Relationship between floral longevity and sex allocation among flowers within inflorescences in Aquilegia buergeriana var. oxysepala (Ranunculaceae)

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RELATIONSHIP BETWEEN FLORAL LONGEVITY AND SEX ALLOCATION AMONG FLOWERS WITHIN INFLORESCENCES IN *AQUILEGIA BUERGERIANA* VAR. *OXYSEPALA* (RANUNCULACEAE)[1]

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Understanding the fitness of plants with inflorescences requires examining variation in sex allocation among flowers within inflorescences. We examined whether differences in the duration of the male and female phases of flowering lead to variation in sex allocation and reproductive success among flowers within inflorescences. In 2002 and 2003, we quantified floral longevity, floral sex allocation, and reproductive success between the first and the second flowers within inflorescences in a protandrous species, *Aquilegia buergeriana* var. *oxysepala*. Floral longevity was greater in the first flowers than in the second ones in both years. The male phase lasted longer, and the initial number of pollen grains and the number of pollen grains removed were greater in the first flowers than in the second ones in both years. Within first flowers, the number of pollen grains removed was greater in flowers that had longer male phases, thus duration of the male phase may positively affect male reproductive success in the first flowers. The female phase lasted longer and the number of ovules was greater in the first flowers than in the second only in 2002. However, seed production per flower and female phase duration in both years were not significantly related. The variation in the number of pollen grains among flowers in this species may be caused by the variation in male phase duration.

Key words: female phase duration; floral longevity; floral sex allocation; inflorescence; male phase duration; ovule; pollen grain.

Understanding fitness of plants with inflorescences requires examining the variation in sex allocation (ratio of ovules, pollen grains, and pollen to ovules) among flowers within inflorescences because fitness is the sum of the reproductive success of individual flowers. Variations in sex allocation among flowers within inflorescences have been reported for numerous hermaphroditic plants (Holtsford, 1985; Thomson, 1989; Nishikawa and Kudo, 1995; Brunet, 1996; Nishikawa, 1998; Kudo and Molau, 1999; Vogler et al., 1999; Ashman and Hitchens, 2000; Guitián et al., 2001; Kudo et al., 2001; Huang et al., 2002; Ishii and Sakai, 2002; Garcia, 2003; Huang et al., 2004; Kliber and Eckert, 2004). For example, Ishii and Sakai (2002) reported that early opening flowers have more ovules and lower pollen to ovule (P : O) ratios than late opening ones in *Narthecium asiaticum* (Liliaceae). A survey of the literature by Thomson (1989) revealed a decline in the number of ovules per flower from early to late opening or from proximal to distal position flowers in 13 of 15 species.

Variation in the mating environment (opportunity for pollen donation and/or receipt) and in resource availability selects for different sex allocation among flowers within inflorescences. Several factors have been proposed to explain the variation in sex allocation among flowers within inflorescences: dichogamy (Brunet and Charlesworth, 1995; Brunet, 1996; Huang et al., 2004), pollinator directionality (Wyatt, 1982; Brunet and Charlesworth, 1995; Kudo et al., 2001), temporal variation in floral display (Ishii and Sakai, 2002), variation in the risk of herbivores (Kliber and Eckert, 2004), architectural effects of inflorescences (Diggle, 1995, 1997; Wolfe, 1992, 2001), and loss of resources by maintenance respiration (Sakai and Harada, 2004). For example, Brunet and Charlesworth (1995) suggested that opportunities for pollen donation and/or receipt should differ among flowers within inflorescences in dichogamous species because the proportion of staminate-(or pistillate-)phase flowers should increase in the population with progress of the flowering season. Indeed, in *Aquilegia caerulea*, a protandrous species, Brunet (1996) reported that early opening flowers had more ovules than late opening ones. In the protogynous species *Aquilegia yabeana*, the pattern of floral sex allocation contrasts with that in *A. caerulea*: early flowers have more pollen grains than later ones (Huang et al., 2004).

However, although floral longevity influences the number of pollen grains removed or received (Primack, 1985), the effects of floral longevity on sex allocation have not yet been examined. Floral longevity is assumed to reflect a balance between the benefit of successful pollination and the cost of flower maintenance (Primack, 1985; Ashman and Schoen, 1996). The cost of flower maintenance includes nectar production following anthesis, floral respiration, and transpiration (Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995). The temperature during flowering should influence the degree of the last two costs: high temperature promotes floral respiration and transpiration (Primack, 1995), and floral longevity may decrease at high temperatures. Holtsford and Ellstrand (1992) have reported evidence suggesting shortened floral longevity due to high temperature. Such variation in floral longevity would occur among flowers within sequentially blooming inflorescences and, consequently, lead to a difference in the opportunities for pollen donation and/or receipt among the flowers.

The differences in floral longevity among flowers should differentially affect the reproductive success between the male

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and the female functions. In general, the female function of a flower is rapidly completed because most ovules are fertilized by relatively few pollinator visits (Bell, 1985; Bell and Cresswell, 1998). In contrast, pollen removal requires many visits by pollinators (Bell, 1985; Richardson and Stephenson, 1989; Yong and Stanton, 1990; Bell and Cresswell, 1998). Consequently, an increase in floral longevity may enhance male rather than female fitness through increased visits by pollinators. Thus, a difference in floral longevity among flowers within inflorescences might cause variation in floral sex allocation among those flowers.

In this study, we quantified the variation in floral longevity, duration of male and female phases, floral sex allocation (the numbers of pollen grains and ovules), and reproductive success (pollen grains removed and seed production) of flowers of inflorescences in *Aquilegia buergeriana* Sieb. et Zucc. var. *oxysepala* (Trautv. et Mey.) Kitam. Because flowers bloom from June to August in this species, flowers that open early are expected to last longer than ones that open later because of an increase in temperature as the flowering season progresses. *Aquilegia buergeriana* var. *oxysepala* is an ideal species in which to independently examine male and female functions because it is protandrous. Because a dichogamous species has relatively discrete pistillate and staminate phases, we can determine the durations of these phases of a flower in the species. Thus, we examined the relationship between the floral longevity and floral sex allocation among flowers within inflorescences.

MATERIALS AND METHODS

Study site and species—This study was conducted from June through August 2002 and 2003 in a natural population of *A. buergeriana* var. *oxysepala* (Ranunculaceae) at the Mt. Hakkoda Botanical Laboratory of Tohoku University in Aomori Prefecture, northern Honshu, Japan (40°38′ N, 140°51′ E, about 890 m a.s.l.).

Aquilegia buergeriana var. *oxysepala* is a perennial herb that grows at the edges of mountainous forest in Japan. One mature plant produces 2.7 ± 1.2 racemes (mean ± SD, $N = 142$ in 2002). The conspicuous purple flower consists of five petals with long nectar spurs that produce copious nectar. During June to August, each inflorescence bears 1.6 ± 0.5 flowers (mean ± SD, $N = 142$ in 2002). In this study, we examined the first inflorescences with two blooming flowers within a plant. The distal flowers almost always opened first, followed by the basal flowers. The first (= distal) and second (= basal) flowers within inflorescences discontinuously opened at intervals of 7.3 ± 1.6 days and lasted 5.5 ± 1.1 and 3.7 ± 0.8 days (mean ± SD, $N = 68$ in 2002), respectively. Thus, there was only a slight overlap in anthesis between the first and the second flowers within an inflorescence; in 2002 anthesis of the first and the second flowers overlapped within inflorescences in only two of 68 inflorescences. On the other hand, there were overlaps in the floral longevity in the different inflorescences: The first flowers opened from 22 June to 5 July in 2002 and from 24 June to 8 July in 2003, and the second ones opened from 30 June to 12 July in 2002 and from 25 June to 17 July in 2003 at the study site. Figure 1 shows the daily mean temperature during these periods in 2002 and 2003 at the site. This species is self-compatible and protandrous. The anthers (≥40 anthers) dehisce over 3–5 days, and the stigmas (4–6 carpels) are exposed for 2–4 days after the anthers dehisce. There are a few pollen grains in the anthers at the time that the stigmas are exposed. The 4–6 carpels mature into an aggregate of follicles in August. Flowers abundantly secrete nectar. Their main pollinators are *Bombus diversus diversus*. We observed that they pollinated 1.01 ± 0.05 flowers (mean ± SD, $N = 174$) per visit to a plant at our site in 2003. This result showed that there was little geitonogamous pollination within inflorescences in *A. buergeriana* var. *oxysepala*.

Floral longevity, male and female phase durations—We compared floral longevity and duration of male and female phases between the first and the second flowers within inflorescences in 2002 and 2003. We randomly selected 66 plants for the observation of the first and the second flowers in 2002, respectively, and 42 plants for the first flowers and 19 plants for the second flowers (flowers that aborted due to low temperature were excluded) in 2003. We observed those flowers every day and recorded the date of anthesis (dehiscence of the first anther), exposure of stigmas (end of male phase and start of female phase), and senescence (petal fall) of each flower. We defined the floral longevity as the duration from anthesis to senescence, the male phase of the flower as the duration from anthesis to exposure of the stigma, and the female phase of the flower as the duration from exposure of the stigma to senescence. These data were analyzed with the Mann-Whitney *U* test to compare floral longevity and duration of male and female phases between the first and the second flowers within inflorescences.

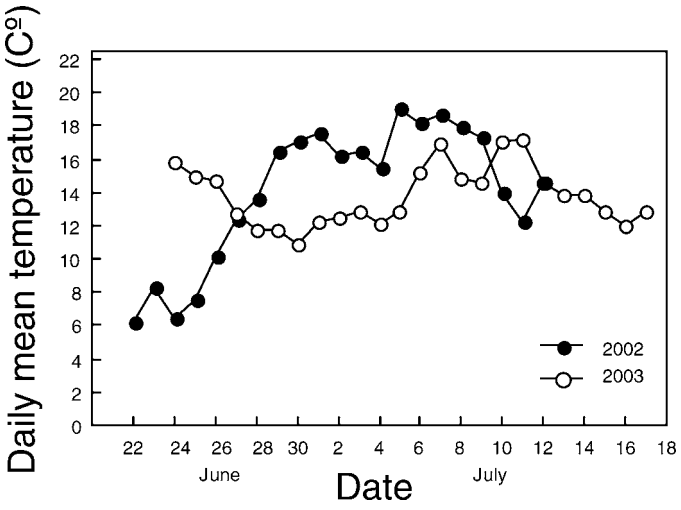

Fig. 1. Daily mean temperature from 22 June to 12 July in 2002 and from 24 June to 17 July in 2003 (flowering of first and second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*) at the study site (40°38′ N, 140°51′ E, about 890 m above sea level).

Floral sex allocation—We compared the number of ovules, pollen grains, and P : O ratios of the first and the second flowers within inflorescences in 2002 and 2003. We randomly selected 14 and 18 inflorescences in 2002 and 2003, respectively, and collected the first and the second flower buds of these inflorescences just before anthesis. We counted the numbers of anthers and ovules of each bud with a dissecting microscope. We estimated the number of pollen grains of a flower as follows: We collected five anthers from each bud because of the existence of numerous anthers (≥40 per bud), and placed these in 1.5-mL tubes with 1 mL of 0.1% saline. We then counted the number of pollen grains in two sets of 0.025-ml subsamples with a dissecting microscope. The number of pollen grains of a flower was estimated based on the average number of pollen grains of the two sets × 20 × the number of anthers of the flower divided by 5. We also calculated the P : O ratio (number of pollen grains/number of ovules) of each flower. To compare the number of ovules, pollen grains, and P : O ratios between the first and the second flowers within inflorescences, these data were analyzed with the Wilcoxon rank test because the first and the second flowers were sampled within an inflorescence.

Number of pollen grains removed—We compared the number of pollen grains removed from the first and the second flowers within inflorescences in 2002 and 2003 by the following procedure. We randomly selected 54 plants for the observation of the first and the second flowers in 2002, respectively, and 18 plants for the first flowers and 14 plants for the second flowers in 2003. We collected these flowers on their dayof stigma exposure (end of male phase) and counted the number of remaining pollen grains in all anthers of the flower with a dissecting microscope using the described methods. We then estimated the number of removed pollen grains for each flower by subtracting the number of remaining pollen grains from the average number of pollen grains per first (second) flower. These data were analyzed with the Mann-Whitney *U* test to compare the number of pollen grains removed between the first and the second flowers within inflorescences.

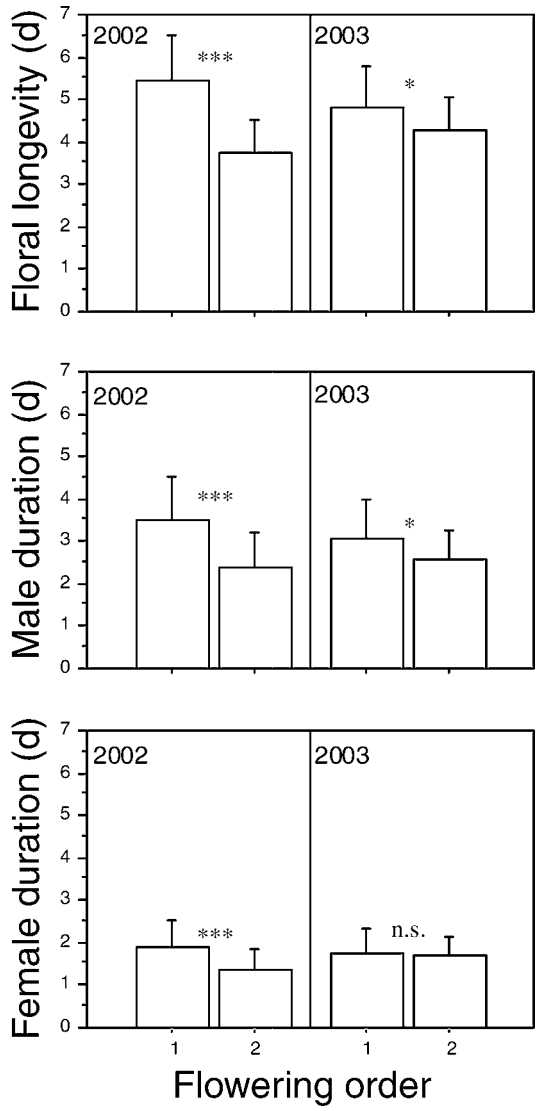

Fig. 2. Floral longevity, male and female phase durations in the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD ($N = 131$ in 2002 and $N = 61$ in 2003). The data were analyzed with the Mann-Whitney U test (*: $P < 0.05$; ***: $P < 0.001$; ns: not significant).

We also examined the effects of the duration of the male phase of a flower on pollen removal in 2002 and 2003. We compared these data between flowers with a male phase lasting 2 days and those with a male phase lasting 3 days. We analyzed these data for the first and the second flowers, respectively, because there were variations in the initial number of pollen grains of a flower between the first and the second flowers. In 2002, the number of pollen grains removed from the first flower and that removed from the second flower were based on 33 and 34 flowers, respectively. In 2003, the numbers for both the first and the second flowers were based on 14 flowers. These data were analyzed with the Mann-Whitney U test to compare the number of pollen grains removed between flowers with a male phase of 2 days and those with a male phase of 3 days.

Moreover, we examined the variation in the rate of pollen removal of the first and the second flowers within inflorescences. We compared these data between the first and the second flowers within inflorescences in 2002 and 2003. We analyzed these data for flowers with a 2-day male phase and those with a 3-day male phase because the length of the male phase may vary. In 2002, the number of pollen grains removed from the flowers with a 2-day male phase and those with a 3-day male phase were based on 51 and 16 flowers, respectively. In 2003, the number of flowers with a 2-day male phase and that for the flowers with a 3-day male phase were based on 10 and 19 flowers, respectively. These data were analyzed with the Mann-Whitney U test to compare the rate of pollen removal between the first and the second flowers.

Variation in the number of seeds produced by a flower—We compared the number of seeds produced by the first and the second flowers within inflorescences under open pollination in 2002 and 2003. We randomly selected 20 inflorescences in 2002 and 2003, respectively, collected all resulting fruits and counted the number of seeds for each fruit. These data were analyzed with the Wilcoxon rank test to compare the number of seeds produced by the first and the second flowers within inflorescences.

We also examined the effects of the duration of the female phase of a flower on the number of seeds produced for the first and the second flowers under open pollination in 2002 and 2003. We compared the number of seeds between flowers with a female phase duration of 1 day and those with a female phase duration of 2 days. In 2002, the numbers of seeds for the first and the second flowers were based on 35 and 26 samples, respectively. In 2003, the numbers of seeds of the first and the second flowers were based on 49 and 41 samples, respectively. These data were analyzed with the Mann-Whitney U test to compare the number of seeds between flowers with a female phase duration of 1 day and those with a female phase duration of 2 days.

Experimental manipulation of constraints of seed production—In 2003, we tested whether pollen quantity constrains seed production by comparing the number of seeds produced between naturally pollinated and hand-pollinated flowers. Hand-pollination of 35 first and 23 second randomly selected flowers was conducted with a mixture of pollen from a set of several pollen donors. The other flowers within the inflorescences were not manipulated.

In 2003 with a flower removal experiment, we tested whether interfloral competition for resources occurs. We randomly selected 40 inflorescences with two flower buds each and removed the first or the second flowers before anthesis (first flower removal, $N = 19$; second flower removal, $N = 21$). The other flowers within these inflorescences were naturally pollinated.

We evaluated the effect of this manipulation by comparing the number of seeds produced by the first (second) flowers and that produced by the control first (second) flowers with the Mann-Whitney U test.

RESULTS

Floral longevity, male and female phase durations—Floral longevity and male phase duration were significantly longer in the first flowers than in the second ones in 2002 and 2003 (Fig. 2). On average, the first flowers lasted 1.7 and 0.5 days longer than the second ones in 2002 and 2003, respectively. The male phase duration of the first flowers was 1.1 and 0.5 days longer on average than that of the second flowers in 2002 and 2003, respectively. A similar trend was observed for the difference in female phase duration between the first and the second flowers, but this trend was significant only in 2002 (Fig. 2). The female phase duration of the first flowers was 0.5 days longer on average than that of the second flowers in 2002.

Floral sex allocation—The number of pollen grains per flower was significantly greater in the first flowers than in the second ones in 2002 and 2003 (Fig. 3). The number of ovules per flower was, however, significantly greater in the first flowers than in the second ones only in 2002 (Fig. 3). As a result, the P : O ratios were significantly greater in the first flowers than in the second ones only in 2003 (Fig. 3).

Number of pollen grains removed—The number of pollen grains removed was significantly greater in the first flowers than in the second ones in 2002 and 2003 (Fig. 4).

For first flowers, the number of pollen grains removed was significantly greater in flowers with a male phase lasting 3 days

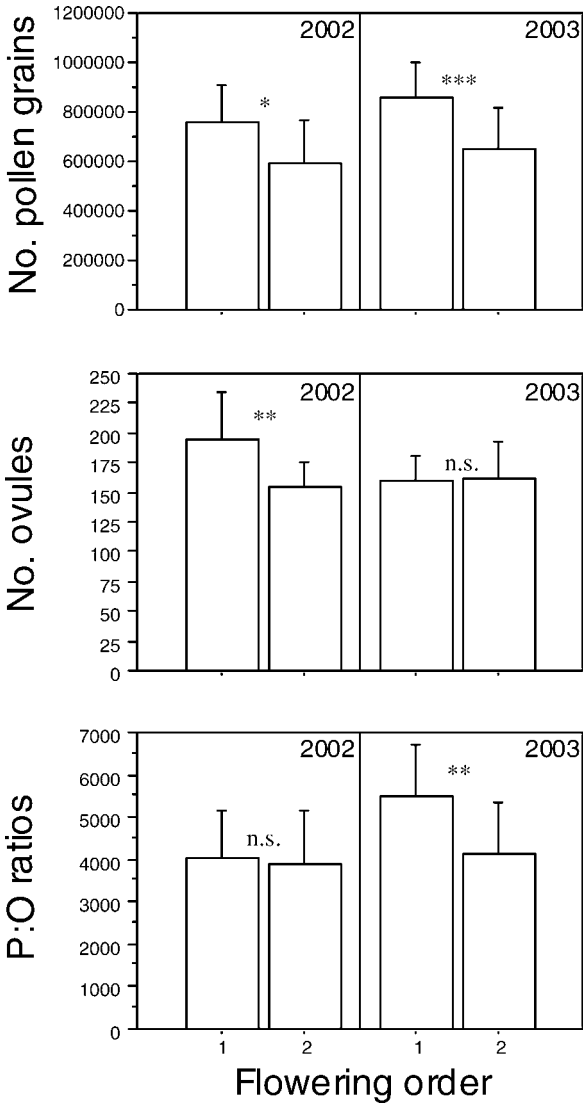

Fig. 3. Number of pollen grains (P) and ovules (O) per flower and P : O ratios in the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD ($N = 28$ in 2002 and $N = 36$ in 2003). The data were analyzed with the Wilcoxon rank test (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant).

than in those lasting 2 days in both 2002 and 2003 (Fig. 5). However, there was no significant relationship between flowers with a 2- vs. a 3-day male phase duration in the second flowers in 2002 and 2003 (Fig. 5).

In 2002, the rate of pollen removal was significantly greater in second flowers than in first ones for both those with a 2-day and those with a 3-day male phase (Fig. 6). In 2003, the rate of pollen removal was significantly greater in first flowers than in second ones for those with a 3-day male phase, while there was no difference for those with a 2-day male phase (Fig. 6).

Number of seeds produced—Significantly more seeds produced were produced for the first flowers than for the second ones in 2002 and 2003 (Fig. 7). However, the duration of the female phase did not significantly affect the number of seeds produced in either the first or the second flowers in 2002 and 2003 (Fig. 8).

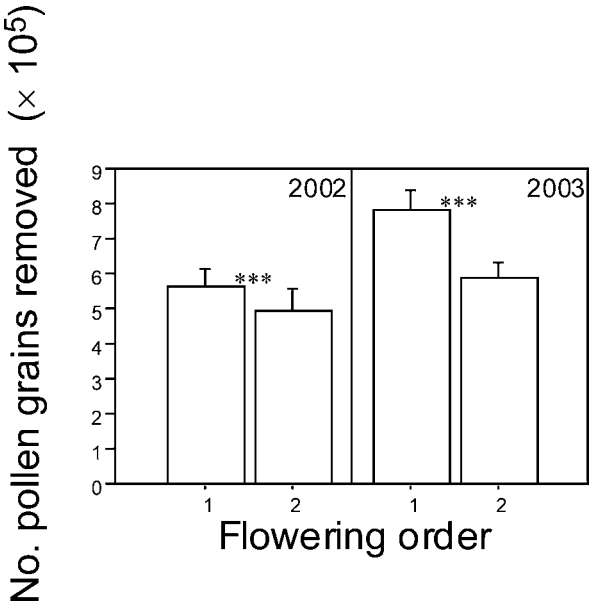

Fig. 4. Number of pollen grains removed from the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD ($N = 67$ in 2002 and $N = 26$ in 2003). The data were analyzed with the Mann-Whitney U test (***: $P < 0.001$).

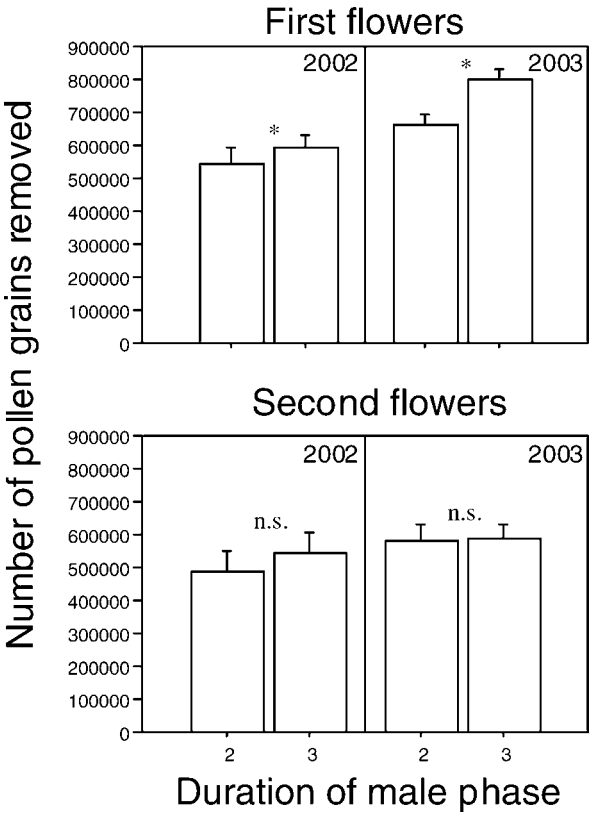

Fig. 5. Number of pollen grains removed during the male phase duration of 2 and 3 days for the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD (the first flowers: $N = 33$ in 2002 and $N = 14$ in 2003; the second flowers: $N = 34$ in 2002 and $N = 14$ in 2003). The data were analyzed with the Mann-Whitney U test (*: $P < 0.05$; ns: not significant).

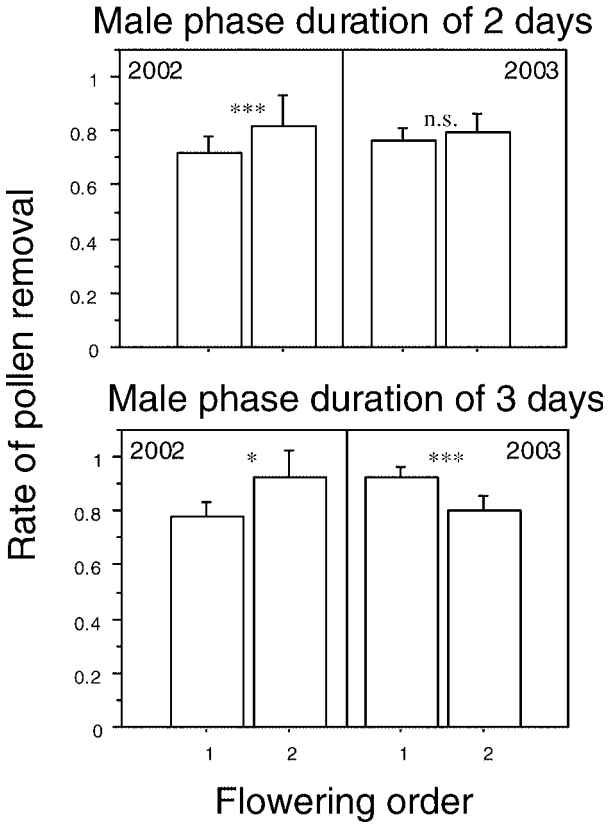

Fig. 6. Rate of pollen grains removed during the male phase duration of 2 and 3 days of the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD (the flowers with a 2-day male phase: $N = 51$ in 2002 and $N = 10$ in 2003; those with a 3-day male phase: $N = 16$ in 2002 and $N = 19$ in 2003). The data were analyzed with the Mann-Whitney U test (***: $P < 0.001$; *: $P < 0.05$; ns: not significant).

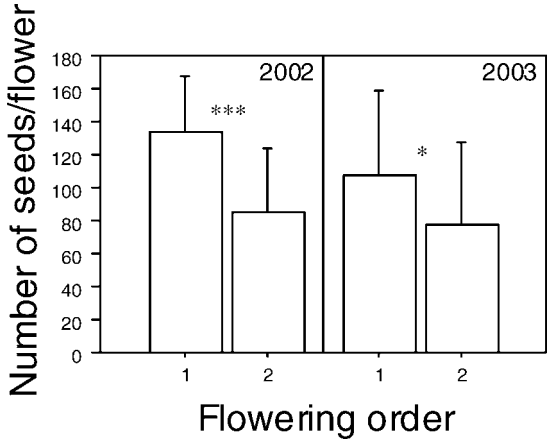

Fig. 7. Number of seeds in the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD ($N = 20$ in 2002 and 2003). The data were analyzed with the Wilcoxon rank test (***: $P < 0.001$; *: $P < 0.05$).

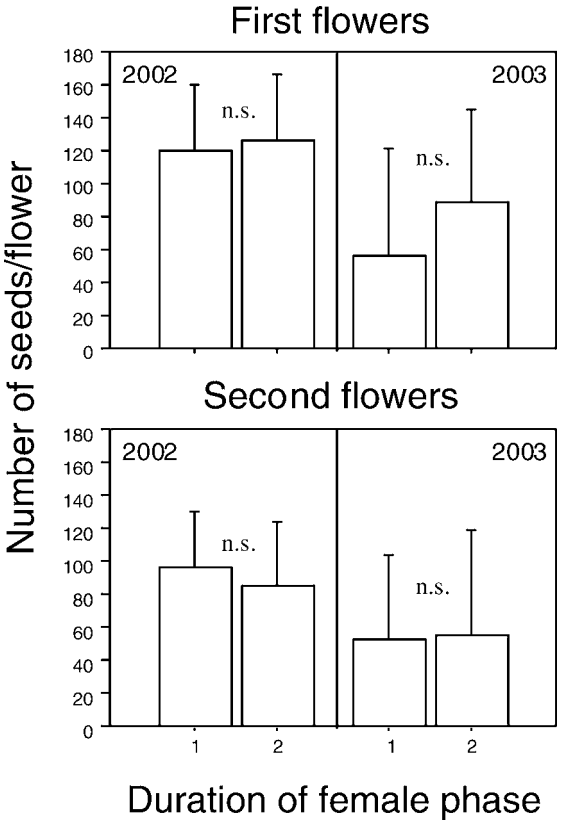

Fig. 8. Number of seeds in 1 and 2 days of the female phase duration of the first and the second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. Values represent means + SD (the first flowers: $N = 35$ in 2002 and $N = 49$ in 2003; the second flowers: $N = 26$ in 2002 and $N = 41$ in 2003). The data were analyzed with the Mann-Whitney U test (ns: not significant).

Experimental manipulation of constraints of seed production—The numbers of seeds produced by naturally and hand-pollinated first flowers were 85.80 ± 56.66 and 95.63 ± 49.60, respectively (mean ± SD), and the numbers of seeds produced by naturally and hand-pollinated second flowers were 60.91 ± 61.74 and 63.89 ± 59.22, respectively (mean ± SD). We found no significant differences in the number of seeds produced by naturally and hand-pollinated flowers in either the first or the second flowers (first naturally pollinated flowers, $N = 49$; first hand-pollinated flowers, $N = 35$, $U = 774$, $P = 0.4474$; second naturally pollinated flowers, $N = 17$; second hand-pollinated flowers, $N = 23$, $U = 194$, $P = 0.9774$). Thus, seed production was not limited by pollination in either the first or the second flowers.

The number of seeds produced in the case of nonmanipulation and that produced in the case of bud removal treatment in the first flowers were 85.80 ± 56.66 and 90.84 ± 57.10, respectively (mean ± SD), and the number of seeds produced in the case of nonmanipulation and bud removal treatment in the second flowers were 63.88 ± 59.22 and 28.14 ± 45.33, respectively (mean ± SD). We found no significant differences in the number of seeds produced in the case of nonmanipulation and bud removal treatment in either the first or the second flowers (first flower bud removal, $N = 19$; second nonmanipulated flower, $N = 49$, $U = 434$, $P = 0.6651$; first nonmanipulated flower, $N = 17$; second flower bud removal, N

$= 21$, $U = 124$, $P = 0.1096$). Thus, seed production was not limited by resource competition in either the first or the second flowers.

DISCUSSION

We have described the relationship between sex allocation and longevity for first and second flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. The results suggest that longer floral longevity may increase male reproductive success and vary the sex allocation between flowers within inflorescences. The variation in longevity among flowers within inflorescences may affect the fitness of a plant through differential male and female reproductive success in this species.

Variation in floral longevity, male and female phase durations—Findings that floral longevity and duration of male and females phases were longer in the first flowers than in the second ones within inflorescences (Fig. 2) may reflect seasonal variation in temperature (Fig. 1). Many previous studies have shown that high temperature decreases floral longevity (e.g., Motten, 1983; Schoen and Ashman, 1995; Ashman and Schoen, 1996; Ishii and Sakai, 2000), possibly due to the acceleration of floral respiration, transpiration (Primack, 1985; Ashman and Schoen, 1994, 1996; Schoen and Ashman, 1995), and deceleration of development of floral organs (Arroyo et al., 1981). Ashman and Schoen (1996) showed that the respiration cost of corollas was not negligible in *Clarkia gracilis tracyi* and *Trillium grandiflorum*. Respiration rates are almost certainly correlated with the temperature of the flower's environment. For example, in arctic species, growth and reproduction were enhanced in plants growing in open-top chambers in which the temperature was high (reviewed in Henry and Molau, 1997; Totland and Nyléhn, 1998). These findings indicate that the variation in floral longevity may be attributable to the seasonal change in temperature. In *A. buergeriana* var. *oxysepala,* flowers are open from June to July. Analysis of the residual variation showed that floral longevity of the first and the second flowers was correlated with daily average temperature (T. Itagaki; unpublished data). Therefore, maintenance of late opening flowers (the second flowers) may be more costly than that of early opening flowers (the first flowers) due to a seasonal increase in temperature (Fig. 1). However, there was a significant difference in the residual variation in floral longevity between the first and the second flowers (T. Itagaki; unpublished data). Thus, it is possible that the variation in floral longevity may not be explained only by temperature.

Variation in male function—In addition to the long duration of the male phase (Fig. 2), the number of pollen grains per flower was greater in the first flowers than in the second ones within inflorescences (Fig. 3). Moreover, we found that the number of pollen grains removed by pollinators was greater in the first flowers than in the second ones within inflorescences (Fig. 4). These results indicate that the first flowers invest more resources in male reproduction and gain higher potential for male reproductive success than the second ones within inflorescences.

The greater number of pollen grains removed in the first flowers than in the second ones within inflorescences was probably due to the long duration of the male phase, as indicated by our finding of a positive relationship between the male phase duration and the number of pollen grains removed only in the first flowers (Fig. 5). On the other hand, the number of pollen grains removed had no significant correlation with the duration of the male phase in the second flowers (Fig. 5). This result could be because pollen removal in the second flowers was completed within the 2 days of the male phase. However, the rate of pollen removal was not necessarily higher in the second flowers than in the first ones (Fig. 6). Therefore, the variation in the rate of pollen removal between the first and the second flowers within inflorescences may not affect the relationship between the duration of the male phase and the number of pollen grains removed in those flowers.

Previous studies have also shown that there are positive effects of the length of the staminate phase on the opportunities for pollen removal (Richardson and Stephenson, 1989; Young and Stanton, 1990; Bell and Cresswell, 1998; Ishii and Sakai, 2000; Sargent and Roitberg, 2000; Evanhoe and Galloway, 2002). These studies indicate that pollen removal requires many pollinator visits. In *A. buergeriana* var. *oxysepala* as well, the first flowers may have higher potential for male reproductive success due to the long duration of their male phase. Thus, the difference in male phase duration between the first and the second flowers within inflorescences may be an adaptive mechanism for the difference in the number of pollen grains per flower between the flowers in *A. buergeriana* var. *oxysepala*.

Variation in female function—The numbers of ovules and seeds per flower were greater in the first flowers than in the second ones within inflorescences (Figs. 2 and 5). However, there was no significant relationship between the duration of the female phase and the number of seeds of the flower (Fig. 8). This last result may agree with those of previous studies (Bell, 1985; Richardson and Stephenson, 1989; Young and Stanton, 1990; Bell and Cresswell, 1998; Ishii and Sakai, 2000; Sargent and Roitberg, 2000). For instance, Bell and Cresswell (1998) showed that the female function of a flower (reception of pollen grains on the flower's stigma) was more rapidly completed after exposure to pollinators than the male function (the proportion of remaining pollen grains in the anthers) in *Brassica napus*. These findings indicate that the length of a pistillate phase of a flower may not greatly affect the opportunities for receipt of pollen grains by the flower.

Other than the female phase duration, there was no evidence indicating that pollen quantity and resource constraints affected the number of seeds produced. Interfloral competition for resources may cause variations in sex allocation among flowers within inflorescences (Wyatt, 1982; Bookman, 1983; Lee, 1988; Diggle, 1995, 1997; Guitián and Navarro, 1996). For instance, early-produced flowers may have a competitive advantage in acquiring limited resources, thus leading to an increased allocation of resources to these flowers within inflorescences (Lee, 1988). If our bud removal treatment reduced interfloral competition, the number of seeds produced by the remaining flowers within these inflorescences could have been expected to increase. However, such removal had little impact on the number of seeds produced by the remaining flowers. Also, the quantity of pollen received may constrain the number of seeds produced. However, no effects of additional pollination on the number of seeds produced were found. These findings suggest that resource availability and pollen quantity

do not explain the variation in female sex allocation between the first and second flowers within inflorescences in *A. buergeriana* var. *oxysepala*. As a result, we were unable to determine the causes of the observed variation in numbers of ovules and seeds between those flowers within inflorescences.

Previous hypotheses—In *A. buergeriana* var. *oxysepala*, the male reproductive component (pollen grains) was greater in the first flowers than in the second ones within inflorescences, with a similar, but weaker, trend for the female reproductive component (ovules) (Fig. 3). The pattern of earlier opening flowers being male-biased is rare (but see Huang et al., 2004; Kliber and Eckert, 2004). The factors previously suggested as possible adaptive mechanisms for the variation in floral sex allocation (Brunet, 1996; Ashman and Hitchens, 2000; Kudo et al., 2001; Ishii and Sakai, 2002; Huang et al., 2004; Kliber and Eckert, 2004) fail to explain the pattern in *A. buergeriana* var. *oxysepala*. For instance, Brunet (1996) suggested that in *A. caerulea* early-opening flowers had more ovules than late-opening flowers because in protandrous species the number of female phase flowers should increase with progress of the flowering season. As a result, early-opening flowers may receive more pollen grains than late-opening ones from many male-phase flowers. However, in *A. buergeriana* var. *oxysepala*, a protandrous species, the greater number of pollen grains per flower in the first flowers compared with the second ones within inflorescences is not consistent with this prediction. Pollinator directionality within inflorescences (Kudo et al., 2001) and temporal variation in floral display size (Ishii and Sakai, 2002) does not occur in *A. buergeriana* var. *oxysepala*: We rarely observed an overlap of anthesis (see Materials and Methods) nor geitonogamous pollination and variation in attractiveness to pollinators between flowers within an inflorescence. Furthermore, architectural effects have not been elucidated in *A. buergeriana* var. *oxysepala*; such effects may result from less vasculature at distal positions within inflorescences (Diggle, 1995, 1997; Wolfe, 1992, 2001). However, in *A. buergeriana* var. *oxysepala* the first flowers rather than the second ones are distal. In addition, we found no indication of differences in overall floral organ mass between the first and the second flowers within inflorescences (T. Itagaki; unpublished data). Thus, we found no evidence for resource constraints due to architectural effects on the floral sex allocation in this species.

Conclusions—Floral longevity and sex allocation were found to vary within inflorescences in *Aquilegia buergeriana* var. *oxysepala*. In this species, a seasonal variation in temperature may cause variation in floral longevity between the first and the second flowers within inflorescences. Our findings also suggest that variation in the male phase duration of a flower may be positively correlated with the number of pollen grains removed from a flower. Therefore, the longer duration of male phase and the greater number of pollen grains per flower in the first flowers compared with the second ones may reflect adaptations to specific mating opportunities. In contrast, the variation in the female phase duration may not affect allocation to the female function. We found that the variation in floral sex allocation was unlikely to be caused by the factors suggested in previous studies, such as dichogamy, pollinator directionality, temporal variation in floral display, and resource constraint. Further study is needed to determine what factors produce variation in the number of ovules and seeds produced among flowers within inflorescences.

LITERATURE CITED

ARROYO, M. T. K., J. J. ARMESTO, AND C. VILLAGRAN. 1981. Plant phenological patterns in the high Andean cordillera of central Chile. *Journal of Ecology* 69: 205–223.

ASHMAN, T.-L., AND M. S. HITCHENS. 2000. Dissecting the causes of variation in intra-inflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *American Journal of Botany* 87: 197–204.

ASHMAN, T.-L., AND D. J. SCHOEN. 1994. How long should flowers live? *Nature* 371: 788–791.

ASHMAN, T.-L., AND D. J. SCHOEN. 1996. Floral longevity: fitness consequences and resource costs. *In* D. G. Lloyd and S. C. H. Barrett [eds.], Floral biology: studies on floral evolution in animal-pollinated plants, 112–139. Chapman and Hall, New York, New York, USA.

BELL, G. 1985. On the function of flowers. *Proceedings of the Royal Society of London, B, Biological Sciences* 224: 223–265.

BELL, S. A., AND J. E. CRESSWELL. 1998. The phenology of gender in homogamous flowers: temporal change in the residual sex function of flowers of oil-seed rape (*Brassica napus*). *Functional Ecology* 12: 298–306.

BOOKMAN, S. S. 1983. Effects of pollination timing on fruiting in *Asclepias speciosa* Torr. (Asclepiadaceae). *American Journal of Botany* 70: 897–905.

BRUNET, J. 1996. Male reproductive success and variation in fruit and seed set in *Aquilegia caerulea* (Ranunculaceae). *Ecology* 77: 2458–2471.

BRUNET, J., AND D. CHARLESWORTH. 1995. Floral sex allocation in sequentially blooming plants. *Evolution* 49: 70–79.

DIGGLE, P. K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics* 26: 531–552.

DIGGLE, P. K. 1997. Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. *International Journal of Plant Sciences* 158 (Supplement): S99–S107.

EVANHOE, L., AND L. F. GALLOWAY. 2002. Floral longevity in *Campanula americana* (Campanulaceae): a comparison of morphological and functional gender phases. *American Journal of Botany* 89: 587–591.

GARCIA, M. B. 2003. Sex allocation in a long-lived monocarpic plant. *Plant Biology* 5: 203–209.

GUITIÁN, J., P. GUITIÁN, AND M. MEDRANO. 2001. Causes of fruit set variation in *Polygonatum odoratum* (Liliaceae). *Plant Biology* 3: 637–641.

GUITIÁN, J., AND L. NAVARRO. 1996. Allocation of reproductive resources within inflorescences of *Petrocoptis grandiflora* (Caryophyllaceae). *Canadian Journal of Botany* 74: 1482–1486.

HENRY, G. H. R., AND U. MOLAU. 1997. Tundra plants and climate change: the International Tundra Experiment (ITEX). *Global Change Biology* 3 (Supplement 1): 1–9.

HOLTSFORD, T. P. 1985. Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. *American Journal of Botany* 72: 1687–1694.

HOLTSFORD, T. P., AND N. C. ELLSTRAND. 1992. Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* 46: 216–225.

HUANG, S.-Q., S.-G. SUN., Y. TAKAHASHI, AND Y.-H. GUO. 2002. Gender variation of sequential inflorescences in a monoecious plant *Sagittaria trifolia* (Alismataceae). *Annals of Botany* 90: 613–622.

HUANG, S.-Q., L.-L. TANG, Q. YU, AND Y.-H. GUO. 2004. Temporal floral sex allocation in protogynous *Aquilegia yabeana* contrasts with protandrous species: support for the mating environment hypothesis. *Evolution* 58: 1131–1134.

ISHII, H. S., AND S. SAKAI. 2000. Optimal timing of corolla abscission: experimental study on *Erythronium japonicum* (Liliaceae). *Functional Ecology* 14: 122–128.

ISHII, H. S., AND S. SAKAI. 2002. Temporal variation in floral display size

and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae). *American Journal of Botany* 89: 441–446.

KLIBER, A., AND C. G. ECKERT. 2004. Sequential decline in allocation among flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology* 85: 1675–1687.

KUDO, G., T. MAEDA, AND K. NARITA. 2001. Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency or resource limitation? *Journal of Ecology* 89: 48–56.

KUDO, G., AND U. MOLAU. 1999. Variations in reproductive traits at inflorescence and flower levels of an arctic legume, *Astragalus alpinus* L.: comparisons between a subalpine and an alpine population. *Plant Species Biology* 14: 181–191.

LEE, T. D. 1988. Patterns of fruit and seed production. *In* J. Lovett-Doust and L. Lovett-Doust [eds.], Plant reproductive ecology: patterns and strategies, 179–202. Oxford University Press, New York, New York, USA.

MOTTEN, A. F. 1983. Reproduction of *Erythronium umbilicatum* (Liliaceae): pollination success and pollinator effectiveness. *Oecologia* 59: 351–359.

NISHIKAWA, Y. 1998. The function of multiple flowers of a spring ephemeral, *Gagea lutea* (Liliaceae), with reference to blooming order. *Canadian Journal of Botany* 76: 1404–1411.

NISHIKAWA, Y., AND G. KUDO. 1995. Relationship between flower number and reproductive success of a spring ephemeral herb, *Anemone flaccida* (Ranunculaceae). *Plant Species Biology* 10: 111–118.

PRIMACK, R. B. 1985. Longevity of individual flowers. *Annual Review of Ecology and Systematics* 16: 15–37.

RICHARDSON, T. E., AND A. G. STEPHENSON. 1989. Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. *American Journal of Botany* 76: 532–538.

SAKAI, S., AND Y. HARADA. 2004. Size-number trade-off and optimal offspring size for offspring produced sequentially using a fixed amount of reserves. *Journal of Theoretical Biology* 226: 253–264.

SARGENT, R. D., AND B. D. ROITBERG. 2000. Seasonal decline in male-phase duration in a protandrous plant: a response to increased mating opportunities? *Functional Ecology* 14: 484–489.

SCHOEN, D. J., AND T.-L. ASHMAN. 1995. The evolution of floral longevity: resource allocation to maintenance versus construction of repeated parts in modular organisms. *Evolution* 49: 131–139.

THOMSON, J. D. 1989. Deployment of ovules and pollen among flowers within inflorescences. *Evolutionary Trends in Plants* 3: 65–68.

TOTLAND, Ø., AND J. NYLÉHN. 1998. Assessment of the effects of environmental change on the performance and density of *Bistorta vivipara*: the use of multivariate analysis and experimental manipulation. *Journal of Ecology* 86: 989–998.

VOGLER, D. W., S. PERETS, AND A. G. STEPHENSON. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in *Campanula rapunculoides* (Campanulaceae). *American Journal of Botany* 86: 482–494.

WOLFE, L. M. 1992. Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae)? Developmental constraints vs. resource limitation. *American Journal of Botany* 79: 1286–1290.

WOLFE, L. M., AND W. DENTON. 2001. Morphological constraints on fruit size in *Linaria canadensis*. *International Journal of Plant Sciences* 162: 1313–1316.

WYATT, R. 1982. Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. *American Journal of Botany* 69: 585–594.

YOUNG, H. J., AND M. L. STANTON. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* 71: 536–547.