

# EXPERIMENTAL EVIDENCE FOR PREDOMINANT NOCTURNAL POLLINATION DESPITE MORE FREQUENT DIURNAL VISITATION IN *ABRONIA UMBELLATA* (NYCTAGINACEAE)

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**Abstract**—Different suites of floral traits are associated with historical selection by particular functional groups of pollinators, but contemporary floral phenotypes are not necessarily good predictors of a plant's effective pollinators. To determine the extent to which plant species specialize on particular functional groups of pollinators, it is important to quantify visitation rates for the full spectrum of flower visitors as well as to experimentally assess the contributions of each functional group to plant reproduction. We assessed whether attracting both diurnal and nocturnal flower visitors corresponded to pollination generalization or specialization in the Pacific coastal dune endemic *Abronia umbellata* var. *umbellata*. In multiple populations over two years, we observed flower visitors during the day and at night to assess visitation rates by different insect groups and conducted pollinator exclusion experiments to assess the contributions of diurnal and nocturnal visitors to seed production.

Flower visitation rates were 8.67 times higher during the day than at night, but nocturnal visitation resulted in significantly higher seed set, suggesting that nocturnal noctuid and sphingid moths are the chief pollinators. Most diurnal visitors were honey bees, with tongues too short to reach *A. umbellata* nectar or contact stigmas and effect pollination. The prevalence of honey bees, combined with the lack of successful seed production resulting from diurnal pollination, suggests that honey bees are pollen thieves that collect pollen but do not deposit it on stigmas. Our results underscore the need to experimentally assess the contributions of different groups of flower visitors to plant reproduction.

**Keywords:** *Abronia umbellata*, moth pollination, Nyctaginaceae, pollination ecology, pollination syndromes, seed set, specialization

## INTRODUCTION

The remarkable phenotypic diversity of flowering plants is thought to be due largely to selection exerted on flower morphology and development by pollen vectors (Fenster et al. 2004). One of the most compelling pieces of evidence for the importance of pollinator-mediated selection is the nonrandom association of floral traits among species that differ in the types of animals that mediate cross-pollination, a phenomenon generally referred to as pollination syndromes (van der Pijl 1961). For example, plants that have historically experienced selection exerted predominantly by nocturnal moths typically have medium to long narrow corolla tubes that contain relatively dilute nectar, are white or pale in colour, lack nectar guides, and emit a sweet fragrance in the evening (Willmer 2011).

While pollination syndromes can provide insight into the historical pollinator-mediated selection that helped to shape contemporary floral phenotypes, they do not necessarily predict which flower visitors are currently effective pollinators

(Ollerton et al. 2009), nor can they be used to infer the degree of specialization of a plant's current pollinator fauna (Waser et al. 1996). The number of functional groups observed visiting a plant species is often a better indicator of that plant's degree of specialization than the number of visiting taxa alone (Fenster et al. 2004; Armbruster 2017), but it remains critical to distinguish between visitors that effect pollination and those that do not, especially because many animals that visit flowers perform little to no pollination (Hargreaves et al. 2009; Irwin et al. 2010).

The important distinction between floral visitation and effective pollination makes experimental approaches critical for determining the importance of particular visitors or visitor guilds to pollination and plant fitness. Different visitor guilds often visit specific plant taxa at different times of day, thus temporal pollinator exclusion can be used to determine the relative importance of temporally-divergent visitors (Brunet & Holmquist 2009; Walter 2010; Bustamante et al. 2010). Combining such experiments with observations of flower visitors can determine which functional groups are the most effective pollinators. Such experiments should be replicated across populations and years, given that many studies have demonstrated pronounced spatiotemporal variation in pollinator faunas (Herrera 1988; Wolfe & Barrett 1988;

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Received 14 August 2017, accepted 14 February 2018

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TABLE 1. Locations of *Abronia umbellata* study sites in California, USA, activities conducted at each site, and dates visited. “Coll” = pollinator collections, “Exp” = pollinator exclusion experiments, and “Obs” = pollinator observations.

Site	Code	County	Latitude (°N)	Longitude (°W)	Activities	Dates Visited
(1) McGrath State Beach	CMGA	Ventura	34.21876	119.25853	Coll, Exp, Obs	2010: May 23, July 9–10; 2011: May 22–27, June 11, July 6–7
(2) San Buenaventura State Beach	CBVA	Ventura	34.26788	119.27815	Coll	2010: July 10
(3) Coal Oil Point Reserve	CCOA	Santa Barbara	34.40824	119.87909	Coll, Exp, Obs	2010: May 24, June 1–7; 2011: June 2–8, July 3–4
(4) Coreopsis Hill	CGN2A	San Luis Obispo	35.02181	120.62203	Coll	2010: July 13
(5) Montaña de Oro State Park	CSPA	San Luis Obispo	35.30072	120.87560	Coll, Exp, Obs	2010: July 2–8; 2011: June 26–30, July 28–30
(6) Manresa Uplands State Beach	CMNA	Santa Cruz	36.91531	121.85155	Coll, Exp, Obs	2010: June 11–17; 2011: June 14–17, July 24–25
(7) Seacliff State Beach	CSEA	Santa Cruz	36.96854	121.90492	Coll	2011: July 25

Fishbein & Venable 1996; Eckert 2002). However, spatiotemporal replication is logistically demanding and difficult to achieve in many systems. We are aware of only four studies that include multiple years of study at more than one site (Morse & Fritz 1983; Fleming et al. 2001; Holland & Fleming 2002; Bustamante et al. 2010), just three of which combined temporal exclusion experiments with pollinator observations.

The Pacific coast dune plant *Abronia umbellata* Lam. var. *umbellata* (Nyctaginaceae) possesses some floral traits seemingly specialized for moth attraction at night, but other traits that are attractive to diurnal insects. It provides an excellent opportunity to study the extent to which floral traits associated with attracting visitors from multiple functional groups translate into a generalized vs. specialized pollinator fauna. *Abronia umbellata* var. *umbellata* is self-incompatible (SI) and obligately outcrossing, in contrast to the self-compatible and highly autogamous var. *breviflora* (Doubleday et al. 2013). *Abronia umbellata* var. *umbellata* (hereafter SI *A. umbellata*) exhibits some traits that typify the moth-pollination syndrome: it has reverse herkogamous narrow, tubular flowers with anthers close to the mouth of the floral tube and stigmas recessed deeply within (Fig. 1B) and, in the evening, emits a sweet fragrance containing benzenoid compounds typical of moth-pollinated species (Doubleday et al. 2013). However, unlike canonically white “moth flowers,” the flowers are pink-purple with contrasting white “eyespot” encircling the floral tube opening (Fig. 1A), suggesting attraction of diurnal visitors. The flowers are uniovulate and borne on umbellate inflorescences, and fruits are tough, winged diclesia, a type of anthocarp that appears to be

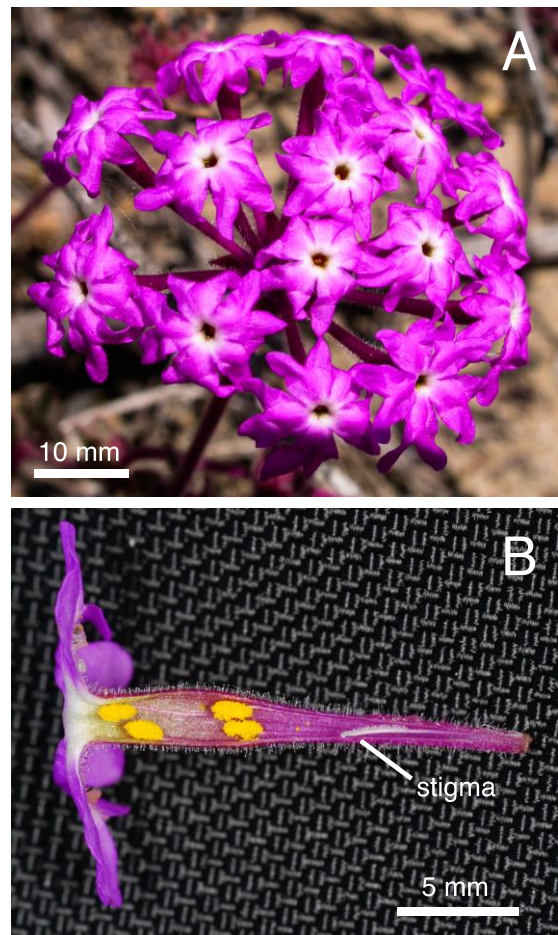


FIGURE 1. Morphology of inflorescences (A) and flowers (B) of *Abronia umbellata* var. *umbellata* (Nyctaginaceae).

TABLE 2. Mean flower visitation rates (visits (v) per flower (f) per hour (h)), standard errors of the means, number of observation periods ( $n_{\text{obs}}$ ), total observation time (Time, in h) at each *Abronia umbellata* study site for pollinator observations during the day and night pooled across two years (2010 and 2011). Pollinator observations consisted of recording visitation to all of the flowers in  $\sim 2$  m diameter patch for 3 or 5 min. Sampling effort is the product of number of flowers observed during each observation period and the length of that observation period in hours ( $f^*h$ ). Populations are listed by increasing latitude. Site codes are as in Table 1.

Site	Day (0600–1800h)					Night (1800–0600h)				
	Visit rate (v/f/h)	SE	$n_{\text{obs}}$	Time (h)	Effort ( $f^*h$ )	Visit rate (v/f/h)	SE	$n_{\text{obs}}$	Time (h)	Effort ( $f^*h$ )
(1) CMGA	0.0000	0.0000	15	0.75	293.5	0.0012	0.0012	143	9.62	1918.9
(3) CCOA	0.0519	0.0109	159	7.95	7394.8	0.0033	0.0015	220	13.27	6144.5
(6) CSPA	0.0228	0.0072	202	12.47	4494.6	0.0105	0.0075	119	7.02	2670.1
(7) CMNA	0.0013	0.0008	116	5.80	3794.7	0.0020	0.0009	389	22.12	8955.3
Mean	0.026	0.003	–	–	–	0.003	0.001	–	–	–
Total	–	–	492	26.97	15977.60	–	–	871	52.03	19688.80

dispersed by wind (Darling et al. 2008). An umbel's flowers open individually over the course of 1–4 days, each umbel bears apparently functional flowers for 7–10 d, and individual flowers remain open day and night (L. A. D. Doubleday, pers. observation). The timing of pollen presentation and stigma receptivity are unknown. The chief pollinators of SI *A. umbellata* have not been determined, although Tillett (1967) speculated that crepuscular and nocturnal noctuid and sphingid moths are likely the most important pollinators.

This study has two main objectives. First, we document patterns of visitation by different insect guilds to flowers during the day and at night. Second, we use temporal pollinator exclusion experiments to determine which visitors are effective pollinators by directly quantifying the relative importance of nocturnal vs. diurnal flower visitors to seed set. We assess SI *A. umbellata*'s pollination ecology in multiple natural populations in two years.

## MATERIALS AND METHODS

### Pollinator observations

At each of four sites on the Pacific coast of California, USA (Tab. 1) we estimated the rates of diurnal and nocturnal

insect visitation to individual SI *A. umbellata* flowers during standardized 3- or 5-min observation periods. For each period, we randomly selected a patch of inflorescences within a circular plot of  $\sim 2$  m diameter and simultaneously observed all flowers without regard to individual plants (plants are prostrate and intermingle such that individuals that cannot be separated without damaging them). We recorded each flower visitor's identity (to the lowest taxonomic level possible in the field), the number of umbels visited by each flower visitor, and the number of flowers probed on each umbel visited. Our presence, standing  $\sim 1$  m from the plot, did not seem to influence flower visitor behaviour. We used red headlamps (Petzl® TIKKA PLUS2®) for evening (sunset – 0200 h) observations because nocturnal insects are relatively insensitive to red light (Briscoe & Chittka 2001). We conducted observations across all times of day and night except between midnight and 0500 h (Fig. 2). We saw insects probing flowers only between 1000 and 2200 h, with peaks in flower visitation between 1100 and 1600 h and 2000 and 2200 h (Fig. 2). The timing of these flower visitation peaks, combined with a lull in visitation between 1600 and 2000 h (Fig. 2), sunrises between 0545 and 0630 h, and sunsets between 2000 and 2030 h made 0600 and 1800 h reasonable dividing times

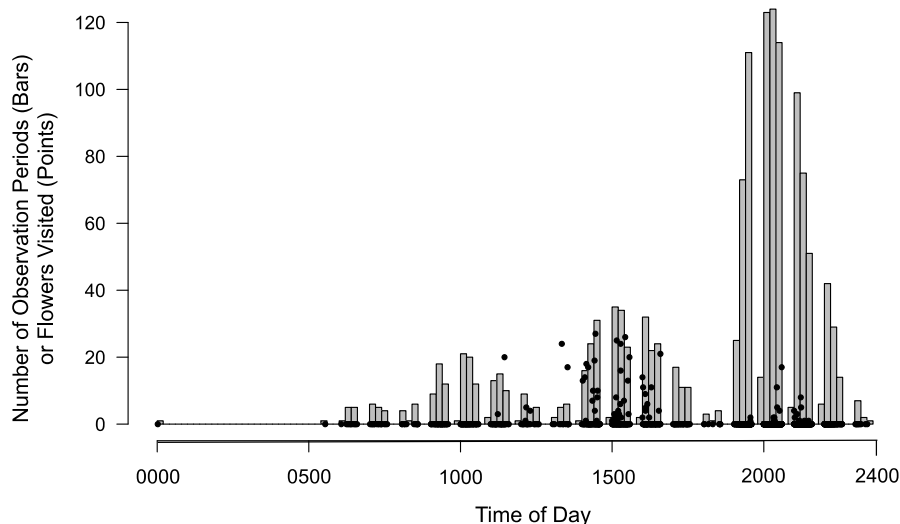


FIGURE 2. Sampling effort and temporal patterns of flower visitation to *Abronia umbellata* var. *umbellata* flowers in natural populations. Bars represent the number of pollinator observation periods conducted at a given time and points represent the total number of flowers visited during an observation period at a given time. Data is pooled across days and sites.

TABLE 3. Insects collected while visiting flowers of *Abronia umbellata* at sites in California, USA. Site numbers are in Table 1.

Order	Family	Species	Day- or	Year	Sites	Number of specimens
Hymenoptera	Apidae	<i>Apis mellifera</i>	Day	2010	1, 3, 5, 6	16, 12, 3, 2
				2011	1, 2, 6	1, 1, 2
		<i>Bombus</i> spp.	Day	2010	3, 7	1, 1
	Unknown	Small, long-tongued solitary bee	Day	2011	6	1
Lepidoptera	Hesperiidae	<i>Hylephila phyleus</i>	Day	2010	1	2
	Nymphalidae	<i>Vanessa cardui</i>	Day	2010	1	1
				2011	8	1
	Unknown	Small brown and orange butterfly	Day	2011	6	1
	Geometridae	<i>Euphyia</i> sp.	Night	2010	2, 7	2, 1
	Noctuidae	<i>Trichoplusia ni</i>	Night	2010	7	2
				2011	6	1
		<i>Copablepharon robertsoni</i>	Night	2010	6	1
		<i>Copablepharon sanctaemonicae</i>	Night	2010	2, 6	3, 6
				Night	2011	6
	<i>Autoplusia egenoides</i>	Night	2010	7	3	
Pyrilidae	<i>Phobus funerellus</i>	Night	2010	7	11	
Sphingidae	<i>Hyles lineata</i>	Night	2010	1, 7	1, 1	
Diptera	Acroceridae	<i>Eulonchus</i> sp.	Day	2010	6	1

between day and night. We made 492 observation periods (26.97 h) between 0600 and 1800 h (“day”) and 871 (52.03 h) between 1800 and 0600 h (“night”). There were two reasons why we conducted more observations at night: daylight was important for other sampling activities, so we could not devote as much daytime effort to pollinator observations, and we were determined to learn the taxonomic identities of nocturnal moth pollinators, even though they were relatively rare. Because of the logistic challenges involved in studying multiple sites separated by hundreds of km, while conducting experiments lasting 7–10 d at some sites, sampling effort was unbalanced across sites and sites were visited on different dates (Tab. 1 & 2). Pollinator faunas often fluctuate seasonally (Herrera 1988), and sampling date may have affected which flower visitors were present at a given site. Whenever possible, we collected a sample of flower-visiting insects and identified these to the lowest taxonomic level possible given the expertise available to us (Tab. 3). We calculated total visitation for each insect group as visits per flower per hour.

#### Temporal pollinator exclusion experiment

At four sites over two years we quantified the relative importance of diurnal vs. nocturnal pollination by excluding pollinators from individual umbels at different times of day. The experiment involved one site in 2010 (CMNA) two others in 2011 (CMGA, CSPA) and a third in both years (CCOA; Tab. 1). Individual umbels were allocated to one of three treatments: (i) exposed to pollination at night but enclosed in a wire cage covered with fine bridal veil during the

day (0600–1800 h); (ii) exposed to pollination during the day but enclosed at night (1800–0600 h); or (iii) exposed to pollination always (i.e. never enclosed). In 2011, we added an additional treatment: (iv) enclosed always. Any seed produced by these umbels would have to result from either autonomous selfing and/or pollinators accessing enclosed flowers. Only a very small number of continuously enclosed umbels set seed: one at CCOA (mean proportion of flowers setting seed  $\pm$  ISE =  $0.0018 \pm 0.0033$  seeds/ovule), two at CMGA ( $0.010 \pm 0.0081$ ) and seven at CSPA ( $0.029 \pm 0.012$ ) suggesting that autonomous selfing and/or the failure of the enclosures to exclude pollinators were infrequent. It is possible that stigmas frequently received self-pollen, but fertilization rarely occurred because of strong genetic self-incompatibility (Doubleday et al. 2013). Experimental crosses on both enclosed and exposed plants did not reveal any negative effects of enclosure on seed set (Doubleday 2012).

We randomly assigned  $\geq 30$  umbels (only one umbel per plant) just at anthesis (1–2 flowers beginning to open) to each of the treatment groups. Because *A. umbellata* infructescences shatter at maturity, we bagged them with bridal veil to capture all mature diclesia. For each umbel, we assessed whether each flower produced a seed by counting the number of flowers on each umbel and the number of filled seeds produced. Failure to set seed included flowers from which a diclesium never developed, and those from which a diclesium developed but did not contain a filled seed. At our study sites, only about 30% of developed diclesia contain seeds (mean  $\pm$  1SD:  $29.52 \pm 30.00\%$ ; L. A. D. Doubleday and C. G. Eckert, unpublished data). Diclesia routinely expand but contain no seeds under

pollinator-free glasshouse conditions (L. A. D. Doubleday and C. G. Eckert, unpublished data), indicating that successful pollination is not a prerequisite for fruit expansion.

### Statistical analyses

We used R (version 3.1.3, R Core Team 2017) for all statistical analyses. Because flower visitation occurred in only 6% of observation periods, visitation rates were zero-inflated. Accordingly, we tested for a difference in visitation rate between night and day using randomization tests. We computed the mean difference between day and night visitation rates, randomized the data without respect to when an observation was conducted using the “sample()” function in R, and calculated mean visitation rates for day and night for each of 10,000 randomizations. The proportion of differences calculated from randomized data that were equal to or greater than the observed difference is equivalent to a  $P$  value ( $P_{\text{rand}}$ ). We report these as approximate because each randomization run returns a slightly different value.

We tested for differences in seed set among temporal enclosure treatments by fitting generalized linear models (GLMs, `glm()` function in R) with binomial error structure (logit link function) to variation in seed set (the number of flowers making a seed vs. the number not making a seed). Because we studied different populations and performed a different set of treatments in each year, data for 2010 and 2011 were analyzed separately. Hence, we evaluated site and enclosure treatment and their interaction as potential predictors. We used quasi-likelihood estimation because data were overdispersed, and performed likelihood ratio tests to evaluate the significance of each term in the model using  $F$  tests following Buckley (2015). We used the `lsmeans()` and `contrast()` functions in the `lsmeans` R package (version 2.25, Lenth 2016) to perform post-hoc, pairwise contrasts among enclosure treatments.

## RESULTS

### Flower visitation

Visitation was 8.67-times higher during day than night observation periods for populations and years combined (Tab. 2;  $P_{\text{rand}} < 0.0001$ ), 5.09-times higher in 2010 ( $P_{\text{rand}} < 0.0001$ ), and 13.10-times higher in 2011 ( $P_{\text{rand}} \sim 0.0008$ ). When we separately analysed each of three sites with more than 100 observation periods for each time period, day visitation was greater at CCOA ( $P_{\text{rand}} < 0.0001$ ), but not CSPA ( $P_{\text{rand}} \sim 0.13$ ) or CMNA ( $P_{\text{rand}} \sim 0.50$ ). Most diurnal visitors were introduced honey bees (*Apis mellifera*: Apidae, Tab. 3). Other diurnal visitors included bumble bees, butterflies, flies (Tab. 3), and, on one occasion, a diurnal sphingid (possibly *Hemaris* sp.) (L. A. D. Doubleday, personal observation), but these other visitors were infrequent. When we excluded honey bee visits from the analysis (pooling years and populations), mean night visitation (mean  $\pm$  I SE:  $0.0030 \pm 0.0011$ ) was higher than day visitation ( $0.0008 \pm 0.0009$ ) but not significantly so ( $P_{\text{rand}} \sim 0.13$ ). The differences for individual sites analysed separately were also not significant (all  $P_{\text{rand}} > 0.11$ ). When observations were pooled by year with honey bee visits excluded, night visitation

was more frequent than day visitation for 2010 ( $P_{\text{rand}} \sim 0.037$ ) but not 2011 ( $P_{\text{rand}} \sim 0.18$ ).

We caught 81 visitors that probed SI *A. umbellata* flowers and identified 70 to species, nine to genus, and two to order (Tab. 3). The 49 day visitors represented at least seven species (though 33 visitors were honey bees) and three orders. The 32 night visitors, all of which were Lepidoptera, represented at least seven species from four families. Noctuid moths were most common (50% of night visitors), with three of four species collected from only one population and the fourth species collected from two populations.

### Temporal pollinator exclusion

Seed set was generally very low, even among open-pollinated umbels always exposed to pollinators (mean  $\pm$  I SE:  $0.071 \pm 0.012$  seeds/ovule, Fig. 3). In 2011, umbels exposed to pollinators only at night set 3.8-times more seed than those exposed only during the day and did not differ from umbels exposed all the time (Fig. 3, Tab. 4). Seed set was somewhat higher at sites CSPA and CMGA than CCOA, but there was no difference in treatment effects between sites. Results from 2010 suggest 3.4-times higher seed set by night-exposed than day-exposed umbels (Fig. 3), but the difference among treatments was not quite significant ( $P = 0.073$ , Tab. 4). Again, there was variation among sites in mean seed set (CCOA > CMNA), but not in the effects of enclosure treatment on seed set. At CCOA for 2010 and 2011 combined, seed set did not vary among treatments or between years, nor did the treatment effect vary between years (Tab. 4).

## DISCUSSION

Self-incompatible, outcrossing *A. umbellata* var. *umbellata* possesses some traits suggestive of historical selection by nocturnal moths but does not completely conform to the classic moth-pollination syndrome. Our pollinator observations suggest that several functional groups of insects visit the plant during the day and at night, with much higher rates of flower visitation during the day in some populations. However, our temporal pollinator exclusion experiments suggest that nocturnal visitors are more effective pollinators than diurnal visitors. In 2011, night-pollinated umbels had significantly higher seed set (3.8-times higher) than day-pollinated umbels, and the difference in 2010 was of similar magnitude, but not statistically significant (Fig. 3). Taken together, these results suggest predominant nocturnal pollination despite more frequent visitation during the day.

Of the taxa we observed visiting flowers at night, noctuid moths were most common, but we also observed sphingid, pyralid, and geometrid moths. Average tongue lengths were available for members of three of the four moth families we collected from SI *A. umbellata* flowers: 25–33mm for *Hyles lineata*, the sphingid species we observed; 10–20 mm for most temperate noctuids; and 4–9 mm for a different pyralid species than the one we observed (Willmer 2011). Stigmas are recessed  $14.07 \pm 1.01$  mm (mean  $\pm$  I SD, C. G. Eckert, unpublished data) from the floral face in SI *A. umbellata*, suggesting that sphingids and many noctuids would be

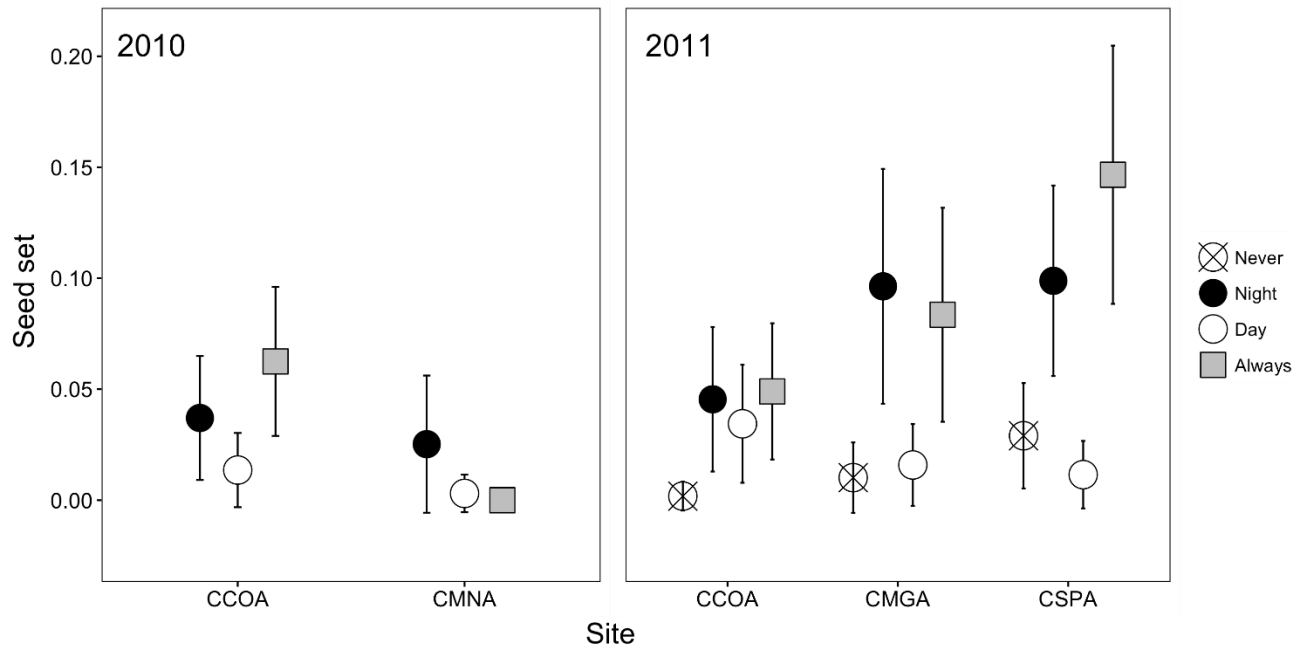


FIGURE 3. Mean seed set of umbels subjected to different pollinator exclusion treatments in natural populations of *Abronia umbellata* var. *umbellata*. Umbels were available to pollinators all the time ("Always"), from 1800 – 0600h ("Night"), from 0600 – 1800h ("Day") or always enclosed ("Never"). Sample sizes in 2010: CCOA Night = 18, Day = 19, Always = 19; CMNA Night = 10, Day = 16, Always = 11; 2011: CCOA Never = 26, Night = 24, Day = 28, Always = 30; CMGA Never = 27, Night = 22, Day = 28, Always = 22; CSPA Never = 28, Night = 29, Day = 29, Always = 21. Each point is a back-transformed least-squares treatment mean, and error bars are 95% confidence intervals.

effective pollinators because their tongues are long enough to successfully contact the stigma.

Many species of *Abronia* may be predominantly moth pollinated, as the floral display of most species is typified by umbels of fragrant, tubular flowers with recessed stigmas, requiring pollinators with long, narrow tongues. Jabis et al. (2011) suggested that the sphingid moth *Hyles lineata* was the most effective potential pollinator of *A. alpina*, but that diurnal taxa also contributed substantially to pollination. Saunders and Sipes (2006) suggested that several species of noctuid and sphingid moths were likely the most important pollinators of *A. ammophila*, but that butterflies were also likely to affect some pollination. Similarly, Williamson et al. (1994) speculated that noctuid and sphingid moths were key

pollinators of *A. macrocarpa* as multiple moth species were observed visiting flowers and bearing *A. macrocarpa* pollen in the field.

During the day, we observed much higher frequencies of visitation by honey bees than by other taxa and, when performing pollen supplementations as part of another experiment, we found it increasingly difficult to obtain sufficient donor pollen from open flowers, which we suspect was due to pollen theft by honey bees (L. A. D. Doubleday, personal observation). The combination of recessed stigmas in flowers of *A. umbellata*, the relatively short glossae of *A. mellifera* (mean  $\pm$  1 SD = 5.15  $\pm$  0.47mm,  $n$  = 28; L. A. D. Doubleday, unpublished data), and our observation of low

TABLE 4. Analyses of variation in seed production by umbels of *Abronia umbellata* after experimental isolation from pollinators. Cells present  $F$  and  $P$  values from likelihood ratio tests comparing generalized linear models fit with binomial errors to the number of flowers setting seed vs. not setting seed. Contrasts among treatment means are shown when the effect of treatment was significant (A = umbels always exposed to pollinators, N = exposed only during the night, D = exposed only during the day, X = never exposed). All four treatments were applied in 2011, whereas only three were used in 2010 (A, N, D). For the analysis of CCOA including both years, the full model did not fit the data better than a null model ( $F_{3,132} = 1.08$ ,  $P = 0.38$ ). NS = not significant at  $P < 0.05$ . Least squares means by treatment and site are in Fig. 2.

Sites	Years	Treatment (T)	Site (S)	T x S	Contrasts
CCOA, CMNA	2010	$F_{2,89} = 2.70$ , $P = 0.073$	$F_{1,89} = 6.86$ , $P = 0.010$	$F_{2,87} = 2.52$ , $P = 0.086$	NS
CCOA, CMGA, CSPA	2011	$F_{3,308} = 15.89$ , $P < 0.0001$	$F_{2,308} = 4.64$ , $P = 0.010$	$F_{6,302} = 1.90$ , $P = 0.080$	A = N > D = X
CCOA	2010, 2011	Treatment (T) $F_{2,132} = 1.70$ , $P = 0.18$	Year (Y) $F_{2,132} = 0.13$ , $P = 0.72$	T x Y $F_{2,132} = 0.90$ , $P = 0.42$	NS

seed set among day-pollinated umbels makes it extremely unlikely that honey bees vector substantial outcross pollen. It is also unlikely that they are successfully extracting nectar from flowers, because available nectar is deeply recessed within the flower at the base of the floral tube (L. A. D. Doubleday, personal observation). This, and the close proximity of dehiscing anthers to the mouth of the floral tube suggests that these visitors are gathering pollen as pollen thieves, but further study would be required to confirm this as we were unable to assess pollen loads on honey bees foraging on SI *A. umbellata* flowers. It is likely that pollen theft has negative effects on plant fitness: for example, Hargreaves et al. (2010) demonstrated that pollen theft by bees decreases reproductive success in *Aloe maculata*. Bees are the most commonly reported pollen thieves (Hargreaves et al. 2009).

Introduced pollinators like honey bees have significant, but varied, effects on native ecosystems (Goulson 2003). It would be premature to generalize about the effects of honey bees across diverse native ecosystems, but honey bees have been shown to dominate the spectrum of flower-visiting taxa in certain fragmented habitats in the Neotropics (Aizen & Feinsinger 1994), and meta-analysis has shown that habitat loss has negative effects on the abundance of unmanaged bee species but not on honey bee abundance (Winfree et al. 2009). Honey bees are ineffective pollinators of some native plants but effective pollinators of others (reviewed by Butz Huryn 1997) and introduced honey bees may compete for nesting cavities with native bees, birds, and mammals, but definitive studies are lacking (reviewed by Goulson 2003). Adding hives of Africanized honey bees to habitats in French Guiana reduced visitation to certain plants by native stingless bees, due to honey bees outcompeting native bees for limited floral resources, and removing the honey bee hives reversed the trend (Roubik 1978). Most studies of the effects of honey bees on native pollinators focus on introduced vs. native bees, and less is known about the effects of honey bees on moth pollinators and moth-pollinated plants. The presence of honey bees in Pacific coastal dune systems is likely to have a negative effect on SI *A. umbellata*'s reproductive success, because pervasive pollen theft would increase pollen limitation and *A. umbellata*'s seed set is partly limited by pollen (L. A. D. Doubleday & C. G. Eckert, unpublished data).

#### ACKNOWLEDGEMENTS

We thank A. Boag, D. Denley, S. Lynskey, and B. Gresik for help in the field; N. Derieg, J.E. Dugan, B. Gresik, S. Hodges, M. King, M. Jacobs, H.M. Page, G. Rochon-Terry, C. Willis, and J. Viengkone for lab assistance; D. Hubbard, J.E. Dugan, and C. Sandoval for support in the field; D. Lafontaine, J.-F. Landry, and C. Schmidt of the Canadian National Collection of Insects, Arachnids and Nematodes for insect identifications; California State Parks, the University of California Natural Reserve System, and the United States Fish and Wildlife Service for research permits and access to study sites. This work was supported by the Government of Ontario (Ontario Graduate Scholarship to L.A.D.D.) and the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to C.G.E. and Canada Graduate Scholarship to L.A.D.D.).

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