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Conditional strategies in an animal-pollinated plant: size-dependent adjustment of gender and rewards

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ABSTRACT

Question: How do hermaphroditic flowering plants that must attract animal pollinators allocate to flower production, seed production and nectar production among flowers, as functions of their individual size and the size of their floral display?

Organism: *Chamerion* (= *Epilobium*) *angustifolium*; vertical inflorescences display male-phase flowers above female-phase flowers.

Site: Silver Star Mountain, Vernon, British Columbia, Canada.

Methods: We measured nectar production from male- and female-phase flowers across a range of display sizes. By experiment, we determined the effect of alternative nectar distributions on bumblebee foraging. We measured total flower production (male investment), seed production (female investment) and plant size.

Results: Large floral displays allocated extra nectar production to female-phase flowers. Bumblebees probed fewer male-phase flowers per visit when extra nectar was added to female-phase flowers than when nectar was added more evenly among gender phases. Large plants produced about a third fewer flowers per gram of plant biomass than did the smallest plants. But large plants matured a nearly proportional number of seeds.

Keywords: bumblebees, *Chamerion, Epilobium*, inflorescence design, nectar gradient, nectar production rate, patch departure, pollen dispensing, sex allocation.

INTRODUCTION

Individual size differences within a population will often reflect variation in resource state and current fitness attainment [‘winnings’ (Williams, 1992)]. A positive relation between size and phenotypic fitness may be especially evident among flowering plants. Relative to smaller individuals, large plants can display a greater number of rewarding flowers at once (e.g. Chaplin and Walker, 1982; Worley *et al.*, 2000) and mature a greater number of seeds (e.g. Solbrig and Solbrig, 1984; Dudash, 1991). Large floral displays should be particularly beneficial for outcrossing,

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animal-pollinated plants because pollinators visit large displays more frequently than smaller conspecifics (reviewed by Ohashi and Yahara, 2001).

Although a frequent rate of pollinator visits (herein 'attractiveness') should generally benefit the male function of the floral display (i.e. pollen dispersal), further attractiveness can yield diminishing increases in pollen dispersal (reviewed by Harder *et al.*, 2001). For example, as each individual visitor removes an increasing amount of pollen per visit, a greater fraction of that pollen can be lost to saturation on the pollinator body or to increased pollen grooming behaviour (see also Lloyd, 1980; Klinkhamer *et al.*, 1994). Pollinators typically probe more flowers per visit to large floral displays (Ohashi and Yahara, 2001), and hence the cost of excess pollen removal is especially apparent for the most attractive plants in a population. Here, we explore reproductive tactics that might vary with a plant's ability to attract pollinators.

Size-dependent nectar production

The largest floral displays in a population could maximize pollen dispersal if many individual pollinators were to remove only small amounts of pollen or probe only a few pollen-bearing flowers at a time (Harder and Thomson, 1989; Iwasa *et al.*, 1995). Plants with less attractive displays must balance the benefit of removal restriction with the even larger cost associated with pollen removal failure; hence, pollen dispersal from smaller displays is maximized with fewer restrictions on pollen removal (Harder *et al.*, 2001). One mechanism by which the optimal pollen dispensing schedule might be adjusted to attractiveness is by varying the distribution of nectar production rates within inflorescences (Biernaskie and Cartar, 2004). The flowers of vertical inflorescences are often protandrous and open so that the youngest (male-phase) flowers are positioned above older (female-phase) flowers. Many displays of this type produce nectar in a gradient that decreases from bottom to top. Accordingly, nectarivores approach the lowermost flowers, move upwards, and leave when energy intake declines to a threshold rate (e.g. Pyke, 1978; Best and Bierzychudek, 1982; Hodges, 1985). Plants might mediate the number of pollen-bearing flowers probed per visit by adjusting the rate at which energy gains reach the departure threshold (Ohashi and Yahara, 2001). In particular, if plants can adjust the nectar gradient with respect to display size, then we expect large floral displays to produce a stronger nectar gradient than smaller, competing conspecifics. This should promote earlier departure, limit the pollen removal of each visitor, and thus maximize the male fitness advantage of large floral displays.

Size-dependent gender allocation

Given a difference in the shape of male and female fitness gain curves (fitness with respect to investment in each gender function), simultaneous hermaphrodites that differ in relative size should also differ in the proportion of energy that is allocated to each gender function (Charnov, 1982; Lloyd and Bawa, 1984). For example, if both male and female gain curves show diminishing returns but the male gain curve decelerates faster, then an emphasis on female function is evolutionarily stable for relatively large individuals. Smaller individuals, in this case, gain a greater male fitness return per unit of investment because their absolute investment in reproduction is also small [the 'budget effect' of Klinkhamer *et al.* (1997)]. Relatively small individuals should therefore emphasize male function.

Female allocation may generally increase with size in animal-pollinated plants (see Klinkhamer *et al.*, 1997 and references therein; Sarkissian *et al.*, 2001) if investment in the male function of the floral display (i.e. attractiveness) yields strongly diminishing returns (due to the cost of excess pollen removal, for example, discussed above). If, however, the largest individuals have special mechanisms to limit pollen wastage, then allocation to male function may increase nearly proportionately with plant size (Ishii, 2004).

Objectives

We investigate size-dependent allocation tactics in *Chamerion angustifolium* – a simultaneous hermaphrodite with vertical inflorescences and structured dichogamy. We determine:

- whether the strength of the nectar gradient varies positively with floral display size;
- the flexibility of bumblebee departure, and expected pollen removal, from alternative nectar gradients;
- the pattern of size-dependent gender allocation, measured as flower production [an upper estimate of male investment (after Klinkhamer and deJong, 1997)] versus seed production (female investment).

METHODS

Chamerion (= *Epilobium*) *angustifolium* (L.) Holub. is a perennial wildflower, commonly found in disturbed areas of North America and Europe. Newly opened flowers gradually expose pollen from eight anthers that dehisce sequentially. The male phase lasts about 2½ days, at which time the stigma becomes receptive. Flowers are female-phase for an additional 1½ days before wilting (Sargent and Roitberg, 2000). Floral displays at our site ranged from 6 to 27 flowers. The most common pollinators observed were nectar-collecting worker bumblebees (*Bombus* spp.; mainly *B. flavifrons* and *B. bifarius*).

In the summer of 2003, we studied *C. angustifolium* on Silver Star Mountain, near Vernon, British Columbia, Canada (50.3°N, 119.3°W), where large populations are found along roadsides and in the clearings of Silver Star Resort. At our study sites, ramets flower for about 4–6 weeks in July and August, after which dehiscent fruits release hundreds of wind-dispersed seeds.

Size-dependent nectar production

Field observations

Ramets for nectar measurements were haphazardly sampled to span the range of observed floral display sizes. We sampled ramets throughout four large focal patches, where the display size distributions were not obviously different. At each observation period, at least two ramets from each size category – small (<9 flowers), medium (≈12 flowers) and large (>15 flowers) – were selected. We chose plants that were at peak flowering stage (those with flower buds and developing fruits) and that were not obviously part of the same genet.

We quantified floral nectar production as the amount of energy invested in nectar over four daylight hours. Once a ramet was selected in the field, we carefully removed any standing nectar in its flowers with a 2 μl microcapillary tube. The whole inflorescence was then enclosed in a bag of bridal veil to exclude nectar foragers. After 4 h, we collected bagged inflorescences and randomly selected three male- and three female-phase flowers per inflorescence for nectar measurements. [We did not select intermediate flowers (no pollen remaining and stigma not yet receptive) or newly opened flowers (no exposed pollen).] We extracted nectar with 2 μl microcapillary tubes and measured its volume. The sugar concentration (mg solute/mg solution) of each sample was measured with a hand-held refractometer (ATAGO, Japan) and later converted to mg solute/ml solution. Total production per flower was calculated in Joules (J) of energy, where J = (density of sucrose at 20°C × energy value of sucrose × concentration × volume).

Analysis. For each ramet ($n = 45$), we calculated the mean 4 h nectar production of male- and female-phase flowers. We tested whether nectar production (J) varied with the main effects of 'gender phase', 'display size' and their interaction (SAS, PROC MIXED). 'Ramet' was included as a random subject variable to account for the paired nectar production measures (mean male-phase, mean female-phase production) within each ramet.

Nectar manipulations and bumblebee behaviour

Gradient strength. Bumblebee bout lengths (number of flowers probed before departure) were compared on inflorescences to which the same volume of nectar was added in alternate distributions. We first trimmed all floral displays to 11 flowers (three female-phase, eight male-phase flowers above). Two treatment levels – a 'strong' gradient (0.5 μl of 40% sucrose solution added to each of three lowermost flowers) and a 'weak' gradient (0.25 μl added to each of six lowermost flowers) – were assigned in random order to each of 17 ramets. Non-enriched male-phase flowers offered only the amount of nectar that could be produced between visits (much less than our artificial additions and not measurable with microcapillary tubes). Each treatment level lasted for at least five consecutive bumblebee visits (with nectar levels replenished between visits), but were extended until at least two visitors probed all enriched flowers in a single bout. During each visit, we recorded the total number of flowers probed and the number of those that were male-phase.

Analysis. We determined the mean number of flowers probed per bumblebee visit for each ramet × treatment level combination ($n = 34$), using only those visits in which all enriched flowers were probed (conclusions did not differ when all visits were included). We used analysis of variance (ANOVA) to test the effect of nectar treatment on mean bout length, with 'ramet' included as a (random) blocking factor; each treatment level appeared once within each block.

Pollen removal. We compared the time bumblebees spent on male-phase flowers that were or were not enriched with nectar, and the amount of pollen removed from those flowers. On each of 15 different ramets, two flowers with undehisced anthers were enclosed with small bridal veil bags to exclude visitors. When the anthers had dehisced, we carefully removed the bags and emptied flowers of all standing nectar without contacting the anthers. Two anthers with fully exposed pollen were chosen from each flower; all others were clipped off. One of the focal flowers on each ramet was randomly selected for a 2 μl (40% sucrose) nectar addition; the second flower served as a non-enriched control. Trials lasted for two foraging bouts by (presumably) two different bees that each probed both the enriched and control flower. We checked for nectar removal and replenished the enriched flower after the first visit. During most visits (save three, missing at random), we recorded the handling time of focal flowers (time between landing and leaving a flower) with a stopwatch. Following the second bout, the anthers of each flower were collected in separate vials. We estimated the number of remaining pollen grains per flower by suspending pollen in 25 µl of (3 : 1) lactic acid : glycerine solution, and counting two 5 µl samples on a haemocytometer under a compound microscope.

Analysis. We calculated the difference in handling time of the nectar-enriched and control flower for each individual bee visit ($n = 27$). We used a paired t-test to evaluate the null hypothesis H_0: $\mu_{\text{difference}} = 0$. In addition, we compared the estimated survival function of each treatment group (control, nectar-enriched) using a Weibull-based model. The qualitative results were not different from the paired t-test, however, and we report only the latter here. To test the effect of nectar treatment on the mean number of pollen grains remaining per flower, we used an ANOVA model with 'ramet' included as a random blocking variable; as above, each treatment level appeared once within each block.

Size-dependent gender allocation

We erected a 4 × 2 m sampling frame in each of two dense patches of *C. angustifolium* at Silver Star Resort. Three random points were selected along the length of each frame, where transects were initiated to span the 2 m width. At 50 cm intervals along each transect, we selected the largest and smallest ramets (based on stem thickness) within at most 30 cm of the selected point and perpendicular to the transect. Thus, a total of 60 ramets was selected by six repeated systematic samples. Our selection criteria were designed to include a large range of ramet sizes in each transect sample.

Five undehisced fruits per ramet were collected over two dates in late September 2003. On the first date, two or three fruits were randomly selected from those mature enough to collect, depending on whether less than or more than half, respectively, of the ramet's fruits were available. The remaining fruits were collected approximately 2 weeks later by a random selection of the fruits that were unavailable on the first collection date. Above-ground biomass of each ramet was also collected at this time.

Under a stereoscope, we determined seed set per fruit by counting the number of inflated ovules (see Weins *et al.*, 1987) from those fruits collected at positions 1, 3 and 5 of our random sample from each ramet. For each ramet, we recorded the height, total fruit production and total flower production (= # fruits + # undeveloped ovaries). Total seed production was estimated as the average inflated ovules/fruit × total fruits. Vegetative biomass was dried at 70°C for 48 h and weighed to the nearest one-hundredth of a gram.

Analysis. We used a simple allometric model to examine the relation between ramet biomass (x) and seed, fruit or flower production (y's) (after Klinkhamer and deJong, 1997):

$$y = a\,x^{b} \qquad \text{or} \qquad \log y = a + b \log x$$

Linear regression of log-transformed data gives an estimate of the slope b, which indicates a more than ($b > 1$) or less than ($b < 1$) proportional increase in allocation with ramet biomass.

In accordance with repeated systematic sampling methods (Levy and Lemeshow, 1999), an estimated slope was determined for each transect and then used to calculate the overall mean estimate, b^* (the overall effect size, from six transects) and its 95% confidence interval. The overall mean estimate was also used for hypothesis testing (e.g. H_0: $b^* = 1$). We had little power to detect differences between the two patches; hence, this effect was ignored in our analyses. Where pooled data are presented (Fig. 3), we use leverage plots (JMP Start Statistics, SAS Institute, Inc.) that account for variation in the data due to 'transect' (random factor). Leverage plots are interpreted in the same manner as a simple regression plot. Reported statistics, however, were calculated from the mean of each transect's slope, as above.

We used JMP 4.0 for all analyses, except where noted. For all ANOVA models, we checked for similar standard deviations within treatment groups and examined residual plots to check that the distribution of errors was not skewed. Mixed models were estimated with the REML method. Throughout the paper, estimated means and least squares (LS) means are reported with 95% confidence intervals as: estimate (lower limit, upper limit).

RESULTS

Correlates of resource state

Above-ground biomass was our estimate of a plant's resource state. In the sample of plants collected for biomass measures, ramet height correlated positively with mass (Pearson correlation coefficient, $r = 0.90$, $P < 0.0001$, $n = 58$). Thus, height may be considered a surrogate for biomass. In the field, the height of ramets selected for nectar measurements correlated positively with observed display size ($r = 0.71$, $P < 0.0001$, $n = 45$ ramets), and (marginally) with mean 4 h nectar production (J) per flower ($r = 0.25$, $P = 0.098$, $n = 45$).

Size-dependent nectar production

Field observations

Across all display sizes, female-phase flowers produced more nectar, on average, than male-phase flowers [estimated mean difference = 5.30 J (4.28, 6.31), $n = 45$ inflorescences]. However, the mean difference between female- and male-phase nectar production increased with floral display size. We detected a display size-by-gender phase interaction (ANOVA, $F_{1,43} = 9.12$, $P = 0.0042$) because large displays allocated differentially to nectar production in female- relative to male-phase flowers (Fig. 1). In our study populations, large inflorescences displayed a larger absolute number of female-phase flowers, although the proportion of open flowers that were in female-phase decreased slightly with increased floral display size ($r = -0.28$, $P = 0.064$, $n = 45$).

Nectar manipulations and bumblebee behaviour

Gradient strength. On average, bumblebees probed a larger number of flowers before departure from 'weak' gradients compared with 'strong' ones ($F_{1,16} = 54.9$, $P < 0.0001$). The mean difference in the number of flowers probed can be accounted for by an increased number of flowers probed on upper, male-phase flowers (Fig. 2).

Pollen removal. Bumblebees spent on average 6.12 s (5.01, 7.22) longer on nectar-enriched flowers than on non-enriched controls (paired t-test, $t_{26} = 11.4$, $P < 0.0001$). The mean

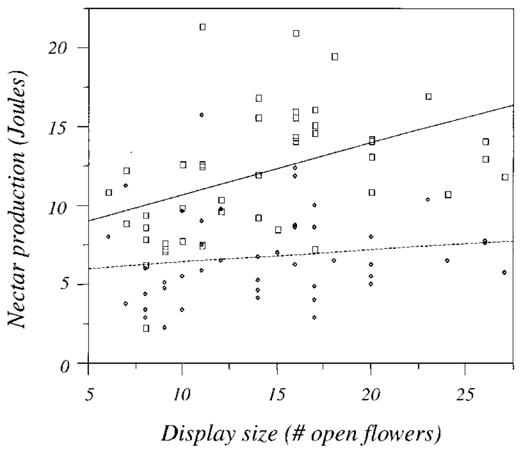

Fig. 1. Interaction of floral display size and gender-phase (◊ = male phase, □ = female phase) on the mean 4 h nectar production of *C. angustifolium* flowers. Although raw data are plotted, analyses in the text accounted for having paired measures (male- and female-phase production) within each ramet.

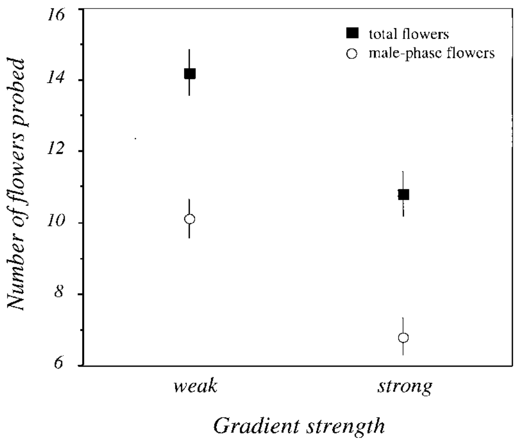

Fig. 2. Least squares mean (±95% CI) number of flowers probed by bumblebees on 11 flowered *C. angustifolium* displays with alternative nectar gradients added (see text). Estimated differences: total flowers probed (■), 3.38 flowers (2.41, 4.35); number of male-phase flowers probed (○), 3.24 flowers (2.43, 4.04).

amount of pollen remaining in nectar-enriched flowers was significantly less than that remaining in control flowers ($F_{1,14} = 31.3$, $P < 0.0001$); the estimated mean difference in pollen remaining per flower pair was 288.7 grains (178.0, 399.5).

Size-dependent gender allocation

Gender allocation, measured by total flower and seed production, varied considerably with ramet biomass. The largest ramets in the population produced disproportionately fewer flowers and fewer fruits than smaller ramets; for both measures, the estimated mean slope b^* was significantly less than unity (Table 1; one sample t-test, $t_5 = -7.5$ and -7.8, respectively; both $P < 0.001$). The relation between ramet biomass and total seed production per ramet was, however, not detectably different from unity (Table 1; $t_5 = -0.75$, $P = 0.49$). Thus, larger plants produced, on average, fewer flowers per gram of biomass, but a greater number of seeds per flower (Fig. 3).

The higher per-flower seed set in large ramets, relative to smaller ramets, may reflect greater pollination success (i.e. the large number of non-inflated ovules in the flowers of small ramets may have been unfertilized). To test this possibility, we randomly selected 30 of the collected ramets from which to determine the inflated : non-inflated ovule ratio, using the remaining fruits (positions 2 and 4) from each ramet. Data were treated as a simple random sample. We could detect no relation between ramet biomass and the mean inflated : non-inflated ratio per flower (linear regression, $F_{1,28} = 1.3$, $P = 0.27$), however, because total ovule number per flower (inflated + non-inflated) increased with ramet size ($F_{1,28} = 37.7$,

Table 1. Estimated slopes (b) in the regression: $\log y = a + b \log x$, where x = ramet biomass (grams) and y = total flower, fruit or seed production of *C. angustifolium* ramets

Response variable (y)	Transect 1	2	3	4	5	6	b^* (95% CI)
Total # flowers	0.66	0.58	0.75	0.71	0.72	0.44	0.64 (0.52, 0.77)
Total # fruits	0.65	0.57	0.76	0.74	0.74	0.5	0.66 (0.55, 0.77)
Total # seeds	1.05	0.73	1.11	1.24	0.83	0.58	0.92 (0.65, 1.19)

Note: The slope was calculated for each randomly selected transect (from 10 ramets) and the mean is taken as the overall effect size (b^*).

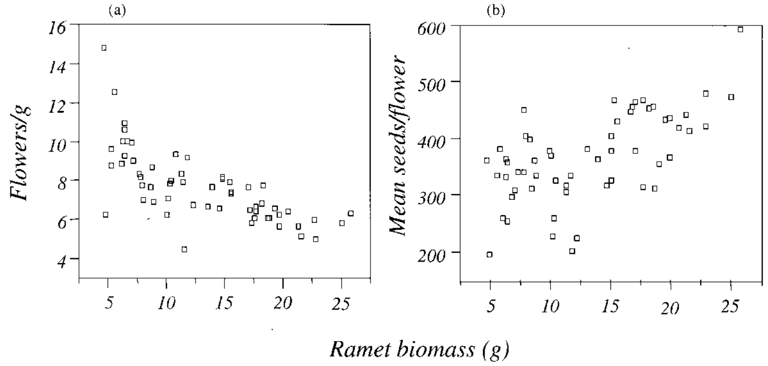

Fig. 3. Leverage plots of the relation between ramet biomass and (a) flower production per gram biomass and (b) mean seed number per flower in *C. angustifolium*, after accounting for variation due to 'transect'. The mean regression slope across all transects was −0.23 (−0.35, −0.11) for the relation in (a) and 9.04 (3.69, 14.39) for (b).

$P < 0.0001$). Hence, there was a negative relation between the total number of non-inflated (potentially aborted) ovules per gram biomass and ramet biomass ($F_{1,56} = 125.4$, $P < 0.0001$), because small ramets produced more flowers per gram biomass than larger ramets.

DISCUSSION

Within *Chamerion angustifolium* populations, the largest ramets should experience the highest reproductive potential through both male and female function. Large ramets produce the largest floral displays, most total nectar and most seeds (this study), and pollinators visit large displays at highest frequency (Schmid-Hempel and Speiser, 1988; Sargent and Roitberg, 2000; J.M. Biernaskie, unpublished). If the success of alternative allocation tactics depends on size, then selection should favour allocation strategies that specify the optimal tactic in relation to size [or quality of environment (e.g. Zhang and Jiang, 2002)]. We observed variation in nectar production and gender allocation phenotypes that may be components of adaptive, size-dependent strategies in *C. angustifolium*.

The nectar distribution as a pollen-dispensing mechanism

The distribution of nectar production in vertical inflorescences with structured dichogamy – typically decreasing from bottom to top – has long been presumed to increase the efficiency of cross-pollination (e.g. Pyke, 1978; Best and Bierzychudek, 1982). Specifically, because pollinators tend to move upwards on inflorescences regardless of rewards (Waddington and Heinrich, 1979; Corbet *et al.*, 1981; Orth and Waddington, 1997), high nectar production rates in lower flowers may ensure that pollinators enter low enough on the display to import foreign pollen to female-phase flowers and stay long enough to remove pollen from the male-phase flowers above.

Our size-dependent hypothesis proposes, more precisely, that the nectar gradient mediates the number of male-phase flowers probed per visit, depending on the attractiveness of the inflorescence. Structured dichogamy alone should benefit pollen dispersal by limiting the amount of pollen that is lost to stigmas within the same plant, as pollinators ascend the display (Harder *et al.*, 2000; Routley and Husband, 2003). Moreover, the distribution of nectar can influence the amount of exposed pollen that is removed by each visitor. The largest, most attractive displays in a population should benefit from any mechanism that maintains attractiveness while also limiting the number of pollen-bearing flowers probed per visit. Smaller displays minimize their disadvantage by dispensing pollen in larger amounts.

Observed variation in the nectar distribution of *C. angustifolium* inflorescences, and its predicted effect on per-visit pollen removal, supports the size-dependent hypothesis. Small inflorescences distributed nectar nearly evenly among female- and male-phase flowers, whereas larger inflorescences allocated extra nectar to female-phase flowers. In our gradient strength experiment, bumblebees probed fewer male-phase flowers per visit when nectar was distributed in a strong, relative to weak, gradient. This behaviour is consistent with known departure rules, which appear to be based on a threshold nectar volume at the current (e.g. Hodges, 1985) or previous two (Kadmon and Shmida, 1992) flowers probed. Hence, on large *C. angustifolium* inflorescences in nature, we expect that bumblebees should (on average) probe fewer male-phase flowers per visit than they would if nectar production was allocated more evenly among flowers. Small inflorescences, on the other hand, are visited less frequently than larger conspecifics, yet produce nectar in their male-phase flowers at a rate similar to larger floral displays (Fig. 1). Hence, if more nectar can accumulate between visits to small inflorescences, then bees are likely to visit several male-phase flowers per visit and remove relatively large pollen loads from each flower probed.

As theory predicts (e.g. Iwasa *et al.*, 1995), the proportion of open flowers probed per visit is in fact negatively related to display size in most plant species, including *C. angustifolium* (Ohashi and Yahara, 2001). Unfortunately, it is difficult to weigh the relative effect of slight differences in the rate of nectar production (reported here) with other factors that may contribute to this behaviour of pollinators (see Ohashi and Yahara, 1999). Nevertheless, our results suggest that the strong nectar gradient, observed in the largest displays, is adaptive in the context of relatively large floral displays (i.e. frequent pollinator visits), and that the weaker nectar gradient, observed in smaller displays, is adaptive in the context of less frequent visitation.

Nectar production as a component of size-dependent gender allocation

On average, the smallest ramets in *C. angustifolium* populations produced nearly three times more flowers per gram of ramet biomass than the largest ones, but matured fewer seeds per flower. It is likely that low seed set in small ramets is a consequence of seed abortion rather than pollen limitation in this species (see Weins *et al.*, 1987). At our sites, bees typically visited inflorescences at mean rates of about once every 3 min (J.M. Biernaskie, unpublished). Furthermore, in other populations of *C. angustifolium*, female-phase flowers of both large and small floral displays receive more than ample pollen loads for full seed set in only hours of open pollination (Schmid Hempel and Speiser, 1988). Thus, by the simplified measure of flower (male) and seed (female) production alone, small ramets appear to emphasize male function and larger ramets appear to emphasize female function.

Our estimate of gender modification is consistent with previous observations of animal-pollinated angiosperms. The observed relation between ramet biomass and total seed production in *C. angustifolium* (Table 1) was admittedly imprecise, but statistically similar to all equivalent estimates reviewed by Klinkhamer and deJong (1997). The less than proportional increase in flower production with ramet biomass was also consistent with available data, but the estimated relationship in *C. angustifolium* ($b^* = 0.64$ flowers per gram) was even smaller than most previous estimates [mean = 0.88, standard deviation = 0.19; $n = 8$ species (Klinkhamer and de Jong, 1997)]. Hence, it may appear that relatively large *C. angustifolium* ramets strongly limit their allocation to male function.

Recognize, however, that simplified measures of gender allocation (i.e. flower and gamete production) ignore additional investments that can modify size-specific gender. In particular, if seed set is not pollen limited, then allocation to pollinator attraction may be assigned mainly to the male function (e.g. Charnov and Bull, 1986). In *C. angustifolium*, the total allocation to nectar production increased with plant (and display) size, due in part to the increased nectar production rate of female-phase flowers. This additional nectar production should increase the attractiveness of large displays, given that the frequency of bumblebee visits to an inflorescence increases with its mean rate of nectar production in this species (Cartar, 2004). We propose that the elevated nectar production of female-phase flowers may be the most efficient way to increase the rate of pollinator visits to the inflorescence (and male fitness), without exposing additional pollen to excess removal [effectively increasing the ‘quality’ of existing pollen grains (Charnov and Bull 1986)]. Hence, a measure of investment based on flower production alone could mask the true allocation to male function – even an emphasis on male function – in the largest ramets of *C. angustifolium* populations.

Models that assume a linear female fitness gain curve and diminishing returns to male investment predict a constant absolute investment in male function for individuals above a threshold size (e.g. Zhang and Jiang, 2002). Given that our estimates of male investment (flower and nectar production) cannot be assigned exclusively to the male function, this remains a difficult prediction to test for *C. angustifolium*. One seemingly supportive example comes from monecious populations of *Sagittaria latifolia*; in that study, female, but not male, flower production increased steadily with ramet size (Sarkissian *et al.*, 2001). One must be careful, however, to assume that female flower production serves only a female function. The addition of extra female flowers to a monecious floral display may be an efficient mechanism to increase the attractiveness of the display, while avoiding the excess pollen removal that may result from further male flower production. As above, this viewpoint highlights the fundamental role of the entire floral display as a single functional mating unit (Harder *et al.*, 2001; see also Elle and Meagher, 2000).

Conclusion

We identified reproductive allocations of an animal-pollinated plant that vary with our estimate of resource state and, presumably, with reproductive winnings. We successfully predicted size-dependent variation in the pattern of nectar production in *C. angustifolium*, and suggest that its effects on bumblebee visitation and the pattern of pollen removal are designed to maximize pollen dispersal from the display. This evidence offers a particularly unique example of how flowering plants might adaptively 'manipulate' the foraging behaviour of their animal pollinators.

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REFERENCES

Best, L.S. and Bierzychudek, P. 1982. Pollinator foraging on foxglove (*Digitalis purpurea*): a test of a new model. *Evolution*, **36**: 70–79.

Biernaskie, J.M. and Cartar, R.V. 2004. Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Funct. Ecol.*, **18**: 125–129.

Cartar, R.V. 2004. Resource-tracking by bumble bees: responses to plant-level differences in quality. *Ecology*, **85**: 2764–2771.

Chaplin, S.J. and Walker, J.L. 1982. Energetic constraints and adaptive significance of the floral display of a forest milkweed. *Ecology*, **63**: 1857–1870.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.

Charnov, E.L. and Bull, J.J. 1986. Sex allocation, pollinator attraction and fruit dispersal in cosexual plants. *J. Theor. Biol.*, **118**: 321–325.

Corbet, S.A., Cuthill, W.E., Fallows, M., Harrison, T. and Hartley, G. 1981. Why do nectar-foraging bees and wasps work upwards on inflorescences? *Oecologia*, **51**: 79–83.

Dudash, M.R. 1991. Plant size effects on female and male function in hermaphrodite *Sabatia angularis* (Gentianaceae). *Ecology*, **72**: 1004–1012.

Elle, E. and Meagher, T.R. 2000. Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae). II. Paternity and functional gender. *Am. Nat.*, **156**: 622–636.

Harder, L.D. and Thomson, J.D. 1989. Evolutionary options for maximizing pollen dispersal in animal-pollinated plants. *Am. Nat.*, **133**: 323–344.

Harder, L.D., Barrett, S.C.H. and Cole, W.W. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proc. R. Soc. Lond. B*, **267**: 1–6.

Harder, L.D., Williams, N.M., Jordan, C.Y. and Nelson, W.A. 2001. The effects of floral design and display on pollinator economics and pollen dispersal. In *Cognitive Ecology of Pollination* (J.D. Thomson, ed.), pp. 297–317. Cambridge: Cambridge University Press.

Hodges, C.M. 1985. Bumble bee foraging: the threshold departure rule. *Ecology*, **66**: 179–187.

Ishii, H.S. 2004. Increase of male reproductive components with size in an animal-pollinated hermaphrodite, *Narthecium asiaticum* (Liliaceae). *Funct. Ecol.*, **18**: 130–137.

Iwasa, Y., deJong, T.J. and Klinkhamer, P.G.L. 1995. Why pollinators visit only a fraction of the open flowers on a plant: the plant's point of view. *J. Evol. Biol.*, **8**: 439–453.

Kadmon, R. and Shmida, A. 1992. Departure rules used by bees foraging for nectar: a field test. *Evol. Ecol.*, **6**: 142–151.

Klinkhamer, P.G.L. and deJong, T.J. 1997. Size-dependent allocation to male and female reproduction. In *Plant Resource Allocation* (J. Grace, ed.), pp. 211–230. San Diego, CA: Academic Press.

Klinkhamer, P.G.L., deJong, T.J. and Metz, A.J. 1994. Why plants can be too attractive: a discussion of measures to estimate male fitness. *J. Ecol.*, **82**: 191–194.

Klinkhamer, P.G.L., deJong, T.J. and Metz, A.J. 1997. Sex and size in cosexual plants. *Trends Ecol. Evol.*, **12**: 260–265.

Levy, P.S. and Lemeshow, S. 1999. *Sampling of Populations*. Toronto: Wiley.

Lloyd, D.G. 1980. Demographic factors and mating patterns in angiosperms. In *Demography and Evolution in Plant Populations* (O.T. Solbrig, ed.), pp. 67–88. Oxford: Blackwell.

Lloyd, D.G. and Bawa, K.S. 1984. Modification of the gender of seed plants in varying conditions. *Evol. Ecol.*, **17**: 255–338.

Ohashi, K. and Yahara, T. 1999. How long to stay on, and how often to visit a flowering plant? A model for foraging strategy when floral displays vary in size. *Oikos*, **86**: 386–392.

Ohashi, K. and Yahara, T. 2001. Behavioural responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In *Cognitive Ecology of Pollination* (J.D. Thomson, ed.), pp. 274–296. Cambridge: Cambridge University Press.

Orth, A.W.E. and Waddington, K.D. 1997. The movement patterns of carpenter bees *Xylocopa micans* and bumblebees *Bombus pennsylvanicus* on *Pontederia cordata* inflorescences. *J. Insect Behav.*, **10**: 79–86.

Pyke, G.H. 1978. Optimal foraging in bumblebees and coevolution with their plants. *Oecologia*, **36**: 281–293.

Routley, M.B. and Husband, B.C. 2003. The effect of protandry on siring success in *Chamerion angustifolium* (Onagraceae) with different inflorescence sizes. *Evolution*, **57**: 240–248.

Sargent, R.D. and Roitberg, B.D. 2000. Seasonal decline in male-phase duration in a protandrous plant: a response to increased mating opportunities? *Funct. Ecol.*, **14**: 484–489.

Sarkissian, T.S., Barrett, S.C.H. and Harder, L.D. 2001. Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters. *Ecology*, **82**: 360–373.

Schmid-Hempel, P. and Speiser, B. 1988. Effects of inflorescence size on pollination in *Epilobium angustifolium. Oikos*, **53**: 98–104.

Solbrig, O.T. and Solbrig, D.J. 1984. Size inequalities and fitness in plant populations. In *Oxford Surveys in Evolutionary Biology* (M. Ridley, ed.), pp. 141–159. Oxford: Oxford University Press.

Waddington, K.D. and Heinrich, B. 1979. The foraging movements of bumblebees on vertical 'inflorescences': an experimental analysis. *J. Compar. Physiol.*, **134**: 113–117.

Weins, D., Calvin, C.L., Wilson, C.A. *et al.* 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia*, **71**: 501–509.

Williams, G.C. 1992. *Natural Selection: Domains, Levels, and Challenges*. New York: Oxford University Press.

Worley, A.C., Baker, A.M., Thompson, J.D. and Barrett, S.C.H. 2000. Floral display in *Narcissus*: variation in flower size and number at the species, population, and individual levels. *Int. J. Plant Sci.*, **161**: 69–79.

Zhang, D.-Y. and Jiang, X.-H. 2002. Size-dependent resource allocation and sex allocation in herbaceous perennial plants. *J. Evol. Biol.*, **15**: 74–83.