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STIMULATION OF FLOWER NECTAR REPLENISHMENT BY REMOVAL: A SURVEY OF ELEVEN ANIMAL-POLLINATED PLANT SPECIES

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Abstract—Understanding the interaction between reward-seeking flower feeding animals and plants requires consideration of the dynamic nature of nectar secretion. Studies on several plants suggest that nectar secretion may increase in response to its removal, but it is not clear whether the phenomenon is widespread. We determined whether 11 species of Colorado mountain wildflowers showed removal-enhanced nectar replenishment (RENR). We measured floral phenology, nectar volumes, rate of replenishment, and compared the cumulative nectar produced following five hourly removals with that accumulated after five hours. Nectar replenishment occurred rapidly, within minutes; statistically significant RENR was observed in 9 of our 11 study species, with the strongest effects in bee-pollinated species. We discuss the implications of RENR in plant species on the measurement of nectar, the adaptive advantage of RENR, and the energetic costs of RENR.

Keywords: Bumble bee, hummingbird, nectar production, pollination, Rocky Mountain Biological Laboratory

INTRODUCTION

The study of nectar secretion schedules is central to pollination ecology. From a plant's viewpoint, flower nectar schedules have the potential to influence the type, schedule, and behaviour of pollinator visits, ultimately affecting pollen transfer and fitness. From a flower-feeding animal's viewpoint, flower nectar schedules have the potential to influence foraging strategies in time and space, and ultimately energetic returns and fitness. For the study of plant-pollinator energetics in particular, some early treatments extolled the relative simplicity of measuring floral nectar and its sugar content. For example, Heinrich (1983, p. 275) considered the economics of bumble bees to be especially tractable because, "they forage for essentially pure energy resources that are *easy to quantify in the lab and in the field"* (emphasis ours). Indeed, by extracting a flower's nectar with a capillary tube at one time point and estimating its sugar content by refractometry, one can establish both volume and approximate energy value in less than a minute. This is considerably easier than determining the dietary value of a leaf or a seed. However, actually calculating the energetic return that a forager can expect while nectar-feeding at a particular flower species is complicated by the variable and dynamic nature of nectar secretion. A flower's nectar characteristics can vary with many intrinsic and extrinsic factors including, but not limited to, flower age and sexual phase, position on a plant, surrounding microenvironment, and time of day or season (reviewed in Pacini & Nepi 2007; Willmer 2011). Furthermore, flowers can dynamically control nectar production: both volume and sugar content can be responsively secreted or reabsorbed by nectaries over the lifetime of a flower (e.g., Nepi et al. 2011).

Nectar secretion can be responsive, in particular, to nectar removal by flower visitors (Castellanos et al. 2002; Ordano & Ornelas 2004). In this paper, we are concerned with removal-enhanced nectar replenishment (RENR), in which a flower that is visited repeatedly over a time period produces more total nectar than a flower visited only once at the end of the period. Particularly when multiple flower-feeders visit flowers at short intervals, and when pollen transfer increases with multiple visits to a flower, RENR seems likely and advantageous for plant fitness, at least to some point (Castellanos et al. 2002). If RENR occurs, a flower's nectar "reward" cannot be considered a simple phenotypic entity like a seed or a leaf; because it interacts dynamically with the behaviour of flower-feeders, it must be treated much as a behavioural variable itself. The plant's constitution and physiology may establish characteristic potentials and limits, but the actual expression of the trait depends on circumstances. The desirability of considering this dynamism has been pointed out for a long time (e.g., Cruden & Hermann 1983).

There is a small but growing literature on the effect of nectar extractions on the total nectar production of flowers. Multiple nectar removals can increase (e.g., Nicolson & Nepi 2005; Ornelas et al. 2007; Munguía-Rosas et al. 2009; Amorim et al. 2013; Bobrowiec & Oliveira 2012), decrease (e.g., Bernardello et al. 1994; Galetto et al. 1997; McDade & Weeks 2004; Carlson 2007), or have no effect (e.g., Nepi et al. 2011; Veiga Blanco et al. 2013) on the amount of nectar produced by flowers, relative to flowers in which nectar has accumulated over the same time period. In a meta-analysis of published studies, Ordano & Ornelas (2004) found that while the responses of different plant species varied widely, overall, nectar removals enhanced the volume produced by

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*Corresponding author; email: jane.ogilvie@utoronto.ca flowers (dataset of 15 species), but nectar removal caused an overall slight decline in the amount of sugar produced (dataset of 31 species). Most of these studies focussed on a single species or genus (e.g., Gill 1988; Rivera et al. 1996; Navarro 1999; Castellanos et al. 2002; Ornelas & Lara 2009), or within a pollinator guild of plants (e.g., McDade & Weeks 2004; Bobrowiec & Oliveira 2012), and the sampling protocols differ widely. Thus, whether RENR is common over a broad range of species and flower traits remains unclear.

To begin broadening and systematizing the study of enhanced nectar renewal, we conducted a survey of RENR in 11 species of animal-pollinated plants in the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (Fig. 1). Chosen for convenience, the plant species were abundant, with large flowers visited mostly by bumble bees or hummingbirds. Many of these plants have received much attention from pollination researchers at the RMBL (e.g., Pyke 1978; Waser 1982; Price et al. 2005). For all species, we first studied the duration and timing of events during floral anthesis, and also nectar volume and concentration, to establish the most suitable flower age to examine RENR. We then adopted a standard protocol for assessing RENR, by comparing multiple removals to single removals over five hour periods. In addition, we also measured how quickly flowers replenished their nectar volumes after being drained. To minimize damage to nectaries from repeated sampling, we blotted up nectar with absorbent paper wicks. This precluded us from obtaining concentration data, so our conclusions about RENR concern nectar volumes only.

MATERIALS AND METHODS

Study system and general protocols

We carried out observations and sampling of the 11 plant species at sites in and around the RMBL in June to August, 2011, in dry rocky subalpine meadows, more mesic meadows, and in the understorey of aspen (*Populus tremuloides*) forests at around 2900 m above sea level. Floral-ecological traits of the plant species are presented in Tab. 1, including their main floral visitors near the RMBL and the time of the season that they were sampled. All species are native to the area, except for the introduced *Linaria vulgaris*. We sampled plants at a particular site for each species, to minimise the variation in nectar due to environmental conditions. To follow known flowers, we marked flowers using permanent markers applied to the calyx or with labelled pieces of coloured tape applied to the pedicel. Because nectar secretion, reabsorption, and concentration may vary throughout the day and with weather conditions (Pleasants 1983; Gill 1988; Herrera 1990; Burquez & Corbet 1991; Rivera et al. 1996), nectar measurements were performed at set times in the early afternoon for all species, and only on warm and sunny days. Although observations at various times of day would have been informative, time constraints associated with doing a broad survey of plant species forced us to choose one generally manageable time. In these cool habitats, early afternoon often represents peak activity of bumble bees (see Fig. 1, Williams & Thomson 1998); hummingbirds become active at first light and continue until dusk. We performed all nectar measurements on plants that were bagged prior to floral anthesis to exclude flower visitors, using either sand-bottom mesh bags (Thomson et al. 2011) or polyolefin Tyvek specimen bags. A separate experiment showed that there was no significant difference in nectar volume and concentration measurements of *Linaria vulgaris* flowers bagged with the two types (E. Y. Luo, unpubl. res.).

Floral scheduling

We first established the duration and timing of events during anthesis in flowers left open to visitors, by following at least 20 individual flowers from each species from bud opening until corolla detachment. We observed the plants one to three times per day and recorded the sexual stage of the flowers. From this, we determined the number of days of floral anthesis, and the order and duration of male and female phases.

Nectar production over the floral lifetime

To determine the most suitable flower age to examine RENR, we measured nectar secretion over the lifetime of flowers for each species. At daily intervals, we measured the volume of nectar that had accumulated in flowers that spanned the range of the flower lifetime. A particular flower was sampled only once. We estimated nectar volumes by introducing one end of a thin paper wick to the nectaries and measuring the moistened length of wick with calipers. We used stiff Whatman paper sold for use as wicks in starch-gel electrophoresis. To cut strips of consistent width, we equipped an Olfa circular cutter with two blades separated by a plastic spacer of 0.71 mm thickness (nominal 0.030 inch). We had previously conducted trials with known volumes of sugar solutions; these indicated that the moistened length varied linearly with volume, although the precise relationship between volume and moistened length depended on nectar concentration. For most plant species in this study, we collected nectar from several flowers with glass microcapillary tubes and determined nectar concentration using a hand-held refractometer suitable for small volumes. We used the mean concentration value to choose the most appropriate species-specific calibration equation for converting the wick "moistened length" to volume, shown in Tab. 2. For *Mertensia fusiformis* and *Delphinium nuttallianum*, we based the "moistened length" calibration conversion on mean nectar concentrations taken from near the RMBL (M. Stang, unpubl. res.). For those species with multiple nectar reservoirs (*Aconitum columbianum*, *Aquilegia* spp., *Delphinium* spp., and *Castilleja linariifolia*), we inserted a paper wick in each and summed the moistened lengths for total nectar volume per flower.

Removal-enhanced nectar replenishment

Following Castellanos et al. (2002), we tested for RENR by comparing flowers sampled only once to flowers sampled repeatedly. For each species, we selected at least 20 pairs of flowers that were matched for location on the plant and for developmental stage. We chose fresh flowers at ages when nectar volumes were near their maximum (see above,

FIGURE 1. Photos of the flowers of the 11 animal-pollinated plant species surveyed for removal-enhanced nectar replenishment (RENR) near the Rocky Mountain Biological Laboratory, Gothic, Colorado. Top row, left to right: *Mertensia ciliata, Mertensia fusiformis* (Boraginaceace), *Distegia involucrata* (Caprifoliaceae); second row, left to right: *Aconitum columbianum, Aquilegia coerulea, Aquilegia elegantula* (Helleboraceae (Ranunculaceae)); third row, left to right: *Delphinium barbeyi, Delphinium nuttallianum* (Helleboraceae), *Ipomopsis aggregata* (Polemoniaceae); fourth row, left to right: *Castilleja linariifolia, Linaria vulgaris* (Scrophulariaceae). Photo credits: *D. involucrata* by D. W. Inouye; *M. fusiformis*, and *L. vulgaris* by E. Y. Luo; remaining by J. E. Ogilvie.

TABLE 1. Floral-ecological characteristics of the study plant species. Nomenclature follows Weber & Wittman (2012), with previous names from Barrell (1969) in parentheses to clarify relationships to older literature. Flowers were considered in male phase if anthers were dehisced, and in female phase if styles were exposed. Common flower visitors are from personal observations by J.D.T. "Bees" typically include bumble bees and a variety of smaller native species (not *Apis mellifera*), with the bumble bees likely being the most effectual pollinators of these generally large flowers (except for *Mertensia fusiformis*, which is visited commonly and effectively pollinated by *Osmia* spp.; Forrest et al. 2011). "Birds" include the common hummingbirds *Selasphorus platycercus* and *S. rufus*. Flower visitors in parentheses are seen less commonly at the flowers.

Plant family Species	Duration of anthesis (d)			Duration of sex phases (range, d)		Common visitors	Time period studied (2011)
	N	Mean	SE	Male	Female		
Boraginaceae							
Mertensia ciliata	20	3.7	0.1	0 to 4	0 to 4	bees	July 15 - 22
Mertensia fusiformis	23	2.4	0.2	0 to 3	0 to 3	bees	June 13 - 16
Caprifoliaceae							
Distegia (*Lonicera*) *involucrata*	30	4.4	0.1	0 to 4	0 to 4	birds, (bees)	July 11 - 17
Helleboraceae (Ranunculaceae)							
Aconitum columbianum	20	6.4	0.2	2 to 6	4 to 6	bees	July 28 - August 9
Aquilegia coerulea (caerulea)	20	4.5	0.1	1 to 4	2 to 4	moths for nectar, bees for pollen	July 8 - 30
Aquilegia elegantula	20	4.0	0.2	1 to 4	2 to 4	birds	June 28 - July 3
Delphinium barbeyi	20	3.6	0.2	1 to 4	3 to 4	bees, (birds)	July 24 - August 3
Delphinium nuttallianum (nelsonii)	20	9.5	0.2	2 to 9	5 to 9	bees, (birds)	June 12 - 23
Polemoniaceae							
Ipomopsis aggregata	21	4.1	0.1	1 to 4	2 to 4	birds	June 28 - July 13
Scrophulariaceae							
Castilleja linariifolia	22	8.6	0.3	1 to 8	3 to 8	birds	July 1 - 24
Linaria vulgaris	20	4.4	0.1	0 to 4	0 to 4	bees	August 4 - 18

Fig. 2, and Tab. 2 for the flower ages tested for each species). In one flower of each pair, we extracted nectar repeatedly by blotting with filter paper wicks as above, at hourly intervals for five hours; in the other we extracted only once, at the end of five hours. Using wicks eliminates the possibility of estimating sugar content refractometrically (although wicks can be dried and stored for subsequent analysis of sugars if desired, see McKenna & Thomson 1988). However, we preferred wicks to glass capillaries for studying RENR because capillaries can easily damage delicate nectary tissues (see Willmer 1980, 2011). We drained the flowers before the experiment to exclude variation in standing nectar volume from the results. To ensure that our sampling method did not damage flowers, on the day after sampling, we checked to see if the flowers were still producing nectar. In most cases, the flowers had measurable nectar. We removed those occasional flowers that did not have nectar from our data set. We summed the five hourly nectar volume measurements from the removal flowers and compared them to the single volume measurement of the paired control flowers using paired *t*-tests. To test for biases in assigning flowers to the control and removal groups, we recorded the initial amounts drained and compared the means of two sample groups using paired *t*-tests. We carried out all analyses in R version 2.15.2 (R Core Team 2012).

Nectar replenishment rate

We examined how quickly nectar replenishment occurred in flowers. We used wicks to initially empty flowers and then re-sampled them at intervals, ranging from 5 to 140 minutes, and recorded the nectar volumes. We sampled these flowers at the same age as flowers tested for RENR, and we used the same nectar concentration to convert the moistened length of wick to volume as in the RENR sampling.

RESULTS

Floral scheduling

The duration of floral anthesis and the sex phases of each species are presented in Tab. 1. Overall, mean anthesis duration varied from 2.4 days in *Mertensia fusiformis* to 9.5 days in *Delphinium nuttallianum,* and in seven species the flowers were protandrous while the remaining four species had hermaphroditic flowers with no dichogamy (Tab. 1).

Nectar production over the floral lifetime

Accumulated nectar volumes typically showed marked variation among flowers at any particular flower age; occasional empty or nearly empty flowers were frequent in this group of species (Fig. 2). Inspection of the points in Fig. 2 shows that nectar volume per flower varied continuously from low levels near zero to highs that were characteristic for that species. There is no suggestion of discontinuous variation between discrete "full" and "empty" categories in any species. The most common pattern was for nectar volumes to increase with flower age (*Mertensia ciliata, M. fusiformis, Aconitum columbianum, Aquilegia coerulea,*

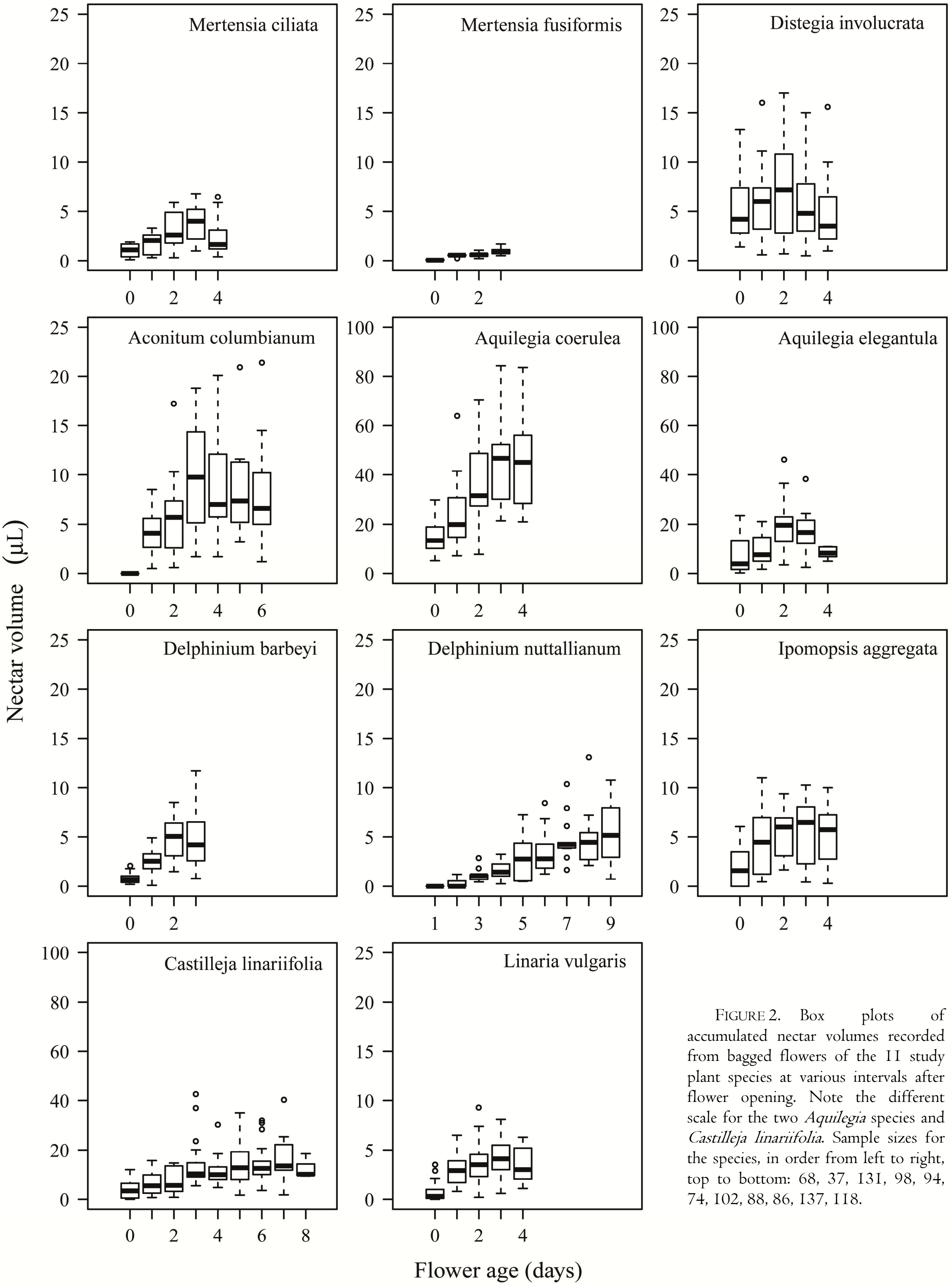

FIGURE 2. Box plots of accumulated nectar volumes recorded from bagged flowers of the 11 study plant species at various intervals after flower opening. Note the different scale for the two *Aquilegia* species and *Castilleja linariifolia*. Sample sizes for the species, in order from left to right, top to bottom: 68, 37, 131, 98, 94, 74, 102, 88, 86, 137, 118.

TABLE 2. Details of the nectar concentration and removal-enhanced nectar replenishment (RENR) sampling of the 11 study plant species. Nectar concentration was measured using microcapillary tubes while RENR was measured using filter paper wicks. The "calibration concentration" values were the concentrations used to calculate the species-specific calibration equation for converting the "moistened length" of wicks to nectar volume, informed by the mean nectar concentrations.

Plant species	Nectar concentration study		Removal-enhanced nectar replenishment study			
	N	Mean concentration (SE) (%w/w)	Calibration concentration (%w/w)	N pairs	Flower age sampled (d)	RENR ratio, 5 samples/single
Mertensia ciliata	22	18.5 (1.5)	20	29	2 – 3	2.88
Mertensia fusiformis	-	-	40	24	2	2.15
Distegia involucrata	53	18.9 (0.7)	20	25	2 – 3	1.52
Aconitum columbianum	21	51.5 (3.2)	50	20	3 – 5	1.62
Aquilegia coerulea	27	18.9 (0.7)	20	20	2 – 3	2.26
Aquilegia elegantula	24	31.3 (1.1)	30	20	2 – 3	1.29
Delphinium barbeyi	22	49.8 (2.0)	50	25	2	1.37
Delphinium nuttallianum	-	-	40	22	5 – 7	2.10
Ipomopsis aggregata	25	16.1 (2.2)	20	25	2 – 3	1.19
Castilleja linariifolia	53	18.7 (1.0)	20	24	3 – 7	2.55
Linaria vulgaris	24	40.9 (4.5)	40	25	2 – 3	1.57

Delphinium barbeyi, D. nuttallianum), producing roughly triangular spreads of nectar volumes. This pattern suggests continuing secretion of new nectar as a flower ages. In *A. columbianum, D. barbeyi* and *D. nuttallianum,* flower openings are sequential and highly structured within inflorescences, so that older flowers occupy lower positions, producing vertical gradients of nectar volumes (see Pyke 1978). In *Ipomopsis aggregata* and *Linaria vulgaris*, nectar volumes tended to be uniform across flower age, as if nectar secretion ceased after flower opening. *Castilleja linariifolia* showed an intermediate pattern in which nectar volume initially increased with flower age but subsequently levelled off. This species was sampled for more days than most others, probably because the colourful bracts persist; the observed levelling off may well have occurred after true anthesis had finished. In two species, *Aquilegia elegantula* and *Distegia involucrata,* nectar volumes peaked at intermediate flower ages, producing unimodal distributions suggestive of initial secretion followed by evaporation or reabsorption.

Removal-enhanced nectar replenishment

In all species except *Aquilegia elegantula* and *Ipomopsis aggregata,* flowers produced significantly more nectar when they were repeatedly drained compared to those sampled only once over the same time period (paired *t*-tests, $P < 0.05$; Fig. 3). For a quantitative index of RENR, we calculated a ratio of the total volume measured from the repeatedly sampled flowers to the total volume measured from the once-sampled control flowers (Tab. 2). Values range from 1.19 in *Ipomopsis aggregata* to 2.88 in *Mertensia ciliata* (Tab. 2). Thus, repeatedly draining a single flower tended to extract about twice the amount of nectar as a flower would accumulate over the same period if left unvisited. The nectar volumes removed from all flowers at the start of the 5-hour sampling period did not differ between removal and control groups for all species (paired *t*-tests, $P > 0.05$), indicating no bias in assigning the flowers to the two groups. Across the 11 study species, there was no correlation between mean or median nectar volumes at the focal flower ages and the RENR ratios (Spearman's rank correlation, $P > 0.05$).

Nectar replenishment rate

In Fig. 4, measures of nectar replenished during various accrual periods after initial emptying indicate the rate of nectar replenishment. Among species, the rate of replenishment generally increased with greater standing nectar volumes. Most flowers exhibited the ability to replenish measurable nectar in minutes rather than hours, suggesting that nectar replenishment occurs rapidly after emptying.

DISCUSSION

In most of our study species, flowers drained repeatedly produced 1.5 to 2.9 times more nectar than flowers drained only once, despite variation in species-specific maximum nectar volumes. Although we have expressed this property in terms of "replenishment," the same pattern has elsewhere been attributed to "reabsorption" (Nicolson 1995). The "reabsorption" viewpoint supposes that nectar is secreted continuously, but that some of it is reabsorbed unless it is removed at frequent intervals. Ascertaining the exact mechanism through which repeated withdrawals harvest more nectar than a single withdrawal would require careful physiological study, but from the standpoint of plant-pollinator relations, the mechanism is less important than the phenomenon. Our measurements suggest that researchers working on flowers adapted for large insect visitors like bumble bees should expect to find removal-enhanced replenishment of nectar; this phenomenon appears to be more the rule than the exception.

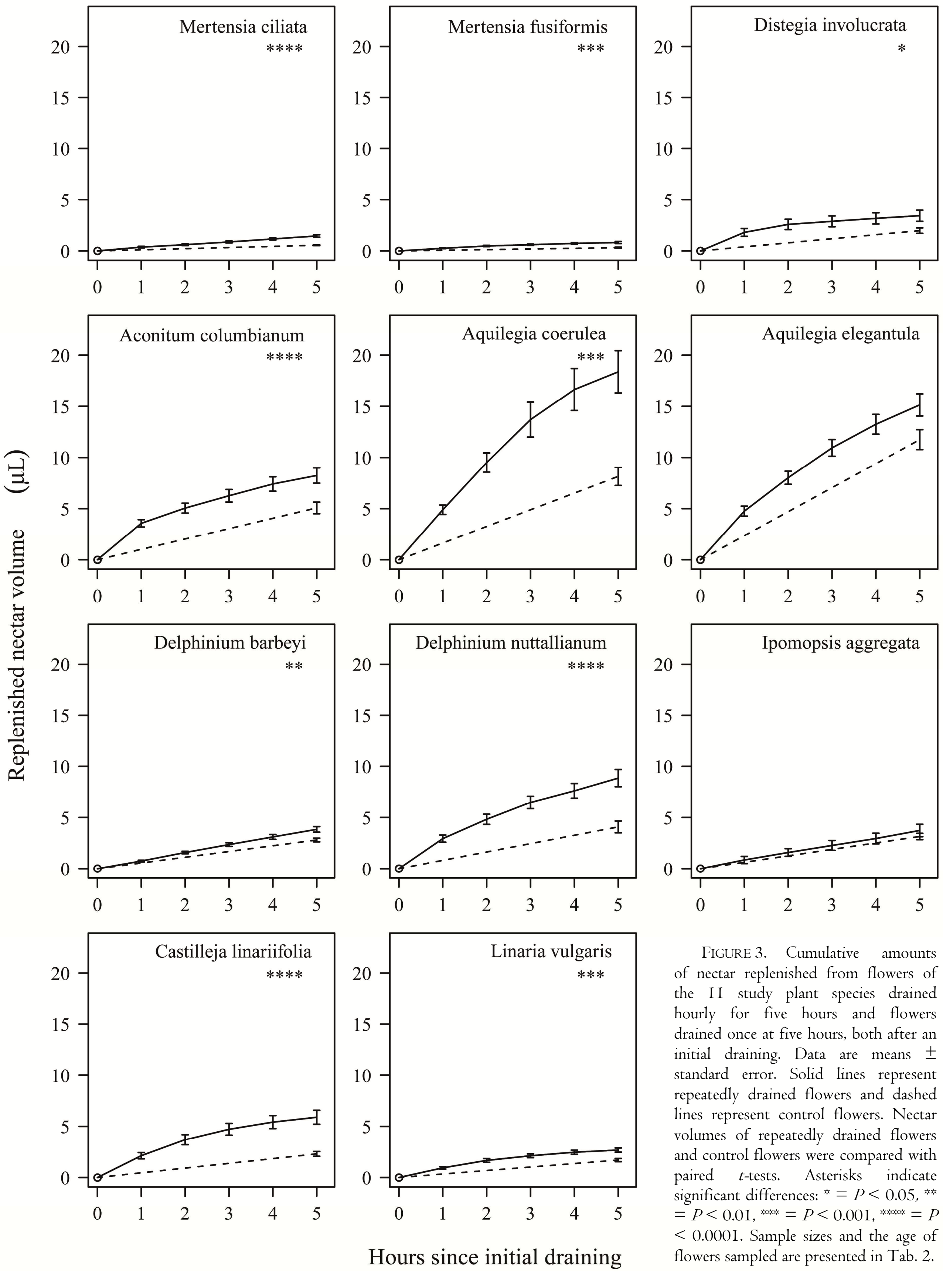

FIGURE 3. Cumulative amounts of nectar replenished from flowers of the 11 study plant species drained hourly for five hours and flowers drained once at five hours, both after an initial draining. Data are means ± standard error. Solid lines represent repeatedly drained flowers and dashed lines represent control flowers. Nectar volumes of repeatedly drained flowers and control flowers were compared with paired *t*-tests. Asterisks indicate significant differences: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Sample sizes and the age of flowers sampled are presented in Tab. 2.

RENR as a complication

Our findings serve to reinforce the strengthening consensus that measuring nectar is not as easy as some earlier treatments assumed (Zimmerman 1988; Willmer 2011). In most of the species we examined, the volume of nectar that flower visitors can extract depends on how many extractions are made. Furthermore, we found that one-time nectar volume measurements were not related to the extent of replenishing that was characteristic of a plant species. Removal-responsive replenishing by flowers must affect investigations of how much energy gain visitors can achieve by visiting a particular species, and how much metabolic cost a plant will incur by providing nectar. Nectar replenishment will be especially important in considering the economics of trapline foraging, in which flower visitors return to plants at frequent intervals and compete with each other to control the nectar flow. Ohashi and others (Ohashi & Thomson 2005; Ohashi et al. 2008) explored the consequences of such competition, theoretically and empirically, but those models did not consider RENR, and the laboratory apparatus used in those experiments produced fixed nectar secretion. Ohashi et al.'s qualitative conclusions regarding the general advantages of traplining would probably be robust, but the quantitative results would surely be modified in plants that display RENR (indeed, plants that are actively visited by traplining animals may be likely to show RENR).

An important caveat of our studies is that we examined how flowers responded to nectar removals by measuring nectar volume and not sugar content. Most plant species that show a significant increase in nectar volume in response to nectar removal also show an increase in total sugar production (e.g., Navarro 1999; Nicolson & Nepi 2005; Ornelas & Lara 2009; Bobrowiec & Oliveira 2012; Amorim et al. 2013, though see Galetto et al. 1994). If sugar concentration remains approximately the same in extracted samples, total sugar content produced by flowers will increase in response to removal (e.g. Navarro 1999). However, we cannot assume that flowers of all species and under varied environmental conditions will replenish their nectar at a constant sugar concentration. Even ignoring environmental effects such as evaporative concentration or hygroscopic dilution, it is easy to imagine that a flower's replenished nectar might be more dilute than the first nectar secreted by that flower. Indeed, in flowers of *Penstemon speciosus*, water seemed to replenish more quickly than sugar (Castellanos et al. 2002). Nectar concentration may be controlled by passive diffusion, while nectar volume may be controlled by hydrostatic pressure differences (Pacini & Nepi 2007). If sugar diffusion is slower than replenishment of water, nectar concentrations may be lower than normal shortly after removal. In addition, continual replenishment of nectar incurs a metabolic cost that can lower female reproduction (Pyke 1991; Ordano & Ornelas 2005; Ornelas & Lara 2009); reducing nectar sugar concentration in situations with continuous visitation may compensate for this cost.

If newly secreted nectar is more dilute, the RENR rate measured for nectar volume (as in our study) would overestimate the rate for sugar content. For questions that hinge on energy content, it would be critical to supplement the sort of measurements we made with additional measurements of sugar concentrations. There is a further complication, too: if replenished nectar is considerably more dilute, it will change the relationship between the moistened length of a paper wick and the volume of solution taken up. Instead of a single regression equation, one would need to devise a more complicated function to account for trends in concentration.

Residual nectar and RENR

Foraging bumble bees frequently leave behind residual nectar in visited flowers (Hodges & Wolf 1981; Zimmerman 1983; Cresswell 1999). Since nectar production may be mediated by a hydrostatic pressure difference (Pacini & Nepi 2007), residual nectar may lower the rate of nectar replenishment in nature compared to that experienced from experimental removal, which might deplete nectar more completely (e.g., our study). This question hinges on how well blotting with filter-paper wicks compares to extraction by flower visitors. If wicks remove more nectar, the rate and metabolic costs of replenishment (Ordano & Ornelas 2005; Ornelas & Lara 2009) may be overestimated in experimental studies relative to natural conditions.

RENR as an object of study

Aside from the negative message that RENR complicates the quantification of nectar energetics, the variation that we observed across species hints that RENR might be an informative trait for comparative studies of flower function. In general, we might expect that the extent and rate of RENR will covary with pollination system. In a parallel example, Castellanos et al. (2006) argued that species of *Penstemon* that were adapted for pollination by hummingbirds differed in their schedules of pollen presentation from those adapted for pollination by hymenopterans. The adaptive function of more gradual pollen presentation by bee-adapted flowers was hypothesized to arise from the grooming behaviour of the bees, which imposed diminishing returns on the relationship between pollen presentation and subsequent delivery. Diminishing returns mean that bee-adapted flowers will achieve the most pollen export if they can present small doses of pollen to many visitors (Harder & Thomson 1989; Thomson & Thomson 1992). In accordance with this prediction, bee-adapted species have anthers that open less widely than those of related bird-adapted species (Castellanos et al. 2006). However, the potential advantages of gradual pollen presentation cannot be realized without a high visitation rate. Therefore, we would predict that bee-adapted flowers would show higher RENR than bird-adapted relatives, because greater nectar replenishment should encourage heavier visitation.

Ideally, such comparisons should be made among near-sister taxa within a strict phylogenetic framework, as in the *Penstemon* anther study. Our opportunistic survey does not allow a rigorous test of the prediction, but there are suggestive indications that the hypothesis is worth pursuing. Consider the eleven species in Fig. 3. Of the three species

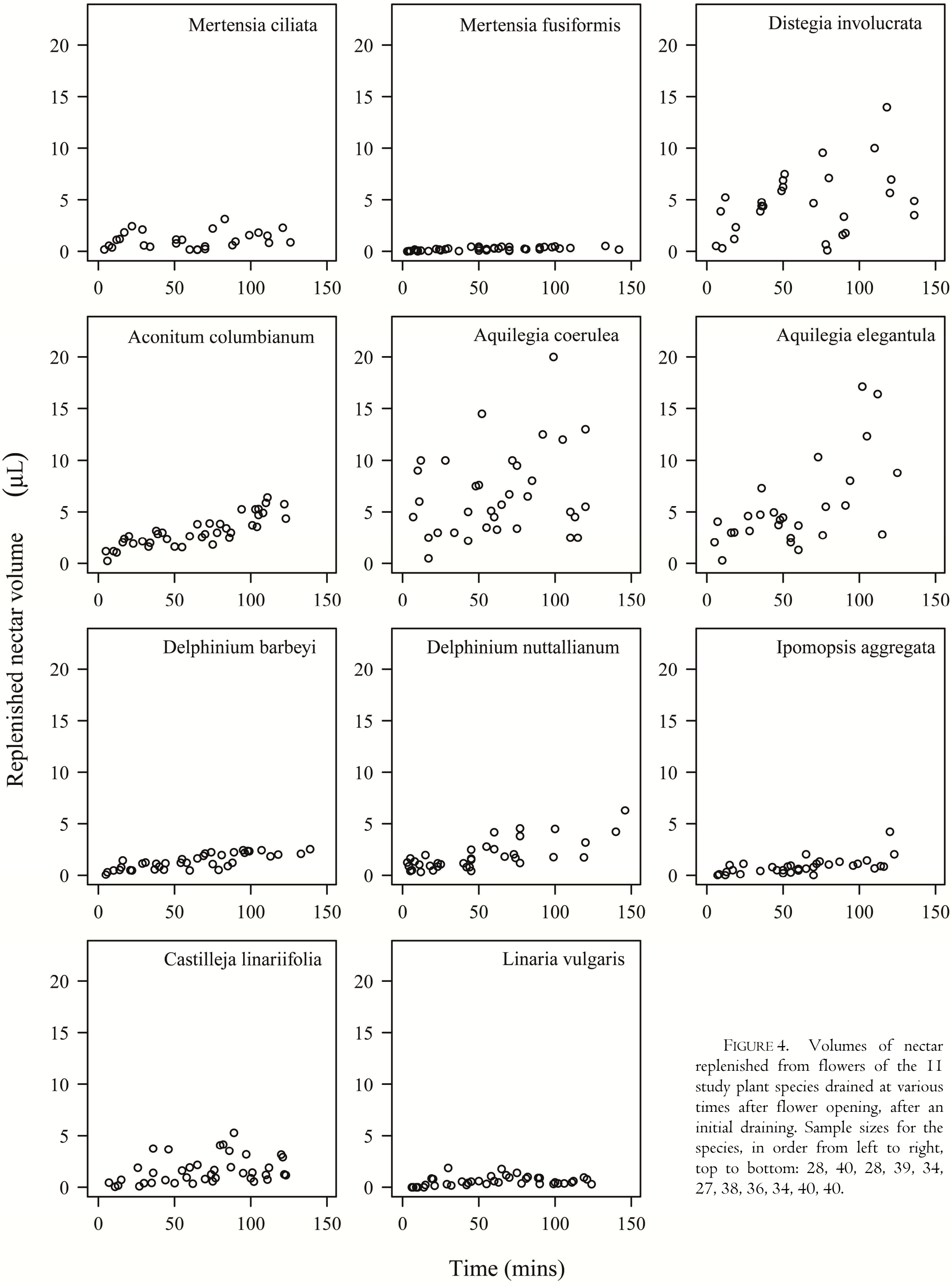

FIGURE 4. Volumes of nectar replenished from flowers of the 11 study plant species drained at various times after flower opening, after an initial draining. Sample sizes for the species, in order from left to right, top to bottom: 28, 40, 28, 39, 34, 27, 38, 36, 34, 40, 40.

most exclusively visited by hummingbirds, two (*Ipomopsis aggregata* and *Aquilegia elegantula*) have the lowest RENR values in the study. Pleasants (1983) also found that nectar production in *I. aggregata* flowers was not stimulated by its removal. The next two lowest values are *Distegia involucrata* and *Delphinium barbeyi*, both of which receive many bird visitors in addition to bees. The only primarily bird-visited species to show high RENR is an Indian paintbrush, *Castilleja linariifolia*. Unfortunately, we lack data from any of the local bee-visited paintbrushes (*C. sulphurea* or *C. occidentalis*), so we cannot tell whether their RENR might be even higher. Nevertheless, there is variation in the RENR ability of bird-visited plants in different genera: some show decreased nectar production in response to nectar removals (e.g., Gill 1988; Bernardello et al. 1994; Rivera et al., 1996; McDade & Weeks 2004; Carlson 2007), others increased production (e.g., Nicolson & Nepi 2005; Ornelas & Lara 2009), and others have similar nectar production to controls (e.g., McDade & Weeks 2004). Pursuing this hypothesis would seem to be more worthwhile within a single genus like *Penstemon* that shows numerous bee-to-bird transitions that can be phylogenetically localised.

Conclusion

Our study confirms that replenishment of nectar volume is commonly a rapid, dynamic process that may be mediated by the frequency of pollinator visits. It appears that most of our study species possessed some ability to regulate replenishment, demonstrating RENR. A full understanding of secretion dynamics would require coordinated investigation of sugar concentration changes in addition to replenishment of volume, but such measurements were beyond the scope of this study. It would be interesting to see if our hypothesized bee-bird difference holds up in more comparisons of related flower species adapted to different pollinators. Potentially common RENR by plants complicates key questions in plant-pollinator interactions, though further investigation will help illuminate these questions.

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REFERENCES

Amorim FW, Galetto L, Sazima M (2013) Beyond the pollination syndrome: nectar ecology and the role of diurnal and nocturnal pollinators in the reproductive success of *Inga sessilis* (Fabaceae). Plant Biology 15:317-327.

Barrell J (1969) Flora of the Gunnison Basin: Gunnison, Saguache, and Hinsdale counties, Colorado. Natural Land Institute, Rockford.

Bernardello L, Galetto L, Rodriguez IG (1994) Reproductive biology, variability of nectar features and pollination of *Combretum fruticosum* (Combretaceae) in Argentina. Botanical Journal of the Linnean Society 114:293-308.

Bobrowiec PED, Oliveira PE (2012) Removal effects on nectar production in bat-pollinated flowers of the Brazillian Cerrado. Biotropica 44:1-5.

Burquez A, Corbet SA (1991) Do flowers reabsorb nectar? Functional Ecology 5:369-379.

Carlson JE (2007) Male-biased nectar production in a protandrous herb matches predictions of sexual selection theory in plants. American Journal of Botany 94:674-682.

Castellanos MC, Wilson P, Thomson JD (2002) Dynamic nectar replenishment in flowers of Penstemon (Scrophulariaceae). American Journal of Botany 89:111-118.

Castellanos MC, Wilson P, Wolfe A, Keller S, Thomson JD (2006) Anther evolution: pollen presentation strategies when pollinators differ in efficiency. American Naturalist 167:288-296.

Cresswell JE (1999) The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). Journal of Ecology 87:670-677.

Cruden RW, Hermann SM (1983) Studying nectar? Some observations on the art. In: Bentley B, Elias T (eds) The biology of nectaries. Columbia University Press, New York, pp 223–241.

Forrest JRK, Ogilvie JE, Gorischek AM, Thomson JD (2011) Seasonal change in a pollinator community and the maintenance of style length variation in *Mertensia fusiformis* (Boraginaceae). Annals of Botany 108:1-12.

Galetto L, Bernardello G, Rivera GL (1997) Nectar, nectaries, flower visitors, and breeding system in five terrestrial Orchidaceae from Central Argentina. Journal of Plant Research 110:393-403.

Galetto L, Bernardello LM, Juliani HR (1994) Characteristics of secretion of nectar in *Pyrostegia venusta* (Ker-Gawl.) Miers (Bignoniaceae). New Phytologist 127:465-471.

Gill FB (1988) Effects of nectar removal on nectar accumulation in flowers of *Heliconia imbricate* (Heliconiaceae). Biotropica 20:169-171.

Harder L, Thomson JD (1989) Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. American Naturalist 133:325-334.

Heinrich B (1983) Insect foraging energetics. In: Jones CE, Little RJ. Handbook of experimental pollination biology. Scientific and Academic Editions, New York, pp 187-214.

Herrera CM (1990) Daily patterns of pollinator activity, differential pollinating effectiveness, and floral resource availability, in a summer-flowering Mediterranean shrub. Oikos 58:277-288.

Hodges CM, Wolf LL (1981) Optimal foraging in bumblebees: why is nectar left behind in flowers? Behavioural Ecology and Sociobiology 9:41-44.

McDade LA, Weeks JA (2004) Nectar in hummingbird-pollinated neotropical plants II: Interactions with flower visitors. Biotropica 36:216-230.

McKenna M, Thomson JD (1988) A technique for sampling and measuring small amounts of floral nectar. Ecology 69:1036-1037.

Munguía-Rosas MA, Sosa VJ, Jácome-Flores ME (2009) Pollination system of the *Pilosocereus leucocephalus* columnar cactus (tribe Cereeae) in Eastern Mexico. Plant Biology 12:578-586.

Navarro L (1999) Pollination ecology and effect of nectar removal in *Macleania bullata* (Ericaceae). Biotropica 31:618-625.

Nepi M, Cresti L, Guarnieri M, Pacini E (2011) Dynamics of nectar production and nectar homeostasis in male flowers of *Cucurbita pepo* L. International Journal of Plant Sciences 172:183-190.

Nicolson SW (1995) Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). Functional Ecology 9:584-588.

Nicolson SW, Nepi M (2005) Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). International Journal of Plant Sciences 166:227-233.

Ohashi K, Leslie A, Thomson JD (2008) Trapline foraging by bumble bees: V. Effects of experience and priority on competitive performance. Behavioural Ecology 19:936-948.

Ohashi K, Thomson JD (2005) Efficient harvesting of renewing resources. Behavioural Ecology 16:592-605.

Ordano M, Ornelas JF (2004) Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. Oecologia 140:495-505.

Ordano M, Ornelas JF (2005) The cost of nectar replenishment in two epiphytic bromelialds. Journal of Tropical Ecology 21:541-547.

Ornelas JF, Lara C (2009) Nectar replenishment and pollen receipt interact in their effects on seed production of *Penstemon roseus*. Oecologia 160:675-685.

Ornelas JF, Ordano M, Lara C (2007) Nectar removal effects on seed production in *Moussonia deppeana* (Gesneriaceae), a hummingbird-pollinated shrub. Ecoscience 14:117-123.

Pacini E, Nepi M (2007) Nectar production and presentation. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar. Springer, Netherlands, pp 167-214.

Pleasants JM (1983) Nectar production patterns in *Ipomopsis aggregata* (Polemoniaceae). American Journal of Botany 70:1468-1475.

Price MV, Waser NM, Irwin RE, Campbell DR, Brody AK (2005) Temporal and spatial variation in pollination of a montane herb: A seven-year study. Ecology 886:2106-2116.

Pyke GH (1978) Optimal foraging in bumblebees and their coevolution with their plants. Oecologia 36:281-293.

Pyke GH (1991) What does it cost a plant to produce floral nectar? Nature 350:58-59.

R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rivera GL, Galetto L, Bernardello L (1996) Nectar secretion pattern, removal effects, and breeding system of *Ligaria cuneifolia* (Loranthaceae). Canadian Journal of Botany 74:1996-2001.

Thomson JD, Forrest JRK, Ogilvie JE (2011) Pollinator exclusion devices permitting easy access to flowers. Journal of Pollination Ecology 4:24-25.

Thomson JD, Thomson BA (1992) Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. In: Wyatt R (ed) Ecology and evolution of plant reproduction: New approaches. Chapman and Hall, New York, pp 1-24.

Veiga Blanco T, Galetto L, Machado IC (2013) Nectar regulation in *Euphorbia tithymaloides* L., a hummingbird-pollinated Euphorbiaceae. Plant Biology 15:910-918.

Waser NM (1982) A comparison of distances flown by different visitors to flowers of the same species. Oecologia 55:251-257.

Weber WA, Wittmann RC (2012) Colorado flora: Western slope. University Press of Colorado, Boulder.

Williams NM, Thomson JD (1998) Trapline foraging by bumble bees: III. Temporal patterning of visits. Behavioral Ecology 9:612-621.

Willmer PG (1980) The effects of insect visitors on nectar constituents in temperate plants. Oecologia 47:270-277.

Willmer P (2011) Pollination and floral ecology. Princeton University Press, Princeton.

Zimmerman M (1983) Calculating nectar production rates: residual nectar and optimal foraging. Oecologia 58:258-259.

Zimmerman M (1988) Nectar production, flowering phenology, and strategies for pollination. In: Lovett Doust J, Lovett Doust L (eds) Plant reproductive ecology: patterns and strategies. Oxford University Press, Oxford, pp 157-178.