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SEX DIFFERENTIAL NECTAR SECRETION IN PROTANDROUS *ALSTROEMERIA AUREA* (ALSTROEMERIACEAE): IS PRODUCTION ALTERED BY POLLEN REMOVAL AND RECEIPT?[1]

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We examined diurnal and nocturnal nectar secretion across sexual stages in protandrous *Alstroemeria aurea*, a bumble bee-pollinated herb with long-lived flowers native to the southern Andes. We found the following patterns: (1) most nectar was produced diurnally and (2) three times more sugar was secreted during the male than female phase, not only because the male phase lasted longer but also because the rate of nectar production was higher. This 3:1 ratio in nectar production matched the ratio of the minimum number of bumble bee visits required on average to saturate male (pollen removal) vs. female (seed set) functions. Standing crop of nectar, on the other hand, did not differ greatly between male- and female-stage flowers left open to visitors, because the high-production male-phase flowers were visited more frequently than female-phase flowers. In an experiment concurrent with the repeated nectar sampling of individual flowers over their life-span, we removed pollen from anthers or deposited pollen on stigmas by hand. Neither treatment, designed to mimic effects of visits by *Alstroemeria*'s native bumble bee pollinator, affected nectar production. The absence of plasticity in nectar secretion in relation to pollination events may reflect a low cost of nectar production, or may result from developmental constraints related to the evolution of the synchronous protandry that characterizes *A. aurea.*

Key words: *Alstroemeria aurea*; Alstroemeriaceae; bumble bees; male and female functions; nectar secretion; plasticity; pollen removal and deposition; protandry.

In most outcrossing plants, nectar is the principal pollinator reward (Simpson and Neff, 1983). Nectar levels often vary widely among flowers of a given plant species or individual. Pollinators alter their foraging behavior in response to such variation in nectar levels, and in turn foraging behavior may determine plant fitness (reviewed in Rathcke, 1992). In natural populations, variation in nectar secretion may result partly from external environmental conditions (e.g., Zimmerman and Pyke, 1988; Wyatt, Broyles, and Derda, 1992; Herrera, 1995) as well as from factors intrinsic to the plant (e.g., Pleasants and Chaplin, 1983; Willson and Ågren, 1989; Hodges, 1993). Whereas the response of nectar secretion to external factors such as temperature, light, and humidity may to a large extent reflect the restrictions these variables impose on plant metabolism, variation in nectar secretion associated with intrinsic flower traits may represent the evolutionary response to diverse selective pressures imposed by the requirements of disparate pollinator regimes. For instance, many plant species signal the lack of nectar in flowers that are already pollinated, but nonetheless retained, with color changes of some flower parts (Gori, 1989; Weiss, 1991; Lunau, 1996). Pollinators in turn respond to these signals by bypassing those flowers and visiting rewarding, presumably nonpollinated ones. More subtle is the evolutionary meaning of variation in nectar production associated with changes in flower sexuality.

Hermaphroditic flowers have two distinct functions: the dispersal of pollen from the anthers to the stigmas of other compatible flowers (male function), and the receipt of compatible pollen on the stigma to set seed (female function). In animal-pollinated species, the number and temporal distribution of pollinator visits needed to maximize reproductive success through each of the two functions are apt to differ (Lloyd and Yates, 1982; Harder and Thomson, 1989; Willson, 1994; Wilson et al., 1994). In general, it has been argued that a greater number of visits is needed to maximize reproductive success through male than through female function. In fact, higher rates of nectar production have been recorded in staminate (male) than pistillate (female) flowers in many diclinous species, and during the staminate phase in species with hermaphroditic flowers but with temporal separation of sexual phases (e.g., Bawa, 1980; Bell et al., 1984; Devlin and Stephenson, 1985; Klinkhamer and de Jong, 1990; Delph and Lively, 1992).

Here, we describe variation in nectar secretion rates over flower life-span in *Alstroemeria aurea* Graham, a south Andean perennial herb with long-lived, protandrous flowers, i.e., flowers that change sex temporally from male to female. We estimate the minimum number of

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pollinator visits needed to saturate male (pollen removal) and female (seed set) functions, respectively, and measure the response of flower visitors to sex-related differences in nectar secretion rates. Because not only the general nectar production pattern but also its plasticity may have been molded by sexual selection, we next ask whether pollen removal and receipt modify basic nectar production patterns. If nectar production is costly and conflicts with other plant functions (Southwick, 1984; Búrquez and Corbett, 1991; Pyke, 1991; but see Bazzaz, Carlson, and Harper, 1979; Harder and Barrett, 1992), then nectar-saving mechanisms may have evolved, with rapid responses to either male or female function. For example, pollen removal and receipt could reduce nectar production during either the male or female phase, respectively, by (1) shortening the duration of that phase or by (2) inducing a decrease in nectar secretion rates during that phase (Cruden, Hermann, and Peterson, 1983; Devlin and Stephenson, 1984; Richardson and Stephenson, 1989). We test for the existence of these two possible, nonexclusive mechanisms.

MATERIALS AND METHODS

Study species and research site—*Alstroemeria aurea,* the amancay, is a clonal plant characteristic of the understory of temperate forests of South America. It reproduces vegetatively by rhizome branching and fragmentation, and sexually via seeds. Here we summarize its major floral features (see details in Aizen and Basilio, 1995).

Each growing season, a plant produces a series of vegetative shoots and usually one flowering shoot, the latter consisting of a leafy stem and a terminal umbelliform inflorescence composed of 1–8 protandrous flowers, which open and change sex synchronously (synchronous protandry). Primary pedicels may branch and produce a second-order, nonoverlapping cycle of flowers. Flowers are large (>5 cm), yellow-orange, and zygomorphic. Nectar is produced at the base of the two inner, upper tepals, which are streaked with reddish nectar guides. One to two anthers, out of a total of six, dehisce daily over a period of ~ 4 d total. Dehiscing anthers occupy a central position within the flower, where they deposit pollen on a pollinator's back, usually as it leaves the flower. Typically after ~ 1 d, dehisced anthers are displaced downwards out of reach of pollinators and the stigma. After the end of the male phase, the flower goes through a ~1-d long neuter phase in which the last anthers shrivel and the style elongates. The start of the female phase is signaled by the spreading of the three stigmatic branches. Flowers remain female for ~3 d before withering. Although sexual stages intergrade in a continuum, flowers may be easily assigned to one of eight different developmental categories each ~1 d long (Aizen and Basilio, 1995): stage 0, premale phase [flowers having just opened, no dehisced anthers]; stage I, young male phase [1–2 dehisced anthers]; stage II, intermediate male phase [3–4 dehisced anthers]; stage III, advanced male phase [5–6 dehisced anthers]; stage IV, neuter phase; stage V, young female phase [stigmatic branches spreading out and start of stigma receptivity]; stage VI, mature female phase [stigmatic branches completely spread]; stage VII, postfemale phase [flowers present signs of senescence such as the curling of the nectariferous tepals].

Bombus dahlbomii, the only bumble bee species native to the south Andean temperate forest, is the most frequent (>90% of all visits) and efficient pollinator of *A. aurea* (M. A. Aizen, unpublished data). Bumble bees forage primarily for nectar in *A. aurea,* discriminating among flowers based on their nectar rather than pollen content.

We conducted this study in a large *A. aurea* population in the upper Challhuaco valley, Nahuel Huapi National Park, Argentina (41°8′S, 71°19′W), in the 1994 austral summer. This population occurs underneath an old-growth *Nothofagus pumilio* forest. Here, > 75% of flowering shoots produce simple umbels by early abortion of secondary or higher order flower buds. In this study, we sampled only those shoots with simple inflorescences, each with 3–5 flowers, the most common inflorescence type.

Regional meteorological data for the 1994 flowering period were obtained from the nearest station at the Instituto Nacional de Tecnología Agropecuaria, EEA Bariloche, 25 km north of the study site.

Nectar sampling—We measured nectar volume by inserting a 4-μL microcapillary tube repeatedly in the two nectaries until no further nectar could be extracted. Sugar concentration, in sucrose equivalence units, was determined with a temperature-compensated hand refractometer (Reichter, Model 10431, Leica Inc., Buffalo, New York). All nectar measurements were made between 13 January and 13 February.

We used two different protocols to measure nectar production across sexual stages. In the ''horizontal'' developmental series, we sampled two flowers per inflorescence repeatedly over their entire life. Through the flowering season, we chose a total of six groups of four flowering shoots located near to each other (nearest neighbor distances 20–50 cm) matched by number of flowers, overall height, and floral developmental stage (~1–2 d before flowers of all four shoots were due to open). We bagged inflorescences with 1-mm mesh mosquito netting. Nectar was sampled at ~12-h intervals, at 0600–0800 and 1800–2000, beginning at anthesis. Early morning samples represented nighttime secretion (including the nectar secreted just at dusk and dawn), and evening samples represented daytime secretion. At each sampling time and for each flower, we recorded the developmental stage with respect to the eight stages described above. In addition, all flowers of a given inflorescence, including the two sampled for nectar, received one of the following treatment combinations of pollen removal and stigmatic pollen deposition: (1) no pollen manipulation, (2) pollen removed only, (3) pollen deposited only, and (4) pollen both removed and deposited. We assigned a different treatment to each shoot of a given group of four (i.e., 4 treatments x 6 replicates). Every time inflorescences were uncovered for nectar sampling, pollen from newly dehisced anthers was carefully and thoroughly removed with a toothpick throughout the male phase (treatments 2 and 4), and pollen collected from several donors, at least 10 m apart, was deposited on the stigma throughout the female phase beginning with stage V (treatments 3 and 4). These treatments result in two events of pollen removal and deposition per day over a flower's male and female phase, respectively, which are well within the range of daily visitation frequencies by bumble bees.

Because prolonged bagging, repeated artificial sampling, and changing environmental conditions may introduce artifacts into nectar measures (reviewed in Rathcke, 1992), we also applied a second sampling protocol. In the ''vertical'' developmental series, we simultaneously bagged groups of eight flowering shoots growing close to each other (under similar microenvironmental conditions) matched by flower number and height. Each shoot of a group was at the start of one of the eight different sexual stages defined above. We sampled a total of 44 groups during the flowering season. Half of the groups were bagged by 0700 and half by 1900, in an alternating fashion. After removing the standing nectar of two flowers per inflorescence, we measured nectar 12 and 24 h later.

We measured extant nectar in flowers open to animal visitors (nectar standing crop) at approximately noon on nine different days over the flowering season. Nectar was haphazardly sampled in flowers encompassing all eight different developmental stages, along 20-m transects.

Pollen removal, pollen deposition, and visit frequencies—Between 5 February and 15 February, at the peak flowering period, we offered a total of 74 male-phase flowers with one recently dehisced, untouched anther to freely foraging bumble bees. After a given number (0–6) of visits over the next 4 h, we collected the anthers and kept each in a 0.5-mm microcentrifuge tube containing 70% ethyl alcohol. The number of pollen grains remaining in each anther was estimated from two

aliquots using a hemacytometer (see details in Aizen and Raffaele, 1996). In similar fashion, we offered a total of 122 virgin female-phase flowers to freely foraging bumble bees. After a given number (0–8) of visits, stigmas were excised, mounted on a slide, and stained with Alexander's solution (Alexander, 1980). Pollen was counted under a stereoscopic microscope at 100×.

From a total of 207 open-pollinated flowers from 50 ramets, we collected stigmas at flower senescence and counted pollen. Stigma excision at this stage does not affect either fruit or seed set (M. A. Aizen, personal observation). We tagged flowers, collected capsules 6 wk later, counted seeds, and related seed output to initial pollen load (e.g., Snow, 1982; Waser and Fugate, 1986; Galen and Newport, 1988).

Over the 1994 flowering season, we recorded bumble bee visits to *A. aurea* during 172 periods, each lasting 10 min (3–5 observation periods per day between 0900 and 1800). At the start of each observation period, we enclosed 100–400 flowers in a 2 × 2 m quadrat frame, counted the number of male- and female-phase flowers thus included and then recorded the total number of male- and female-phase flowers visited by bumble bees during the ensuing period. We excluded flowers in stage 0 or VII from these counts, because bumble bees rarely visit just opened or senescent flowers. Because we could not clearly distinguish the degree of stigma development from our observation posts, neuter-phase (stage IV) flowers were counted as female-phase flowers. From these observations, we estimated the number of visits to male- and female-phase flowers per hour.

Data analysis—We analyzed three variables characterizing nectar production and standing crop: nectar volume, sugar concentration, and their product, nectar sugar content. The two volume and concentration readings for each ramet at each sampling time were averaged before analysis. Sugar concentration readings in sucrose equivalents (i.e., 100 x mg solute / mg solution) were converted to milligrams of solute per microlitre by using tabulated values in Kearns and Inouye (1993, p. 172) and combined with volume values to estimate nectar sugar content in mg.

We used a split-plot ANOVA (Mead, 1988) for the horizontal series data to analyze the effect of time period (day vs. night) on nectar secretion rate. All night- and daytime nectar measurements for each ramet over its life-span were averaged and these averages considered as individual observations. ''Treatment'' (i.e., pollen manipulation) was considered as the whole-plot factor and ''time period'' (i.e., day or night) as the split-plot factor. For the vertical series, we averaged all night- and daytime nectar measurements across the eight ramets of each block and analyzed the effect of time period using a randomized complete block ANOVA.

To analyze the effect of sexual stage on average daytime nectar production (similar qualitative results were obtained if the small amounts of nectar produced at night were included, see Results) and standing crop, we used a split-plot ANOVA for the horizontal series (''treatment,'' whole-plot, ''sexual stage,'' split-plot factor) and a randomized block ANOVA for the vertical series and standing crop measurements. For the standing crop we averaged all measurements for flowers in the same sexual stage measured in the same day, considering ''day'' as the blocking factor. For the horizontal series, we averaged the measurements corresponding to the same sexual stage (although most commonly the ramet remained only one day in a given sexual phase). The sum of squares associated with the ''sexual stage'' main factor was decomposed in three a priori orthogonal contrasts (Sokal and Rohlf, 1981). We compared nectar secretion and standing crop (1) between premale and postfemale (0 and VII) vs. intermediate sexual stages (all others), (2) between the intermediate neuter stage (IV) vs. sexually active stages (I, II, III, V, and VI), and (3) between the sexually active male phase (stages I, II, and III) vs. the active female phase (stages V and VI).

To analyze the effect of pollen removal and deposition treatments on flower life-span and total nectar production (volume and sugar content), we used a randomized complete block ANOVA in which we included treatment as the main factor and the four-ramet clusters as the blocking factor. The effect of treatment on the span of the active male (stages I–III) and female (stages V and VI) sexual phases as well as on the nectar accumulated over those phases (i.e., summed over the days that flowers stayed in each of those two phases) was further analyzed by a split-plot ANOVA. ''Treatment'' (i.e., pollen manipulation) was considered as the whole-plot factor, and ''sexual phase'' (i.e., male or female) as the split-plot factor. Within the ''treatment × sexual phase'' interaction term, we tested two different hypotheses through the construction of orthogonal contrasts. These hypotheses were that (1) pollen removal (treatments 2 and 4) did affect the span of the male phase and/or the total amount of nectar produced during that phase, and that (2) stigmatic pollen deposition (treatments 3 and 4) did affect the span of the female phase and/or the total amount of nectar produced during that phase.

For sugar concentration, we based significance tests on Type III sums of squares (SAS, 1988) because data sets were unbalanced. Nectar volumes and sugar content were $\log(x+1)$-transformed before statistical analysis to increase normality and homoscedasticity. Untransformed means (± 1 SE) are reported, however, for the sake of clarity.

The relationships of number of pollen grains remaining per anther to number of pollen visits, and of number of seeds per flower to number of pollen grains deposited, were characterized by negative exponential curves of the form $Y = a\ \exp(-bX) + c$ (Eq. 1) and $Y = a\ [1-\exp(-bX)]$ (Eq. 2), respectively (procedure NLIN; SAS, 1988). A constant was added to the first equation to improve fit and to account for an observed unremovable fraction of pollen remaining, regardless of the number of visits. Coefficients of determination for these nonlinear fits were larger than for linear fits (Eq. 1, $r^2 = 0.50$ vs. 0.39; Eq. 2, $r^2 = 0.36$ vs. 0.13). The relationship between number of pollen grains deposited on the stigma and number of visits was fitted by a simple linear equation, $Y = a + bX$ (Eq. 3), because there was no evidence, at least within the study range of visits, that this relationship converges on an asymptote.

From Eq. 1, we estimated the number of visits required to remove 90% of the removable pollen per anther (male function), and from Eqs. 2 and 3, the average number of visits required to deposit sufficient pollen to produce 90% of the average number of seeds per flower expected under unlimited pollination (female function). For female function, this calculation assumes that larger pollen loads provide no extra benefits in terms of seed quality due to pollen competition or selective seed maturation (Mulcahy, 1979; Lee, 1984; Marshall and Folsom, 1991).

We analyzed data on visit frequencies using a randomized-block ANOVA. Sexual phase (male or female) was included as the main factor and observation period as a blocking factor.

RESULTS

Both sampling methods (horizontal and vertical) showed that most nectar in *A. aurea* is produced diurnally. The volumes of nectar secreted during daylight hours were not only much higher (X ± 1 SE = 0.56 ± 0.075 vs. 0.12 ± 0.025 μL, $F_{1,20} = 49.4$, $P < 0.0001$ for the horizontal series, and 0.54 ± 0.047 vs. 0.15 ± 0.027 μL, $F_{1,43} = 65.5$, $P < 0.0001$ for the vertical series), but sugar concentration also tended to be higher (27.4 ± 0.87 vs. 21.3 ± 3.36% sucrose equivalents, $F_{1,13} = 2.69$ $P = 0.12$ for the horizontal series, and 36.7 ± 1.07 vs. 27.1 ± 1.72%, $F_{1,43} = 25.08$, $P < 0.0001$ for the vertical series). This resulted in fivefold differences between day and night in the amount of sugar secreted (0.14 ± 0.016 vs. 0.03 ± 0.006 mg for the horizontal series, and 0.23 ± 0.020 vs. 0.04 ± 0.007 mg for the vertical series). Maximum daily temperature also affected diurnal nectar sugar secretion in *A. aurea* (averaged over the eight ra-

TABLE 1. Summary of *F* statistics and significance levels for ANOVAs testing the effect of sexual stage on average daytime nectar production (A, horizontal series; B, vertical series) and standing crop (C). The ''treatment'' factor (i.e., all 2 × 2 combinations of hand-pollen removal and stigmatic deposition) in (A) was tested over the ''block × treatment'' term (i.e., the ''$error_1$'' term, see Data analysis). The ''sexual stage'' factor was decomposed in three orthogonal contrasts: (1) premale (PM) and postfemale (PF) stages vs. intermediate stages (M, N, F), (2) sexually active stages (M, F) vs. intermediate neutral stage (N), and (3) active male (M) vs. female (F) stages. Nectar volume and sugar secreted were log(x + 1)-transformed.

	Nectar volume (μL)		Sugar concentration (%)		Nectar sugar (mg)	
	df	*F*	df	*F*	df	*F*
A) Horizontal series: nectar production						
Treatment	3	1.72	3	0.19	3	1.03
Block	5	11.10****	5	0.08	5	10.55****
$Error_1$ (SS)	15	(0.271)	15	(1 844)	15	(0.040)
Sexual stage	7	7.92****	7	1.02	7	4.58***
PM+PF vs. M+N+F	1	34.33****	1	3.27	1	19.13****
M+F vs. N	1	0.02	1	0.60	1	0.32
M vs. F	1	18.36***	1	0.26	1	4.22*
Treatment × sexual stage	21	0.60	18	1.58	21	0.49
$Error_2$ (SS)	103	(1.085)	55	(4 180)	103	(0.216)
B) Vertical series: nectar production						
Sexual stage	7	20.13****	7	2.10*	7	17.06****
PM+PF vs. M+N+F	1	108.30****	1	1.08	1	98.00****
M+F vs. N	1	1.90	1	0.37	1	0.82
M vs. F	1	6.80**	1	0.29	1	6.21*
Block	43	4.50****	41	6.10****	43	4.80****
Error (SS)	301	(3.003)	184	(7 488)	301	(0.947)
C) Standing crop						
Sexual stage	7	4.47***	7	1.93	7	5.61****
PM+PF vs. M+N+F	1	19.35****	1	0.02	1	22.83****
M+F vs. N	1	0.33	1	0.03	1	0.33
M vs. F	1	0.81	1	9.97**	1	3.66
Day	8	3.30**	8	5.17****	8	3.86**
Error (SS)	55	(0.210)	44	(615.9)	55	(0.056)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

mets of each vertical series cluster; $r = 0.377$, $N = 44$, $P < 0.05$) resulting from the secretion of higher nectar volumes, with no change in concentration during warm days ($r = 0.355$, $N = 44$, $P < 0.05$ and $r = -0.016$, $N = 44$, $P = 0.92$ for nectar volume and sugar concentration, respectively). Most analyses (Tables 1–2) showed significant differences among shoot clusters (''blocks''), indicating spatial and temporal heterogeneity in nectar production.

Both sampling methods (horizontal and vertical) showed similar patterns of variation in nectar production over flower development (Fig. 1; Table 1). Flowers secreted nectar slowly either before the first anthers dehisced (premale stage) or right after the nectariferous tepals curled (postfemale phase). Male stages (I, II, and III) were on the average more productive than female stages (V and VI), both in terms of nectar volume and amount of sugar secreted, while the nectar production rate during the neuter phase (stage IV) was transitional between those of the active male and female phases (Fig. 1; Table 1). The general picture is one in which nectar secretion rate increases over early flower development, peaks at stage II, and gradually declines afterwards (Fig. 1). We did not find any consistent pattern of variation in sugar concentration over flower development, except for a decline during stage II in the horizontal series, but in any event this was overshadowed by the large influence of nectar volume on the rate of sugar secretion. The developmental pattern of nectar standing crop was much less pronounced (Fig. 1). Like the nectar secretion pattern, nectar standing crop was lowest at the beginning (premale) and at the end (postfemale) of a flower's life. Nevertheless, significant differences did not occur in volume or sugar content of the nectar standing crop between the average male and female stage (Fig. 1; Table 1).

By repeated nectar sampling of the 24 flowering shoots in the horizontal series (i.e., six groups of four ramets each), we estimated that an *A. aurea* flower produces a total of 6.7 ± 0.83 μL (X ± 1 SE) of nectar at a concentration of 26.1 ± 1.92% sucrose equivalents, which translates into 1.7 ± 0.19 mg of sugar secreted over its life. Flower life-span, estimated from the permanently bagged flowers, averaged 9.4 ± 0.2 d. The active male phase lasted twice as long as the active female phase (highly significant ''sexual phase'' factor in Table 2; X ± 1 SE = 4.7 ± 0.14 vs. 2.3 ± 0.16 d). Also, the male phase was 3.5 and 3 times as productive as the female phase in terms of the cumulative nectar volume and amount of sugar secreted, respectively (highly significant ''sexual phase'' factor in Table 2; X ± 1 SE = 4.2 ± 0.25 vs. 1.2 ± 0.66 μL, and 1.16 ± 0.075 vs. 0.37 ± 0.102 mg, respectively.). We did not find any evidence that hand-pollination treatments affected either total flower life-span or lifetime nectar production (Table 2A). More specifically, neither pollen removal from the anthers nor stigmatic pollen deposition had an effect on the length of, or on the cumulative amount of nectar secreted during, the male and female phase (Table 2B).

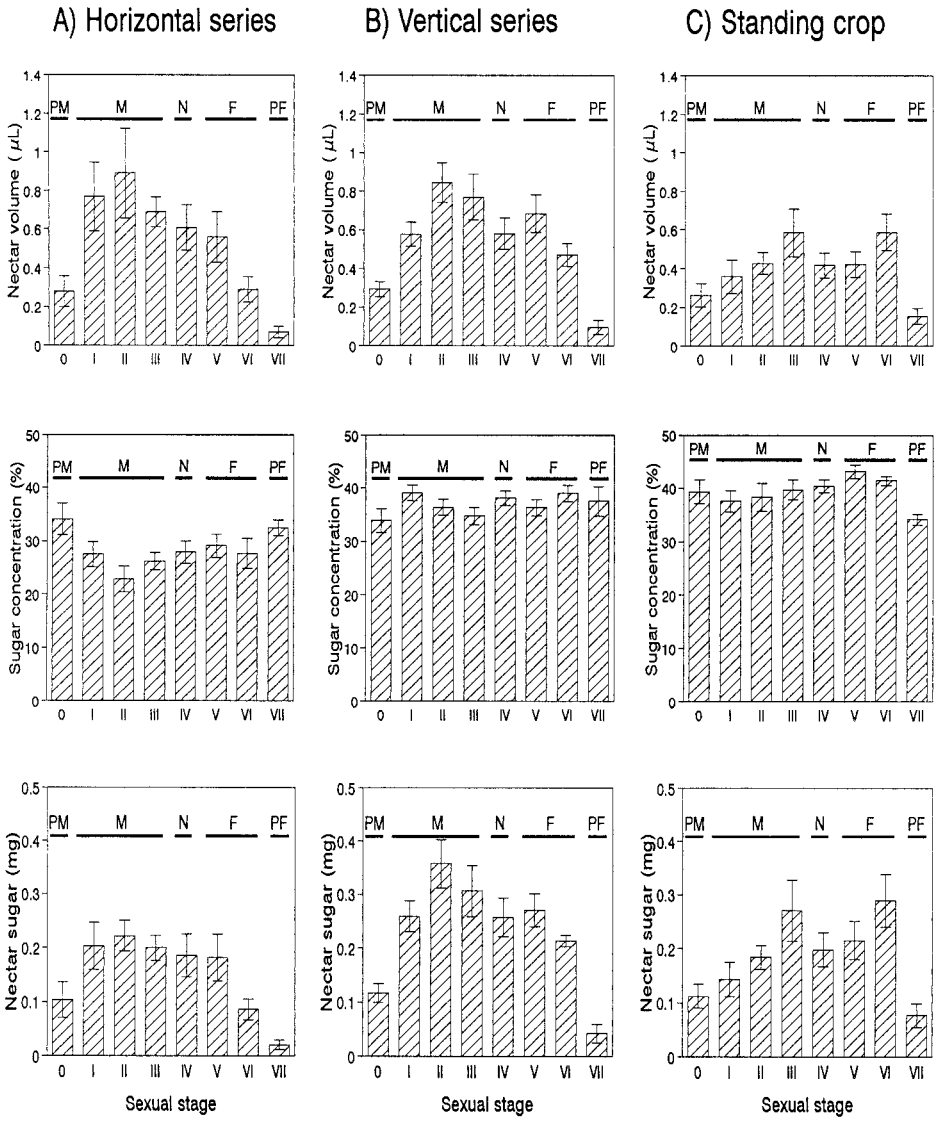

Fig. 1. Average daytime nectar production (A, horizontal and, B, vertical series) and (C) standing crop associated with the different flower developmental stages of *A. aurea* (see Study species and research site). T bars indicate $X \pm 1$ SE values. Stages were grouped as follow: PM = premale phase, M = active male phase, N = neuter phase, F = active female phase, PF = postfemale phase (see Table 1).

A model of exponential decline fitted to the pollen removal data showed that about half of the removable pollen remaining in an anther is removed per bumble bee visit (Fig. 2A). This model also predicts that an average of 3.1 visits is needed to remove 90% of the removable pollen fraction contained in an anther. This is also equivalent to ~3 visits/ d because each anther remains in position to dispense pollen for ~1 d. As one to two anthers dehisce per day over a 4-d period in unbagged flowers (Aizen and Basilio, 1995), we estimated, using the 90% pollen removal criterion, that a minimum of ~12 bumble bee visits during the male phase is needed to saturate male function.

The number of seeds per flower increased steeply with the number of pollen grains deposited for stigmatic loads < 60 pollen grains (at a rate of 4–5 pollen grains per seed), leveling off afterwards (Fig. 2B). Using the negative equation fitted to the seed set vs. pollen load data, we estimated that a stigmatic pollen load of 67 pollen grains produces 90% of the asymptotic seed set value (i.e., 14.2 seeds/ flower). The linear equation fitted to the number of pollen grains deposited vs. number of visits (Fig. 2C) predicts that, on average, a total of 3.6 bumble bee visits (i.e., ~1.5 visits /d) during the female phase will saturate this function.

At any given time, male-phase flowers were more frequently visited than female-phase flowers ($X \pm 1$ SE = 0.69 ± 0.071 vs. 0.56 ± 0.060 visits·flower^{-1}·h^{-1}, $F_{1,171} = 18.2$, $P < 0.0001$).

DISCUSSION

Nectar secretion in *A. aurea* flowers appears to be affected both by environmental factors, such as sunlight and temperature, and by intrinsic factors related to the sexual stages through which a flower passes over its life-

TABLE 2. Summary of *F* statistics and significance levels for ANOVAs testing the effect of treatment (i.e., all 2 × 2 combinations of hand-pollen removal and stigmatic deposition) on (A) total flower life-span and lifetime nectar production, and on (B) sex-phase life-span and nectar production accumulated over the male and female sexual phases. The ''treatment'' factor in (B) was tested over the ''block × treatment'' term (i.e., the ''$error_1$'' term, see Data analysis). Two specific hypotheses (i.e., contrasts) were tested within the ''treatment × sexual-phase'' interaction term: (1) anther pollen removal affects the life-span and total nectar production of the male phase (''pollen removal [male]''), and (2) stigmatic pollen deposition affects the life-span and total nectar production of the female phase (''pollen deposition [female]''). Nectar volume and sugar secreted were $\log(x + 1)$-transformed.

	Life-span (d)		Nectar volume (μL)		Nectar sugar (mg)	
	df	*F*	df	*F*	df	*F*
A) Total						
Treatment	3	0.20	3	0.58	3	0.15
Block	5	8.10***	5	2.18	5	12.65****
Error (SS)	15	(6.375)	15	(0.824)	15	(0.106)
B) Sexual phase						
Treatment	3	1.15	3	0.34	3	0.28
Block	5	3.01*	5	2.74*	5	4.96**
$Error_1$ (SS)	15	(6.708)	15	(0.919)	15	(0.124)
Sexual phase	1	135.90****	1	34.08****	1	26.31****
Treatment × sexual phase	3	0.07	3	0.97	3	0.46
Pollen removal [male]	1	1.03	1	0.29	1	0.09
Pollen deposition [female]	1	0.19	1	0.39	1	0.06
$Error_2$ (SS)	20	(9.958)	20	(0.818)	20	(0.175)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

span. Temperature and irradiance have been shown to be important determinants of secretion rates through their direct influence on plant metabolism (reviewed in Rathcke, 1992), even though diel patterns of nectar secretion may persist under constant environmental conditions (Búrquez and Corbett, 1991). As exemplified by contrasting patterns of nectar production in plants associated with diurnal vs. nocturnal pollinators, the activity period of pollinators may represent ultimate selective factors determining when during the day most nectar is produced, (Cruden, Hermann, and Peterson, 1983). Likewise, diurnal nectar secretion in *A. aurea* might represent an adaptation to bee pollination. Yet, the role of pollinators as selective agents on diel patterns of nectar secretion still awaits formal testing (L. Galetto, Universidad Nacional de Córdoba, personal communication).

As in certain other protandrous plant species (e.g., Bell et al., 1984; Devlin and Stephenson, 1985; Klinkhamer and de Jong, 1990; Snow and Grove, 1995), in *A. aurea* nectar production differs between male and female phases of flowers. This results not only because the male phase is longer than the female but also because nectar secretion rate remains higher throughout the male stages. Our results were not artifacts of prolonged, artificial shading, or of nectary damage through repeated insertion of microcapillary tubes (Rathcke, 1992; Wyatt, Broyles, and Derda, 1992; Galetto and Bernardello, 1993). We found comparable contrasts between male and female nectar production rates when flowers were measured repeatedly over their life (horizontal series) or only twice each (vertical series).

The full male and female fitness contributions of a hermaphroditic flower are attained when sufficient visits occur to remove all the pollen from the anthers and to deposit on the stigma enough pollen for maximum seed set. Therefore, to examine possible adaptation in the developmental pattern of nectar production of a dichogamous flower, criteria to address include (1) whether each of these two functions is saturated at a different minimum rate or total number of animal visits, (2) whether these theoretical rates or numbers are reflected in the observed pattern of nectar secretion, and (3) whether pollinators actually respond to sex-related differences in nectar secretion (cf. Wilson et al., 1994). Here we found that a higher minimum total number of visits (and visits per day) is needed to saturate male than female function. We estimated that an average of 12 visits and 3.6 visits total will minimally be required to saturate male and female functions, respectively. These figures come out to a male: female phase visit ratio of 3.3:1, which closely matches the 3.1:1 ratio in cumulative nectar sugar production between the two phases (see Results). The male:female saturation ratio seems to be quite invariant regarding the exact cutoff saturation criterion: had we used a 95% cutoff value instead of a 90%, a 3.4:1 ratio would have resulted.

The larger cumulative nectar production of the male phase is a consequence not only of longer duration but also of higher daily nectar secretion rates. Here, we estimated that rates of sugar secretion were, on average, 30% (vertical series) and 41% (horizontal series) higher during male stages than female stages, or ~ 25% higher for both series if we include the neuter stage as part of the female phase (as we did to record pollinator visits). Matching this last figure, we also found a similar 23% difference in the rate of bumble bee visits to male-phase vs. female-phase flowers over the 1994 flowering season (see Results). Thus, bumble bees seem to respond to differences in nectar secretion rates between sexual phases by differentially visiting male-phase flowers. The observed lack of differences in nectar standing crop between the male and female sexual phases may then be explained as the result of differential visitation.

In summary, these results support the view that the nectar production pattern has been molded by selection pressures of different strength through male and female sexual functions. Nevertheless, considering the average hourly visitation rates we observed of ~0.6 visits/ flower

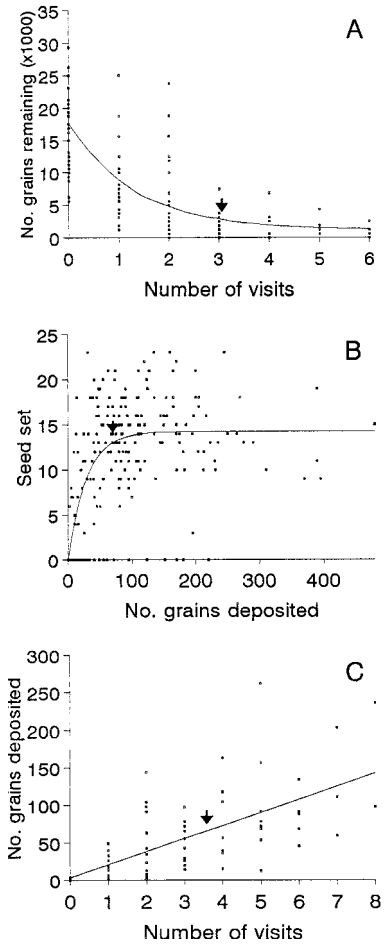

Fig. 2. Relationships between (A) number of pollen grains remaining per anther vs. number of bumble bee visits, (B) number of seeds per flower (seed set) vs. number of grains deposited on the stigma, and (C) number of pollen grains deposited on the stigma vs. number of bumble bee visits. These relationships are described by the equations (A) $Y = 16\ 422 \exp(-0.75\ X) + 1\ 171$ ($r^2 = 0.50$), (B) $Y = 14.25\ (1\text{-}\exp(-0.035\ X))$ ($r^2 = 0.36$), and (C) $Y = 3.5 + 17.4\ X$ ($r^2 = 0.42$). Arrows indicate the estimated (A) number of visits needed to remove 90% of the removable pollen from an anther (note that 1–2 new anthers dehisce per day during the 4-d long male phase, each anther dispensing pollen for ~1 d, so that the depicted estimate must be multiplied by four to obtain the total number of visits needed to saturate male function), (B) stigmatic pollen load needed to produce 90% of average maximum seed set, and (C) number of visits needed to deposit the amount of pollen estimated in (B).

and a 10 h/ d bumble bee activity period, daily visitation greatly exceed the minimum requirement of ~3 and 1.5 flower visits per day to saturate male and female functions, respectively. Average visit frequencies are deceiving, however, because pollinator visits greatly vary in space and time. In particular, bumble bee abundance and visit rates are consistently low during the first 2–3 wk of the *A. aurea* flowering season, among years and across sites (M.A. Aizen, unpublished data). Thus, secretion rates may be molded selectively during periods of pollinator scarcity rather than under ''average'' conditions (Wilson et al., 1994). Additionally, competition among male-phase flowers for prompt pollen deliver to the stigmas may represent another selection pressure for higher rates of nectar secretion during male stages (Stanton, 1994). This could be particularly important in species like *A. aurea* where seed set seems to be rarely pollen limited (Fig. 2B).

We did not find any evidence for plasticity in nectar secretion in response to proximate pollination events. Neither pollen removal nor stigmatic pollen deposition altered nectar secretion rates nor the cumulative amount of nectar produced by shortening the duration of the male or female phase (Table 2). Inflexibility in *A. aurea* even extends to unresponsiveness in nectar production to flowering shoot defoliation (Aizen and Raffaele, 1996). In particular, the lack of flower life-span plasticity in *A. aurea* contrasts with the frequent finding elsewhere that flower life-span shifts in response to pollination events (e.g., Schemske et al., 1978; Devlin and Stephenson, 1984; Richardson and Stephenson, 1989; Gregg, 1991; Preston, 1991; Aizen, 1993; Clayton and Aizen, 1996). Perhaps nectar production in *A. aurea* is ''cheap'' and its precise control not subject to strong selection (cf. Harder and Barrett, 1992). Nevertheless, in a perfectly adaptationist world, the potential benefits of reduced flower longevity would still lead to some plasticity in longevity in response to pollination events, and thereby to plasticity in cumulative nectar production. After all, respiration costs are expected to be high in flowers as large as those of *A. aurea,* suggesting that selective benefits would result from any mechanism that reduced flower longevity after either pollen removal or deposition (Primack, 1985; Ashman and Schoen, 1996; Clayton and Aizen, 1996).

One constraint on plastic responses to pollination events, particularly to pollen removal, may relate to the evolution of synchronous protandry, which necessarily involves a fixed floral developmental pattern. Synchronous protandry in *Alstroemeria aurea* prevents geitonogamous pollination at the ramet level, potentially benefiting a plant by decreasing inbreeding depression and increasing the fraction of exported pollen (Lloyd, 1992). In addition, given fixed flower longevity, plasticity in nectar secretion rates of individual flowers in response to either pollen removal or deposition could be disadvantageous through influences on pollination events in other flowers of the same shoot. Bumble bees will often abandon a plant if they encounter low nectar rewards in a short sequence of its flowers (Dreisig, 1995). A successfully early-pollinated flower that shuts off nectar production might induce the next bumble bee to leave before visiting other same-sex flowers on the same ramet. We conclude that the potential benefits to a ramet of plasticity among its flowers, each responding independently to pollination events, may be offset by the benefits of sharing synchronized developmental clocks and an inflexible nectar production pattern.

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