was higher compared to 2012. In 2012, the relative frequency of pollinators was the same at sites 2 and 3, but Site 1 had a lower percentage of *A. mellifera* and higher percentage of *B. impatiens* and other pollinators (Table 1). There was no difference in the relative frequency of pollinators at sites 2 and 3 in 2014 (Table 1; $\chi^2 = 5.0$, $df = 2$, $P = 0.08$).

Differences in foraging behaviors between *A. mellifera* and native pollinators were evident. *Apis mellifera* visited 1.5 times more flowers within a plant than native pollinators (Fig. 2; $t = 6.46$, $df = 32$, $P < 0.001$). The number of

flowers that *A. mellifera* visited within a plant was not influenced by height of the plant ($F = 1.5$, $df = 47$, $P = 0.08$), but the number of flowers visited within a plant by native pollinators increased with increasing height of the plant ($F = 60.4$, $df = 26$, $P < 0.001$). The frequency of visits by *B. impatiens* and other pollinators was negatively influenced by the height of plants (Table 2). The frequency of visits by *A. mellifera* was not influenced by the height of plants, but was variable across dates and sites (Table 2), as previously discussed.

Reproductive output

Whereas there was some variation in reproductive traits among replications or sites, plant height was the only variable associated with each of the six reproductive traits we measured (Table 3). The number of flowers, seed capsules per flower (i.e., pollinated flowers), and total seeds increased with increasing plant height (Table 3). However, seeds per seed capsule (i.e., number of successful pollination attempts per flower), number of intact seeds, and number of intact seeds per total seeds decreased with increasing plant height (Table 3).

Table 1 Percent abundance (and total number) of pollinators on Lakela's Mint at three sites in South-Central Florida, USA during 2 years

Year	Site	Pollinator		
		<i>Apis mellifera</i>	<i>Bombus impatiens</i>	Other pollinators
2012	1	79 (46)	9 (5)	12 (7)
	2	94 (344)	2 (8)	4 (15)
	3	94 (369)	2 (6)	4 (14)
2014	1	NA	NA	NA
	2	83 (33)	10 (4)	8 (3)
	3	68 (50)	16 (12)	15 (11)

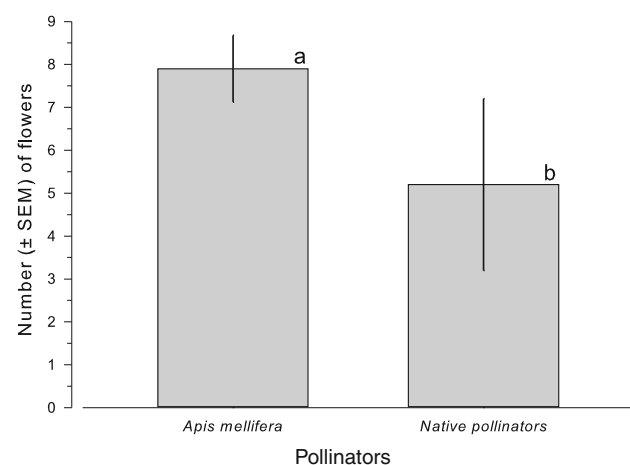


Fig. 2 Mean number (SEM) of Lakela's Mint flowers probed by *A. mellifera* and native pollinators in paired comparisons within 33 single plants across 2 years and three sites in South-Central Florida, USA. Means with different letters indicate that the pairwise comparisons, analyzed with a *t* test, were significantly different

Discussion

Our results suggest that the breeding system of Lakela's Mint is facultative outcrossing because self-pollination is possible even though seed set is highest through outcrossing. Asexual reproduction accounted for a yield of 15 seeds across 7 of the 12 experimental plants. The low number of seeds produced might indicate some unidentified source of experimental error. However, asexual reproduction has been documented within this genus (Evans et al. 2004) and might be a mechanism to overcome

Table 2 Results of general linear models that tested differences in abundances of *Apis mellifera*, *Bombus impatiens*, and other pollinators on Lakela's Mint across six dates in 2012 and 2014, three sites in South-Central Florida, USA, and a continuous gradient of plant heights

Pollinator	Term	Test statistic	<i>df</i>	<i>P</i>
<i>A. mellifera</i>	Date	9.1	1	<0.01
	Site	20.1	1	<0.01
	Plant height	14.9	76	0.15
<i>B. impatiens</i>	Date	21.7	1	<0.01
	Site	0.4	1	0.55
	Plant height	14.2	76	0.01
Other pollinators	Date	0.7	1	0.42
	Site	0.1	1	0.71
	Plant height	15.9	76	0.01

Table 3 Results of general linear and negative binomial models that tested differences in morphology of Lakela's Mint among subsamples (three stems nested within a plant), replications (individual plants), three sites in South-Central Florida, USA, and plant height (continuous variable)

Dependent variable	Term	Test statistic	df	P
Flowers per plant	Subsample(rep)	$\chi^2 = 20.4$	20	0.43
	Replication	$\chi^2 = 31.3$	9	<0.01
	Site	$\chi^2 = 12.3$	2	<0.01
	(⁺)Plant height	$\chi^2 = 84.7$	22	<0.01
Seed capsules per flower	Subsample(rep)	$F = 0.7$	20	0.82
	Replication	$F = 0.9$	9	0.57
	Site	$F = 24.9$	2	<0.01
	(⁺)Plant height	$F = 2.6$	22	<0.01
Seeds per seed capsule	Subsample(rep)	$F = 1.2$	20	0.28
	Replication	$F = 3.2$	9	<0.01
	Site	$F = 10.3$	2	<0.01
	(⁻)Plant height	$F = 2.1$	22	0.01
Intact seeds per plant	Subsample(rep)	$\chi^2 = 21.7$	20	0.36
	Replication	$\chi^2 = 26.4$	9	<0.01
	Site	$\chi^2 = 7.4$	2	0.02
	(⁻)Plant height	$\chi^2 = 53.3$	22	<0.01
Total seeds per plant	Subsample(rep)	$\chi^2 = 19.8$	20	0.47
	Replication	$\chi^2 = 14.2$	9	0.12
	Site	$\chi^2 = 3.3$	2	0.19
	(⁺)Plant height	$\chi^2 = 82.6$	22	<0.01
Intact seeds per total seeds	Subsample(rep)	$F = 0.6$	20	0.91
	Replication	$F = 0.8$	9	0.65
	Site	$F = 0.2$	2	0.82
	(⁻)Plant height	$F = 2.1$	22	<0.01

(⁺) Positive correlation between plant height and dependent variable

(⁻) Negative correlation between plant height and dependent variable

pollen or pollination limitation. The congener *D. frutescens* Shinnery can also produce seeds asexually or autonomously but few of these seeds were viable (Evans et al. 2004). We did not test for viability of the Lakela's Mint seeds that were produced asexually; therefore, follow-up work is needed to determine viability of seeds and whether asexual reproduction plays an important role in the breeding system of this rare species.

Apis mellifera was the most frequent floral visitor followed by *B. impatiens* across 2 years of observations. We did detect some annual variation in the pollinator assemblages, which is common in pollinator-plant mutualisms (Herrera 1988; Cane and Payne 1993). Deyrup and Menges (1997) predicted that Lakela's Mint would be pollinated by bee species, based on morphological characteristics of the flowers, and confirmed this prediction with a brief field observation. Our results matched their prediction that bees would be the most frequent pollinators, but they found the bumble bee *B. pensylvanicus* to be the most frequent pollinator, followed by only a few *A. mellifera*, *B. impatiens*, and other pollinators. Deyrup and Menges (1997) conducted their observations of pollinators of Lakela's Mint in

the same area as in our study, so the difference in pollinator assemblages between their study and ours may be due to the brevity of their observation or long-term variation in pollinator assemblages.

Apis mellifera was the only nonnative pollinator that we observed, and its behavior differed from native pollinators. *Apis mellifera* visited more flowers within plants than native pollinators, which may promote a higher rate of self-pollination and lower rate of seed viability due to inbreeding depression. However, the effects these behavioral differences have on self-pollination and seed viability is unknown. *Apis mellifera* is often a less effective pollinator in areas where it is nonnative and can also disrupt mutualisms between plants and insects, for example through exploitative competition (Dupont et al. 2004; Thompson 2004), which can have consequences for the plants and pollinators (reviewed in Dohzono and Yokoyama 2010). Changing the genetic structure of plant populations is one suggested consequence (Dupont et al. 2004), but has received little attention (but see England et al. 2001).

The height of plants did not influence the attraction of *A. mellifera* to plants or the number of flowers visited by *A.*

mellifera within a plant, whereas plant height influenced both of these behaviors of native pollinators. Once on a plant, native pollinators foraged at more flowers on taller plants than they did on shorter plants. However, the frequency of visits by native pollinators to taller plants was lower than to shorter plants, contrary to the effective pollination hypothesis. Some pollinators visit flowers at a preferred height more frequently than the tallest (Peakall and Handel 1993). In addition, a variety of environmental characteristics, such as the relative amount of sunlight and flowering plant density, are known to influence pollinator behavior in other study systems (e.g., Herrera 1995; Grindeland et al. 2005; Nattero et al. 2011). Dense patches of plants offer clumped resources for pollinators and reduce their energy expenditure (Kacelnik et al. 1986). Lakela's Mint plants are often clustered together in sunny areas (Richardson et al. 2013), so perhaps flowering plant density, or other environmental characteristics, are more important to pollinators in this system than plant height.

Taller Lakela's Mints had more total flowers, which were more likely to experience a pollination event, than shorter plants. However, flowers on shorter plants were more likely to experience multiple pollination events and had a higher output of intact seeds than taller plants. Several underlying mechanisms could explain why the number of intact seeds decreased with increasing plant height. First, we found evidence of seed predators and fungus when dissecting seeds, so perhaps these are more likely to attack taller plants. Second, plants investing in large floral displays may experience an increased rate of self-pollination, which could lower production of viable seeds. We observed native pollinators visiting more flowers on larger plants, but did not test whether that led to an increased rate of self-pollination. Third, there may be a trade-off between producing flowers and producing seeds. Available resources may be limiting and a greater allocation towards flower production may mean fewer resources for seeds (Haig and Westoby 1988; Andersson 2005, 2006). Resource and pollen limitation may occur simultaneously (Campbell and Halama 1993), and such limitation may differ with plant size. For example, larger plants of the ground cherry (*Physalis longifolia*) are limited by both pollen and resources, whereas smaller plants are only limited by pollen (Lawrence 1993). Fourth, native pollinators visit shorter plants more frequently than taller plants, which means that *A. mellifera* may be the primary pollinator of taller plants and less effective, as previously discussed.

In conclusion, some next steps to investigate in this plant-pollinator system are the ability of Lakela's Mint to produce viable seeds via asexual reproduction, the efficiency of *A. mellifera* as a pollinator compared with native

bees such as *B. impatiens* and *B. pensylvanicus*, the rate of self-pollination incurred by *A. mellifera*, the influence of *A. mellifera* on genetic structure of plant populations, and factors influencing the output of intact seeds. Ultimately, understanding the breeding system will help conserve this plant species and provide insight into the underlying factors that influence seed output and mutualisms between rare plants and pollinators.

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