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Whole-plant investment in nectar is greater for males than pollinated females in the dioecious plant *Silene latifolia*

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ABSTRACT

Sexual dimorphism in nectar traits can contribute to sex-differential costs of reproduction in dioecious plants. We examined nectar traits of males and females in paternal half-sibling families of dioecious *Silene latifolia*, a species with sexually dimorphic flower production that is pollinated by nectar-seeking moths. Nectar volume and total sugar content peaked 3 days after flower opening in males and 4 days after flower opening in females. Flowers on females had greater nectar volume and total sugar than did those on males, but nectar in the latter was higher in concentration. Variation among paternal families in nectar volume and total sugar was detected only in females; selection by pollinators may have eliminated genetic variation in nectar production in males. We estimated whole-plant investment in nectar by multiplying family means by the number of reproductive units of each plant. Nectar investment per plant did not differ between males and unpollinated females, but was much lower in pollinated females because they produced fewer flowers. Based on estimated values of flower and fruit number, whole-plant nectar investment for males is expected to be higher than that for females under low to moderate levels of pollination.

Keywords: cost of reproduction, dioecy, nectar, sexual dimorphism.

INTRODUCTION

Outcrossing plants dependent on pollinators for moving pollen to and from plants often offer nectar as their principal pollinator reward (Simpson and Neff, 1983). The unisexual flowers of obligately outcrossing dioecious species differ in their rewards to pollinators, with staminate flowers on males producing both pollen and nectar and pistillate flowers on females producing only nectar. Males and females of dioecious species also typically differ

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in the number of flowers they produce, with males of temperate, animal-pollinated species usually exhibiting more flowers than females (Delph, 1996). These differences in rewards and flower production may lead to selection for sexual dimorphism in investment in nectar, both within individual flowers and at the whole-plant level, and could contribute to sex-differential costs of reproduction. We therefore investigated nectar production in *Silene latifolia*, a dioecious species sexually dimorphic for flower production that offers nectar as a reward to its pollinators.

Production of nectar requires a plant to use carbon (sugars), water and numerous other less abundant constituents (Baker and Baker, 1983; Kearns and Inouye, 1993, and references therein). Estimates for investment in nectar range widely. For example, nectar was estimated to be 30% of the energy invested in reproduction in *Asclepias quadrifolia* (Pleasants and Chaplin, 1983), 4.3–36.6% of daily photosynthate in *Asclepias syriaca* (Southwick, 1984), 20% of the energy in plant biomass in *Medicago sativa* (Southwick, 1984), and 3.3% of the energy content of a flower of *Pontederia cordata* (Harder and Barrett, 1992). Pyke (1991) illustrated a negative effect of investment in nectar: when he experimentally increased nectar-production rate, seed number in *Blandfordia nobilis* was reduced.

Current investment in reproduction is predicted to be inversely correlated with future survival or reproduction (Roff, 1992). The decrease in future fitness is referred to as the cost of reproduction. Sexually dimorphic plants have often been used to investigate the cost of reproduction in plants (Obeso, 2002). In most dioecious species, females allocate proportionally more resources to reproduction than males because they produce fruit in addition to flowers. Consequently, females are expected to, and have been shown to, decrease future growth, reproduction or survivorship relative to males (Delph, 1999; Obseo, 2002). However, the predicted life-history trade-offs are not observed in *S. latifolia*. In this species, even though females allocate more biomass to reproduction, they also allocate as much or more to vegetative growth as males (Lovett Doust *et al.*, 1987; Gehring and Linhart, 1993; Delph and Meagher, 1995).

Of the traits studied to date, flower number is the most sexually dimorphic trait in *S. latifolia* (Delph *et al.*, 2002). Males typically produce 10–15 times more flowers than pollinated females, which curtail or reduce flower production while they are setting fruit (Gehring and Linhart, 1993; Delph and Meagher, 1995; Laporte and Delph, 1996; Meagher and Delph, 2001). To further understand why females do not pay as high a cost of reproduction as males, we investigated the hypothesis that investment in nectar differs between the sexes and, as a consequence, an accurate estimate of each sex's allocation to reproduction requires quantification of investment in nectar. If males allocate more to nectar than females, then allocation to reproduction that is estimated by biomass alone will be underestimated.

Herein, we report nectar volume, nectar concentration and total nectar sugar produced per flower in eight paternal half-sib families of *S. latifolia* grown in a greenhouse. We present evidence of genetic variation in per-flower nectar production and total sugar content in females. We show that estimated whole-plant investment in nectar is greater in males than in fully pollinated females after 8 weeks of flowering. Also, a simple model predicts that whole-plant investment in nectar by males will be higher than that of females under low to moderate levels of fruit-set.

METHODS AND MATERIALS

Study species

Silene latifolia is a dioecious perennial that is native to Eurasia but naturalized in North America (McNeill, 1978). Seeds were collected from 12 females in a population near Blacksburg (Giles County), Virginia. These seeds were grown in a greenhouse and flowers on females were hand-pollinated with pollen from one non-sibling male. One or two fruits were collected from each female, with each fruit containing seeds of a full-sibling family. Seeds from these fruits were grown in a greenhouse at Indiana University, where controlled crosses were conducted between 9 males and 24 females. No crosses between plants with the same paternal or maternal parents were carried out.

Seeds used for this study were from crosses between all nine paternal parents and seven maternal plants. Three maternal parents were crossed with each paternal parent. Poor germination resulted in the elimination of one paternal family (4) from the study.

Seeds were placed on the surface of soil-less Metromix 360 (Scotts Horticultural Products, Marysville, OH) in cavity trays kept in approximately 1 cm standing water on 4 May. On 15 June, seedlings were transplanted into 5-inch plastic pots filled with a 2 : 1 mix of MetroMix 360 and Scotts 3 organic compost. After transplanting to larger pots, we fertilized once per week with Peters 20-20-20 fertilizer. Plants were watered when dry and we randomized the position of all pots weekly.

The first plant began flowering on 30 June. We were interested in determining the effect of pollination on nectar secretion in flowers on females. Females from each cross were therefore either pollinated or left unpollinated, based on when they began flowering. That is, if two females of one cross began flowering in the first week, one was pollinated and one was not. Styles of flowers on pollinated females were rubbed by anthers from two males of a different cross. Usually we pollinated on the first morning the flowers were open but sometimes, if the stigma lobes were not open fully, we waited until the second morning.

Since we wanted to estimate whole-plant investment in nectar, we counted the number of flowers produced by each plant over a given period. We recorded when each plant began flowering, and collected and counted all abscising buds, male and female flowers and dehiscing fruits daily. Eventually, some plants were removed from the study to more or less equalize sample sizes of males, unpollinated females and pollinated females. Plants kept in the experiment were chosen haphazardly based on when they began flowering. Plants were harvested after they had been flowering for approximately 8 weeks. Flowers and immature fruit and flowers were collected at that time. Fruit number is the sum of both mature fruits collected before harvest as well as immature fruit collected at harvest.

Nectar collection

Microcapillary tubes were used to collect as much nectar as possible from each flower. Unfortunately, repeated sampling of flowers was not possible since comprehensive collection of nectar resulted in flower damage. The length of the microcapillary tube occupied by nectar was measured by digital calipers (Mitutoyo Corporation, Japan) and then the nectar was released onto small filter-paper wicks. Wicks were air-dried for later photometric analysis. Since the flowers of *S. latifolia* consistently open in the evening around 20.00 h (L. Delph, unpublished data) and produce nectar in the evening, nectar was

collected early in the morning (between 05.00 and 08.00 h) with the goal of preventing as much evaporation as possible. After nectar was collected from a flower, the flower was placed in a small vial for later determination of dry mass.

Comparisons of the sexes

We paired male and female full-siblings that began flowering at approximately the same time, in order to compare nectar traits between the sexes. Our first set of measurements (Experiment 1) was made on plants that had been flowering for 20 days, and our second set of measurements (Experiment 2) was made on plants that had been flowering for 43 days.

In Experiment 1, fully expanded floral buds were tagged in late afternoon on 28 pairs of males and females. The next morning, tagged buds that had opened the previous evening were haphazardly assigned to a nectar collection time of 12, 36, 60 or 84 h. On females, if there was only one open flower, nectar was collected the second morning after it opened (hereafter referred to as a 36-h flower) or the third morning after it opened (hereafter referred to as a 60-h flower). If there were two open flowers, nectar was collected from one flower at 36 h and one flower at 60 h. If there were three open flowers on a female, nectar was collected at 36, 60 and 84 h. On males, if only one flower opened, nectar was collected after 36 h of flowering. If two flowers opened, nectar was collected after 12 and 36 h, and if three or more flowers opened, nectar was collected at 36, 60 and 84 h. Only rarely were there enough open flowers on a plant to have replicates for any one collection time. We sampled 1–6 flowers per plant (mean = 3 flowers, $n = 56$, standard error = 0.2).

For Experiment 2, our goal was to collect nectar from six flowers on 44 pairs of male and female full-sibs. However, it was not always possible to collect nectar from six flowers on a plant, either because of early flower abscission or a slow rate of flower production. Some plants used in Experiment 1 were also used in Experiment 2. In haphazard order, we selected two 12-h flowers on the first morning they were open on males, two 36-h and two 60-h flowers from each male and female, and two 84-h flowers from females. It took an average of 6.6 days (standard error = 0.2, $n = 87$, range = 3–13) to collect the nectar from a plant.

Effect of pollination

Two bouts of measurements, referred to as Experiments 3 and 4, were designed to determine the effect of pollination on nectar traits of pistillate flowers. For Experiment 3, we collected nectar between 27 July and 19 August from pollinated flowers on 34 females 36 h after their opening. These flowers were from plants which had all of their flowers pollinated on the first morning they were open (i.e. 12-h flowers). Hence, nectar collection occurred 24 h after pollination. On 21 of the plants, we also attempted to collect nectar from a second pollinated flower 60 h after opening and 48 h after pollinating. We compared nectar traits of these pollinated flowers with unpollinated flowers studied between 13 August and 1 September in Experiment 2.

For Experiment 4, which ran from 3 to 6 September, we haphazardly selected both pollinated and unpollinated females that had at least two fully expanded floral buds and tagged them. If both tagged buds were open the next morning, we pollinated one of the flowers and then collected nectar 24 h later (i.e. collected nectar from a 36-h flower). Sample size was 18 plants.

Nectar analysis

We followed the anthrone procedure originally described by McKenna and Thomson (1988) and Thomson *et al.* (1989) and outlined by Kearns and Inouye (1993, pp. 176–177). This procedure estimates total carbohydrate production. Sugar standards with equal amounts of fructose and glucose were prepared in concentrations ranging from 10 to 220 μg sugar per 2 ml. Standards were re-used for approximately 2 weeks. Standard regressions (absorbance versus sugar concentration) were determined separately for each of 39 sample runs.

Filter-paper wicks were placed into 5 ml boiling distilled water for 1 min to dissolve the dried nectar. Two millilitres of the wick solution and 4 ml of anthrone diluted with H_2SO_4 were placed in a test tube and boiled for 10 min. Absorbance was read after tubes cooled to 25°C. For many samples further dilution was necessary to remain within the range of the spectrophotometer; dilutions were either 1 : 1, 1 : 3 or 1 : 9 and final concentrations were multiplied by 2, 4 and 10, respectively.

Reported values are based on linear regressions of absorbance versus sugar concentration of the standards. For 30 of 39 sample runs, absorbance of the standards was determined twice, both before and after the sample run, without any substantial change in the results. For the remaining sample runs, absorbance of the standards was determined once, before the sample run. The mean correlation coefficient of the linear regression of absorbance versus standard sugar concentration was 0.994 ($n = 39$, standard error = 0.001). After determining carbohydrate concentration ($\mu g \cdot ml^{-1}$) of the samples, we multiplied it by 5 to arrive at the total amount of sugar originally collected from the flower. Total sugar was then divided by volume in microlitres to determine nectar sugar concentration in the flower. In nine instances, sugar concentration based on the linear regression of the standards was a negative number. We treated these as missing values.

Whole-plant investment in nectar

First, we estimated whole-plant investment in nectar sugar for males and unpollinated females sampled in Experiment 2 by multiplying the number of flowers by the mean total sugar produced by 60-h flowers for males and 84-h flowers for females, averaged over each plant sampled. Secondly, we estimated whole-plant investment in nectar of all plants in the study by multiplying the number of reproductive units per plant (flowers of males, flowers of unpollinated females and aborted flowers plus fruits of pollinated females) by family means of total nectar sugar content from Experiments 1 and 2. We knew that most flowers had been pollinated by the second morning after opening, so we assumed that maximum nectar investment per pollinated flower was equal to the average 36-h unpollinated flower. Furthermore, we conservatively assumed no sugar reabsorption.

We also developed a simple model to predict the level of fruit-set at which male whole-plant investment in nectar would exceed that of females. Sugar investment by males was calculated by multiplying paternal-family means of flower number and sugar content in 60-h flowers (averaged over all flowers in both Experiments 1 and 2). For females, flower and fruit number at different levels of fruit-set were estimated based on average flower number of unpollinated females and average fruit number of pollinated females in each paternal family. Whole-plant investment in nectar sugar for females was calculated by multiplying mean paternal-family sugar content of 84-h flowers and 36-h flowers by estimated values of flower and fruit number, respectively.

Data analysis

Data from Experiment 2 were analysed using random regression (Lindsey, 1993; Littell *et al.*, 1996; Singer, 1998) in PROC MIXED in SAS (V 9.1; SAS Institute, Inc., 2003). Random regression models were fit individually grouped by plant, with random intercepts and slopes. Flower age and sex were included in the model as fixed factors, while paternal and maternal parents were treated as random factors. Flower mass was included as a continuous variable. Non-significant interactions were removed. If a term was involved in an interaction but was not significant itself, the term was retained to ensure the traditional meaning of an interaction (as a deviation from the main effects).

RESULTS

Nectar volume and total sugar content

Flower mass did not differ significantly between the sampling periods, except for 36-h flowers from females, which were smaller in the second sampling period (Table 1). Flowers

Table 1. Mean values (± standard error) in Experiment 1 (nectar collected between 26 July and 15 August) and Experiment 2 (nectar collected between 13 August and 1 September)

	Females			Males		
Trait	Experiment 1	Experiment 2	*t*	Experiment 1	Experiment 2	*t*
Flower mass (mg)						
12 h				9.1 ± 0.33	9.1 ± 0.33	0.03
36 h	30.7 ± 1.38	26.6 ± 0.68	2.7**	8.7 ± 0.47	8.5 ± 0.19	0.54
60 h	32.4 ± 1.30	29.8 ± 0.71	1.8	7.8 ± 0.41	7.8 ± 0.21	1.5
84 h	28.8 ± 1.13	30.6 ± 0.80	1.2			
Nectar volume (μl)						
12 h				0.5 ± 0.11	0.3 ± 0.04	2.21*
36 h	2.9 ± 0.54	1.9 ± 0.17	0.70	0.8 ± 0.12	0.5 ± 0.06	2.28*
60 h	3.3 ± 0.37	3.0 ± 0.19	1.86	1.1 ± 0.22	0.7 ± 0.09	1.53
84 h	4.5 ± 0.52	3.5 ± 0.20	1.78			
Nectar sugar concentration ($\mu g \cdot \mu l^{-1}$)						
12 h				645 ± 39.4	555 ± 27.4	1.88
36 h	413 ± 31.4	485 ± 21.4	1.90	570 ± 48.8	736 ± 88.5	1.64
60 h	421 ± 27.9	513 ± 19.0	2.73**	586 ± 83.3	710 ± 43.9	1.32
84 h	397 ± 27.7	583 ± 40.1	3.82***			
Total nectar sugar content (μg)						
12 h				327 ± 55.5	177 ± 21.2	2.5*
36 h	1057 ± 148.4	849 ± 77.0	1.25	452 ± 67.7	338 ± 37.4	1.5
60 h	1289 ± 144.3	1511 ± 112.5	1.22	589 ± 103.3	505 ± 65.1	0.68
84 h	1663 ± 189.9	1949 ± 145.3	1.20			

Note: Results of *t*-tests comparing the two data sets are also shown. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

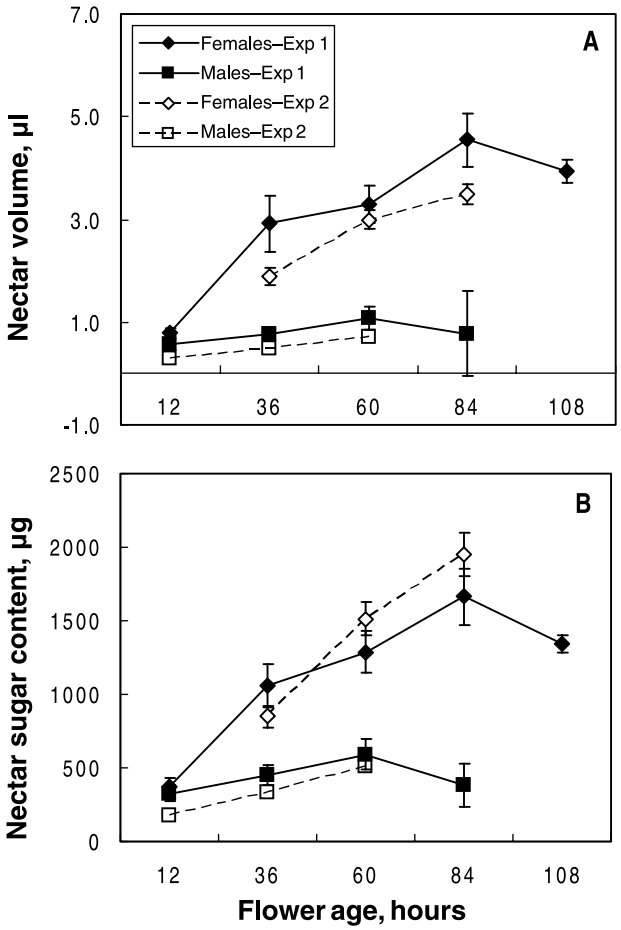

Fig. 1. Change in nectar volume (μl) and sugar content (μg) (± 1 standard error) with flower age in males and females during two sampling periods of *Silene latifolia*. Nectar was collected between 26 July and 15 August for Experiment 1 and between 13 August and 1 September for Experiment 2.

in the first sampling period (Experiment 1) tended to have higher nectar volumes than those in the second sampling period (Experiment 2), but this was only significant for 12-h and 36-h flowers from males (Table 1, Fig. 1A). A tendency for higher total sugar content during the first sampling period was also evident in males but not in females, and this difference was significant for 12-h flowers from males (Table 1, Fig. 1B). Nectar concentration was significantly different between the two sampling periods only for 60-h and 84-h flowers from females (Table 1).

Nectar volume and total sugar content increased with flower age up to a maximum level at 60 h for flowers on males and 84 h for flowers on females (Fig. 1). Nectar volume and total sugar increased at a faster rate in flowers on females than it did in flowers on males, particularly between 12 and 36 h (Fig. 1), resulting in significant interactions between flower age and sex (Tables 2 and 3).

Table 2. Analysis of nectar volume from Experiment 2 using random regression (model $R^2 = 0.814$)

Dependent variable	d.f.	*F*	*P*
Flower age	1, 389	102.20	<0.001
Sex	1, 389	2.85	0.092
Flower age × sex	1, 389	16.72	<0.001
Maternal parent	5, 32	2.68	0.040
Paternal parent	7, 21	1.35	0.278
Paternal parent × sex	7, 389	2.22	0.032
Flower mass	1, 389	43.15	<0.001

Table 3. Analysis of total nectar sugar content from Experiment 2 using random regression (model $R^2 = 0.703$)

Dependent variable	d.f.	*F*	*P*
Flower age	1, 362	90.58	<0.001
Sex	1, 362	3.36	0.068
Flower age × sex	1, 362	11.75	<0.001
Maternal parent	5, 32	1.88	0.125
Paternal parent	7, 21	2.01	0.102
Paternal parent × sex	7, 362	2.33	0.025
Flower mass	1, 362	23.83	<0.001

Based on peak values, an average flower on females produced 4–5 times more nectar that was 1.4–1.5 times less concentrated than an average flower on males (Table 1, Fig. 1). Repeated-measures analysis of variance (ANOVA) of 60-h flowers on males and 84-h flowers on females indicated nectar sugar concentration differed between the sexes ($F_{1,50} = 5.813$, $P = 0.020$). As a consequence of higher volume but lower concentration, females produced 2–3 times more total nectar sugar per flower than did males (Table 1, Fig. 1). Surprisingly, sex was not a significant factor in explaining variation either in nectar volume (Table 2) or total sugar content (Table 3). However, in both cases the effect of sex had a P-value < 0.1. Apparently, a large fraction of the variation could be explained by factors in the models other than sex, particularly flower mass.

Paternal family and sex interacted to affect both nectar volume (Table 2) and total sugar content (Table 3). Repeated-measures ANOVA showed that nectar volume varied among paternal families for females ($F_{7,18} = 3.783$, $P = 0.011$) but not for males ($F_{7,15} = 1.705$, $P = 0.182$). Similarly, paternal family significantly affected total nectar sugar content in 84-h flowers from females ($F_{7,17} = 3.778$, $P = 0.012$) but not in 60-h flowers from males ($F_{7,13} = 0.707$, $P = 0.667$). Nectar volume was also significantly affected by maternal parent (Table 2). Thus, the extent of sexual dimorphism in nectar volume and total sugar content varied among paternal families (Fig. 2).

Flower dry mass had a strong impact on both nectar volume and total sugar content (Tables 2 and 3). The main source of this effect was undoubtedly the large difference in flower mass between the sexes, with flowers from females weighing over three times more than flowers on males (Table 1). However, pistillate flower mass also contributed to the

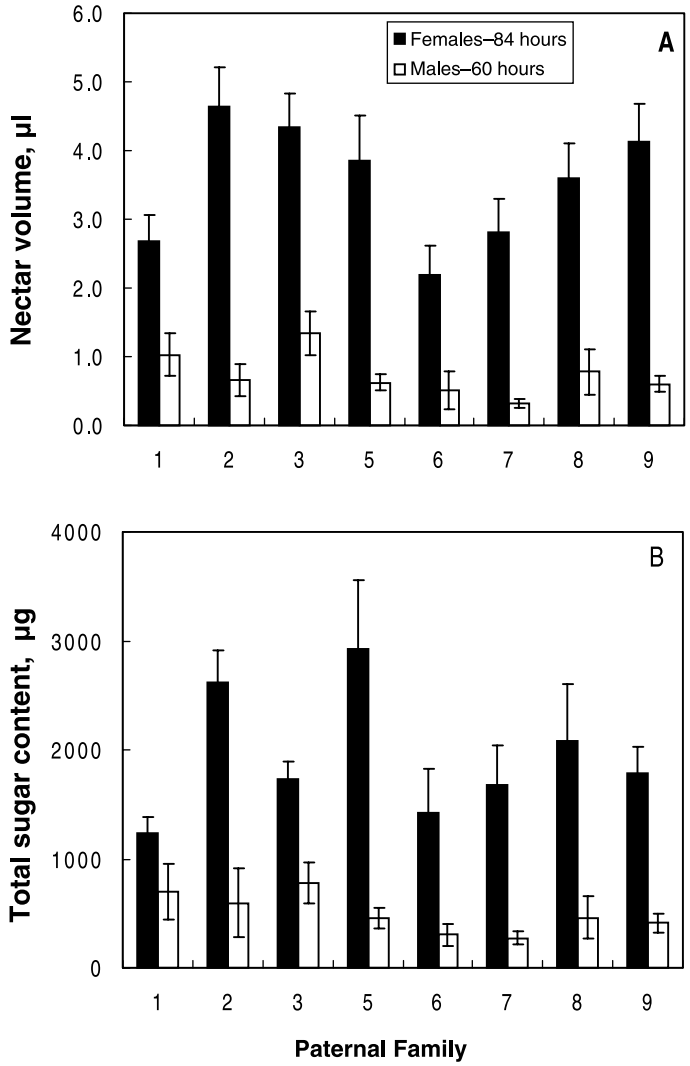

Fig. 2. Nectar volume (μl) and sugar content (μg) (± 1 standard error) of males and females in eight paternal families of *Silene latifolia* in Experiment 2.

effect. Nectar volume increased with flower mass in females (using just one 84-h flower from each female, $r = 0.400$, $n = 39$, $P = 0.012$) but not in males (using just one 60-h flower from each male, $r = 0.202$, $n = 38$, $P = 0.223$). Dry mass of flowers was also correlated with total sugar content in females ($r = 0.447$, $n = 39$, $P = 0.004$) but not in males ($r = 0.167$, $n = 36$, $P = 0.330$).

Effect of pollination on nectar production

Pollination curtailed nectar production in flowers on females. In Experiment 3, flowers were pollinated on the first morning they were open. Twenty-four hours after pollination, 44% of

the pollinated flowers (15/34) had no nectar at all and mean nectar volume was only 0.3 μl (standard error = 0.11, $n = 34$). In comparison, unpollinated flowers sampled in Experiment 2 contained 1.9 μl of nectar (standard error = 0.17, $n = 86$). This difference between pollinated and unpollinated flowers was significant ($t_{118} = 5.458$, $P < 0.001$). No flowers sampled 48 h after pollination in Experiment 3 had nectar ($n = 18$). In Experiment 4, pollinated flowers had significantly less nectar than did unpollinated flowers from the same female (Table 4). In contrast with Experiment 3, all pollinated flowers had some nectar.

Nectar sugar concentration did not differ between pollinated and unpollinated flowers on females in Experiment 3. Sugar concentration of pollinated flowers was 426 $\mu g \cdot \mu l^{-1}$ (standard error = 49.6, $n = 19$), whereas sugar concentration in unpollinated 36-h flowers from Experiment 2 averaged 485 $\mu g \cdot \mu l^{-1}$. In contrast, sugar concentration of pollinated flowers in Experiment 4 was lower than in unpollinated flowers (Table 4).

The total sugar content of pollinated flowers that contained nectar in Experiment 3 averaged 214 μg per flower (standard error = 59.3, $n = 19$). This value was significantly lower than the total sugar content of unpollinated 36-h flowers from Experiment 2, which averaged 859 μg of sugar per flower ($t_{102} = 6.534$, $P < 0.001$). In Experiment 4, total sugar content was also significantly higher in unpollinated flowers (Table 4).

Flower and fruit number

The average number of flowers produced by males, unpollinated females and pollinated females was 485 (standard error = 17.7, $n = 66$), 125 (standard error = 4.1, $n = 67$) and 28 (standard error = 1.4, $n = 60$), respectively, after 57 days of flowering. Flower number varied significantly among paternal families for both males ($F_{7,65} = 2.910$, $P = 0.014$) and unpollinated females ($F_{7,67} = 2.251$, $P = 0.048$) (Table 5). However, fruit number on pollinated females did not vary among paternal families ($F_{7,60} = 0.686$, $P = 0.683$) (Table 5).

Whole-plant investment in nectar sugar

In the subset of plants from which nectar was sampled, estimated whole-plant nectar sugar production was not affected significantly by paternal family, maternal family or sex. Estimated nectar sugar production was 253 mg (standard error = 45.3, $n = 42$) of sugar for males and 252 mg (standard error = 25.9, $n = 44$) for unpollinated females.

When we broadened our estimation of whole-plant investment in nectar to all plants in the study, the results indicated that whole-plant nectar sugar production differed among males, pollinated females and unpollinated females ($F_{2,152} = 208.7$, $P < 0.001$). A *post-hoc*

Table 4. Nectar volume, sugar concentration and total sugar content in pairs of pollinated and unpollinated flowers (mean ± standard error)

Nectar trait	Pollinated flower	Unpollinated flower	n	Results of paired t-tests (P)
Volume (μl)	0.55 ± 0.089	1.08 ± 0.225	17	2.527 (0.022)
Concentration ($\mu g \cdot \mu l^{-1}$)	281.14 ± 31.90	450.21 ± 59.21	16	3.280 (0.005)
Total sugar content (μg)	173.62 ± 33.62	474.44 ± 133.67	16	3.829 (< 0.001)

Table 5. Flower and fruit number in eight paternal half-sib families of *Silene latifolia* (mean ± standard error)

	Males		Unpollinated females		Pollinated females	
Family	*n*	Mean no. flowers	*n*	Mean no. flowers	*n*	Mean no. fruit
1	6	546 ± 20.5	7	114 ± 13.5	7	32 ± 5.6
2	11	426 ± 36.2	11	128 ± 9.0	9	32 ± 4.4
3	8	509 ± 508.8	8	116 ± 11.5	8	27 ± 3.3
5	10	373 ± 40.2	10	119 ± 7.7	10	37 ± 3.6
6	10	594 ± 41.0	10	136 ± 10.7	8	33 ± 4.4
7	5	395 ± 76.0	5	137 ± 5.9	4	36 ± 4.1
8	9	568 ± 31.8	7	146 ± 20.4	6	33 ± 2.9
9	7	460 ± 43.0	9	111 ± 11.5	8	28 ± 5.3

test with a Bonferroni adjustment showed that the values for pollinated females were significantly different from those for males and unpollinated females, which did not differ from each other. Estimated values indicated that males produced about 254 mg (standard error = 22.9, $n = 66$ plants) of sugar in their floral nectar and unpollinated females produced about 242 mg (standard error = 14.3, $n = 67$) during 8 weeks of flowering, whereas pollinated females produced only about 33 mg sugar (standard error = 2.6, $n = 60$).

Modelling how fruit-set influences whole-plant investment in nectar suggested that fruit-set effects on nectar investment would differ among paternal families. For some families, whole-plant investment in nectar would be higher in males than in females regardless of whether females set fruit or not (Fig. 3). These families (1, 3 and 8) had males that produced large numbers of flowers (Table 5). At the other extreme, moderately high fruit-set would be necessary in some families before whole-plant investment in nectar of males exceeded that of females (Fig. 3). These families (5 and 7) had males that produced relatively few flowers (Table 5).

DISCUSSION

Nectar volume and total nectar sugar content increased over the lifespan of flowers on males (3 days) and for the first 4 days for flowers on females (the lifespan of unpollinated pistillate flowers in this experiment was typically 5 or 6 days). As flowers aged, nectar volume and sugar content increased to a much greater extent in flowers on females than those on males. Differences between the sexes in per-flower nectar production may reflect the relatively high cost of producing an individual pistillate flower. Pistillate flowers of *S. latifolia* females are typically two to three times larger in terms of dry mass than staminate flowers of males (Gehring and Linhart, 1993; Delph and Meagher, 1995; current study), and they contain more nitrogen and phosphorus (Carroll and Delph, 1996). We suggest that selection may have resulted in prolonged nectar production in the longer-lived pistillate flowers as a way of improving the odds of having these relatively expensive flowers fertilized. The most effective pollinators of *S. latifolia* are night-flying moths such as hawkmoths (Young, 2002). While olfactory and visual cues are essential for hawkmoth attraction (White *et al.*, 1994; Kelber, 2002; Raguso and Willis, 2002), Hodges (1995) showed that

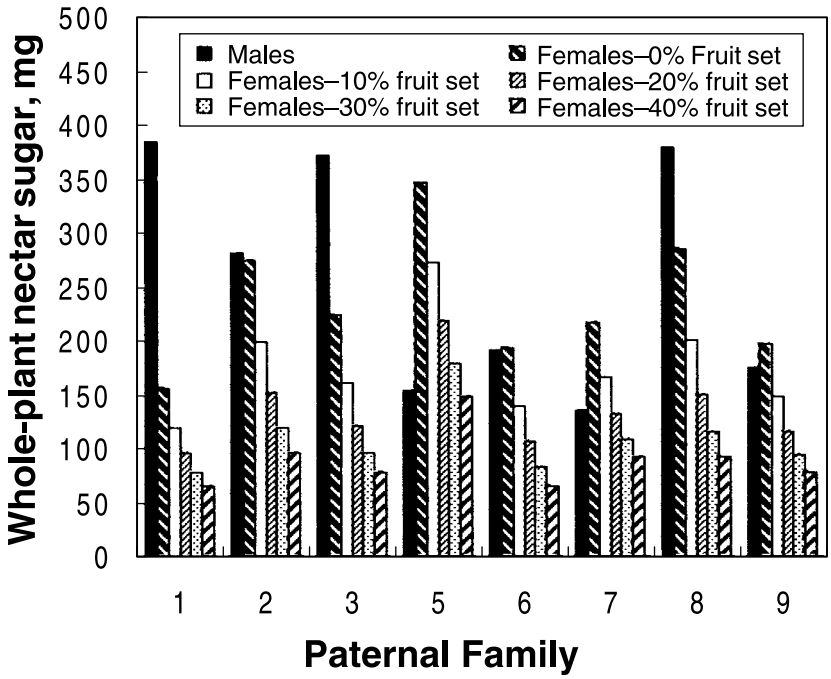

Fig. 3. Predicted values of per-plant nectar cost (mg), based on paternal family means of flower sugar content and flower and fruit number. Numbers of flowers and fruits of pollinated females were estimated based on family means of flower number of unpollinated females and fruit number of pollinated females.

hawkmoths visit more flowers on plants with more nectar. Furthermore, if visit duration increases with nectar volume, more nectar may result in greater pollen deposition.

Flowers on females secreted more nectar with higher total sugar content than did those on males in this study. Our results contrast with those of Shykoff and Bucheli (1995), who reported that nectar volume did not differ between the sexes of *S. latifolia* and also that flowers on males had more sugar than those on females. Although methodological differences may partially explain the differences in results, sexual differences in total nectar sugar per flower have been found to vary dramatically among populations of this species (M. Arntz, E. Vozar and L. Delph, unpublished data). Differences between populations in nectar production or sugar content may evolve as a consequence of direct selection by pollinators or indirect selection on correlated traits. We detected genetically based variation even though we sampled only eight paternal families from one population. Genetic variation of this sort would allow the evolution of among-population differences in nectar production.

We observed higher sugar concentration in nectar of flowers on males and this was also observed by Shykoff and Bucheli (1995) for *S. latifolia* and Kay *et al.* (1984) for *Silene dioica*. One explanation for the similarity between these two closely related species could be that the trait is phylogenetically conserved. An alternative, non-mutually exclusive, hypothesis is that selection by pollinators results in the sexual difference in nectar concentration. The sexes may differ in their need to attract pollinators since female fitness is

expected to be limited by resources rather than pollen, whereas male fitness is limited by a male's ability to attract pollinators and export pollen (Bateman, 1948; Bell, 1985; Delph, 1996). Selection by pollinators may result in higher sugar concentration of flowers on males. Moreover, plant species that are hawkmoth-pollinated typically secrete large amounts of nectar because hawkmoths have high energy requirements (Heinrich and Raven, 1972; Cruden *et al.*, 1983; Opler, 1983; Haber and Frankie, 1989; Willmott and Burquez, 1996). Since individual staminate flowers have relatively small amounts of nectar, hawkmoths may discriminate against males unless they provide a more concentrated reward on a per-flower basis. Furthermore, female *S. latifolia* have few or no open flowers for much of the reproductive season while they are setting fruit. Thus attracting hawkmoths to a population may depend on the flower production of male plants. Higher nectar concentration in many small flowers may be how male *S. latifolia* provide a large visual display (there is a flower size–number trade-off; Delph *et al.*, 2004) and a rich enough nectar patch to provide hawkmoths with adequate energy intake to ensure continued visitation. Selection by pollinators may also explain the lack of genetic variation for nectar production in males. Genetic variation for nectar production may have been eliminated in males if attracting pollinators is more important to their reproductive success than it is for females.

Previous studies have documented a genetic component to nectar production (Hawkins, 1971; Hodges, 1993; Mitchell and Shaw, 1993; Shykoff and Bucheli, 1995; Klinkhamer and van der Veen-van Wijk, 1999; Leiss *et al.*, 2004), which is a difficult task given that nectar production or concentration is influenced by so many non-genetic factors (Mitchell, 2004). Environmental factors that influence nectar traits include flower age, position in an inflorescence, flower size and plant size (these factors were reviewed by Rathcke, 1992), rate of pollen removal (Castellanos *et al.*, 2002; but compare with Aizen and Basilio, 1998), plant water status (Zimmerman, 1983; Zimmerman and Pyke, 1988; Wyatt *et al.*, 1992; Boose, 1997; Carroll *et al*., 2001), humidity (Sampson and Cane, 1999) and nutrient availability (Campbell and Halama, 1993). The impact of many of these factors was decreased in our study, allowing us to detect genetic variation in per-flower nectar volume and sugar content in females. Although we did not detect a genetic correlation between nectar volume or sugar content and average dry mass of flowers in either males or females (results not shown), both nectar volume and sugar content were phenotypically correlated with flower mass in females. Future studies with more families may reveal a significant genetic correlation between flower size and nectar traits. Alternatively, genetic variation in nectar production in females may be linked to other differences between families, such as those that affect carbon gain or plant water status.

Our comparisons of pollinated and unpollinated females suggest nectar reabsorbtion in flowers after pollination, which has been reported in several species (Cruden *et al.*, 1983; Burquez and Corbet, 1991; Koopowitz and Marchant, 1998). During two separate sampling periods for females, nectar volume in pollinated flowers was significantly less than in unpollinated flowers. This may mean that nectar secretion ceased soon after pollination or that nectar was reabsorbed after pollination. Because we pollinated flowers in the morning, the plant may have received the signal of a full pollen load by evening, when nectar secretion would normally occur. We can conclude that evaporation was not a factor. Nectar concentration did not differ between pollinated and unpollinated flowers in Experiment 3. If evaporation were a factor, we would expect a higher sugar concentration. In Experiment 4, nectar sugar concentration was in fact lower in pollinated flowers.

One piece of evidence is suggestive of nectar reabsorption. Nectar concentration was lower in pollinated female flowers than unpollinated flowers in Experiment 4. If nectar production had simply ceased, we would not expect lower nectar sugar concentration in pollinated flowers. In any case, nectar reabsorbtion in natural populations will decrease nectar investment by females only if pollinator visitation rate is low, because otherwise the nectar will be removed by pollinators.

This study contributes to an understanding of why females of *S. latifolia* do not exhibit a higher cost of reproduction than males. Given that females allocate more biomass to reproduction (Lovett Doust *et al.*, 1987; Gehring and Linhart, 1993; Delph and Meagher, 1995) and have lower photosynthetic rates than males (Gehring and Monson, 1994; Laporte and Delph, 1996), a higher cost of reproduction might be predicted. Assuming at least some pollinator activity, males will experience a higher whole-plant nectar sugar investment than females, despite the higher per-flower sugar investment by females. Even in paternal families in which males produce relatively few flowers (e.g. paternal families 5 and 7), whole-plant nectar investment is higher in males than in females under conditions of moderate levels of fruit-set. In addition, other features of the reproductive biology of *S. latifolia* contribute to the inaccuracy of biomass allocation in estimating reproductive investment. These include fruit photosynthesis, which results in overestimation of the amount of carbon fixed by leaves that is invested in fruit for females (Laporte and Delph, 1996), and relatively high respiratory losses in the more ramified male inflorescences (L. Delph and M. Levri, unpublished data).

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