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MALE REPRODUCTIVE SUCCESS AND VARIATION IN FRUIT AND SEED SET IN *AQUILEGIA CAERULEA* (RANUNCULACEAE)[1]

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Abstract. The timing of fruit initiation and the proximity of fruits to resources influence the probability of fruit and seed initiation in many hermaphroditic plants. In two populations of *Aquilegia caerulea,* both fruit and seed set decreased significantly between early and late flowers. Low fruit and seed set of late flowers was not due to pollen limitation. Fruit and seed set of late flowers remained low when extra pollen was added to these flowers (pollen quantity), or when all flowers on an inflorescence received self or outcross pollen (pollen quality). While competition for resources occurred among flowers, resource limitation was not responsible for the low fruit and seed set of late flowers. Indeed, preventing pollination of late flowers significantly increased both fruit and seed set of early flowers, but fruit and seed set of late flowers remained low when early flowers were prevented from setting fruits. Late flowers were not just smaller or larger replicates of early flowers, as they allocated more reproductive resources to male function relative to early flowers. Neither herbivory nor architectural constraints could explain the low fruit and seed set of late flowers. While previously published adaptive explanations proposed to explain the low fruit and seed set of late flowers have emphasized a positive aspect to female reproductive success, it is suggested here that both male and female functions should be considered. Morphological data and estimates of male and female reproductive success demonstrate how, both morphologically and functionally, late flowers on inflorescences of *A. caerulea* specialize as males, early flowers as females. It will be argued that protandry in this sequential bloomer is expected to select for an increase in relative male allocation between early and late flowers, and that the observed pattern of resource allocation and the decrease in fruit and seed set between early and late flowers is consistent with this prediction.

Key words: Aquilegia caerulea; *architectural constraints; floral variation; fruit and seed set; male function; pollen limitation; protandry; reproductive success; resource allocation; resource limitation.*

INTRODUCTION

In many hermaphroditic plants, fruit and seed set decline between early-formed or proximal fruits (located closest to the sources of nutrients) and later-formed or more distal fruits (Stephenson 1981, 1984, Bawa and Webb 1984, Holtsford 1985, McKone 1985, Solomon 1985, 1988, Nakamura 1986, Nicholls 1987, Lee 1988, Thomson 1989, Byrne and Mazer 1990, Diggle 1991, Kang and Primack 1991, Karoly 1992, Obeso 1993, Guitian 1994). A decrease in fruit and seed set in later or more distal flowers has often been suggested to be associated with resource limitation. If flowers and fruits compete for resources, such that early or proximal flowers capture most of the resources, resources may be unavailable to later-opening or distal flowers (interfloral competition for resources) (Stephenson 1981, Holtsford 1985, Nakamura 1986, Lee 1988, Solomon 1988, Thomson 1989, Guitian 1994). If fruit abortion occurs and fruits furthest from maturity abort, the pattern is consistent with the idea that a plant is conserving resources (Bookman 1983, Nakamura 1986, Lee 1988). Thus a decline in fruit and seed set between early and late flowers could reflect an adaptive strategy to conserve resources (Lloyd 1980, Bookman 1983, Nakamura 1986, Lee 1988). Alternatively, low fruit and seed set in late flowers may be the direct result of plant architecture and require no adaptive explanation (Wyatt 1982, Watson and Casper 1984, Nicholls 1987, Waller 1988, Lee 1988, Herrera 1991, Diggle 1992, Wolfe 1992). Smaller floral size of late flowers, with lower fruit and seed set, may be due to fluctuations in meristem size during shoot ontogeny (Nicholls 1987, Diggle 1992, Wolfe 1992). If later produced reproductive structures contain less vascular tissue and the quantity of vascular tissue influences fruit and seed development, then changes in fruit and seed set along an inflorescence may be a direct result of plant development. As herbivory has been shown to promote fruit abscission (Stephenson 1980, 1981), greater levels of herbivory or predation in late (vs. early) flowers could also select for low fruit and seed set in these flowers (Stephenson 1980, Hendrix and Trapp 1981, Hendrix 1984, Lee 1988).

Low fruit and seed set of late flowers could also be associated with variation in pollen abundance or quality of pollen receipt (Lee 1988, Thomson 1989). Both pol-

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len quantity and quality have been shown to influence the probability of fruit and seed maturation in plants (Bertin 1982, 1990, Bookman 1984, McDade and Davidar 1984, Schemske and Pautler 1984, Ellstrand and Marshall 1986, Marshall and Ellstrand 1986, 1988, Mazer et al. 1986, Winsor et al. 1987, Lee 1988, Marshall 1988, 1991, Waser and Price 1989, Cruzan 1990, Bertin and Peters 1992, Montalvo 1992, Rigney et al. 1993, Waser 1993). If the quantity or quality of pollen received declines between early- and late-opening flowers, and lower quality offspring are aborted, the decline in fruit and seed set between early and late flowers could be explained through an increase in offspring quality, resulting from the abortion of lower quality offspring (Stephenson 1981, Willson and Burley 1983, Bookman 1984, Marshall and Ellstrand 1986, Nakamura 1986, Lee 1988). An increase in the level of geitonogamy (selfing among flowers on a plant) between early and late flowers, a decrease in the number of mates available to late relative to early flowers, or degradation in the quality of pollen reaching late vs. early flowers due to environmental factors (Young and Stanton 1990) could all explain the low fruit and seed set of late flowers. Directionality in pollinator movement could also influence the proportion of self and outcross pollen that reach each flower. If pollinators tend to move down inflorescences on plants with distal early flowers, basal flowers may receive less outcross pollen than apical flowers and have lower fruit and seed set (Lee 1988). The influence of pollinator directionality on fruit and seed set will depend on the order of floral development, the degree of dichogamy, and the level of inbreeding depression.

Alternatively, the low fruit and seed set of late flowers could reflect the fact that late flowers specialize as males. Factors such as protandry can modify the probability of pollen transfer among flowers and select for male specialization of late flowers (Brunet and Charlesworth 1995). With protandry, an emphasis on male allocation in late flowers will enhance reproductive success (Brunet and Charlesworth 1995). Thus, while reduction in resources may be an obvious explanation for reduced fruit and seed set of late or distal flowers, there are plausible alternatives that have not been fully investigated.

In this study, a decline in fruit and seed set between early and late flowers on inflorescences of *Aquilegia caerulea* was documented in two populations. In this species, early flowers are distal, late flowers proximal, a situation which allows one to distinguish between the effects of the physical location of the flower (basal vs. distal) and the timing of fruit initiation (early vs. late). This contrasts with the case found in many plant species where early flowers are proximal flowers and where the two variables (position on the inflorescence and time of fruit initiation) are perfectly correlated (Lee 1988). The effects of pollen and resource availability, and pollen quality on the low fruit and seed set of late flowers were determined experimentally. The influence of variation in selfing rate or in the level of herbivory among flowers on the low fruit and seed set of late flowers will be discussed. Morphological and functional estimates of floral gender will be presented for early and late flowers to examine whether late flowers specialize as males. The relationship between protandry and the degree of gender specialization of different flowers will be examined. Using these data, I asked the following questions: Are the fruit and seed set of late flowers low because: (a) resources are used up by early flowers? (b) late flowers receive less pollen than early flowers (pollen quantity)? (c) late flowers receive lesser quality pollen than early flowers? (d) herbivory is greater in late relative to early flowers? (e) late flowers are on smaller reproductive structures as a mere consequence of plant architecture? (f) late flowers specialize as males? One or more of these factors could explain the low fruit and seed set of late flowers observed in *A. caerulea.*

MATERIALS AND METHODS

The populations

Data were collected from two populations of *A. caerulea* in Colorado, one population (BRM) near Gothic (altitude 2880 m), and the other at Bellview (altitude 3030 m), 3.5 km northwest of the BRM population. Experimental manipulations were performed at Bellview, where *A. caerulea* grows on dry mountain slopes under intense sunlight. The habitat of the BRM population was more shady; *A. caerulea* occurred in the understory of coniferous trees or in small meadows surrounded by coniferous trees.

The species

Aquilegia caerulea James, Ranunculaceae, or blue columbine, is a widely distributed perennial herb of the southern and central Rocky Mountains of western North America. It occupies montane and subalpine habitats at elevations of 2100–3700 m. The plant is self-compatible with strongly protandrous flowers; the anthers dehisce over 2–3 d; stigmas become receptive 1–2 d later, and remain receptive for at least 5 d (personal observation). Stigma receptivity can be determined with the naked eye as the papillae swell on the stigmatic surface. Plants vary in number of flowering stalks (inflorescences) (1–10) and flowers per inflorescence (1–10) with an average of 3–4 flowers per inflorescence and 3–4 inflorescences per plant. Flowers open sequentially on inflorescences with distal flowers opening first. The female reproductive organ or gynoecium consists of 5–10 unfused carpels. The gynoecium is surrounded by 50–130 stamens. Bumble bees and hawk moths are the primary pollinators (Miller 1978, 1981).

Pattern of fruit and seed set

Patterns of fruit and seed set were examined on 35 randomly chosen plants at the BRM population during the summer of 1986, and 40 randomly chosen plants at Bellview during the summer of 1987. A preliminary study detected trends in fruit and seed set with order of flower opening on inflorescences, but not on the whole plant. Flowers were grouped according to their position on an inflorescence; first-position flowers opened first on an inflorescence and were apical flowers; second-position flowers opened next, and so on. A temporal rather than a spatial position was chosen for *A. caerulea,* as it was a more suitable criterion given the growth pattern of the species.

Inflorescences and flowers were marked with adhesive labels and the order in which a flower opened on an inflorescence was recorded. At the BRM population, labels were placed on each flower on the first three inflorescences with blooming flowers (or all flowers on plants with less than four inflorescences). At Bellview, flowers were labeled on all but two inflorescences on each plant (which were used to measure pollen and ovule production and level of autogamous selfing). At the beginning of the season, the number of carpels per flower was recorded for each marked flower on all selected plants. Later in the season, mature and aborted fruits were collected and the number of mature seeds per fruit counted (all follicles combined). Seeds from the BRM population were weighed after drying them for 36 h in an oven. As flowers of *A. caerulea* have many carpels, each with the potential of developing into a mature follicle, I examined fruit set two ways. First, I considered the percentage of flowers that did not set fruits at different positions. A fruit is set if at least one carpel develops into a mature follicle. This variable will be referred to as the percentage aborted fruits. Second, I determined the percentage of carpels that developed into mature follicles for flowers at different positions. The term fruit set will be used to describe the percentage of carpels that develop into mature follicles.

Patterns of variation in fruit and seed sets and in seed mass were examined, separately for each population, using a mixed-model analysis of variance, with floral position as a fixed effect and plant and the interaction term, plant × floral position, as random effects, using the GLM procedure of SAS (Type III sum of squares) (SAS 1985). An angular transformation was performed on the percentages prior to analyses. Tukey tests determined significant pairwise differences among floral positions. For fruit and seed set, the trends were first examined on all fruits, including the fruits that set no seed, and then only on the fruits that set at least one seed.

Pattern of ovule and pollen production

To determine whether morphological gender varied among flowers, patterns of ovule and pollen production were examined on 26 randomly chosen plants at the BRM population and on 40 plants at Bellview (described in *Pattern of fruit and seed set* above). Flowers were marked with adhesive labels and order of flower opening on an inflorescence was recorded. Undehisced anthers were collected, counted and placed collectively into 1.5 mL microcentrifuge tubes. After anthers had finished dehiscing in each tube, anthers and pollen were preserved in 70% ethanol (EtOH). All carpels within each flower were collected at the end of stigma receptivity, placed into microcentrifuge tubes and preserved in 70% EtOH. Undehisced anthers and carpels were collected for each flower on the first three inflorescences with blooming flowers (or all flowers on plants with fewer than four inflorescences) at the BRM population and from one inflorescence per plant at Bellview.

Pollen counts were made for the BRM samples using a 16 channel Coulter Counter (Model TA II). Each sample was diluted with 500 mL of 0.1% saline and pollen counts were taken on four 2-mL subsamples. The total numbers of pollen grains were estimated from a size frequency distribution across five Coulter Counter size channels, where pollen diameter ranged between 10 and 32 μm. The numbers of carpels per flower were recorded and the number of ovules per carpel counted under a dissecting microscope for all carpels within each flower of 22 of the 26 marked plants (some samples were misplaced) at the BRM population and all marked plants at Bellview. Patterns of variation in anther number, total pollen count, and number of ovules per flower, were examined with a mixed model analysis of variance with floral position as a fixed effect and plant and the interaction term (plant × floral position) as random effects, using the GLM procedure of SAS (Type III sum of squares) (SAS 1985). Tukey tests were used to test for significant pairwise differences among floral positions.

Estimates of reproductive success and functional gender

Measures of functional gender (Lloyd 1980) estimate the proportion of reproductive success a flower, individual, or any subset of a population, achieved through male relative to female function. Lloyd (1980) proposed a functional gender estimate that has been used commonly to determine gender of individual plants (Primack and Lloyd 1980, Thomson and Barrett 1981, Devlin and Stephenson 1987, Delesalle 1989, Mazer et al. 1989). While Lloyd's method can be used to estimate floral gender, it does not consider the fact that mating opportunities vary throughout the flowering season. While some studies of functional gender in protandrous hermaphroditic plants have considered how temporal and spatial variation in floral gender over the flowering season could influence the functional gender of individuals in the population, they did not consider the functional gender of the different flowers themselves (Thomson and Barrett 1981, Devlin and Stephenson 1987). The method introduced below is specifically designed to calculate floral gender rather than plant gender and considers the fact that not all flowers in a population open simultaneously. It provides a more accurate estimate of floral gender for plants with sequential flowering.

Male reproductive success.—Male reproductive success for each floral position is an estimate of the average number of seeds sired by pollen from a flower at that position. The method introduced below assumes that pollen quality and quantity are constant among flowers. Male reproductive success depends on the number of male and female flowers open at any point in time and on the number of seeds set by female flowers throughout the reproductive season.

Flowers in a population can be grouped according to their physical location on the inflorescence (basal vs. distal, i.e., spatial position), or the order in which they open on an inflorescence (temporal position). On each day of the flowering season, flowers from different positions will be shedding pollen or have receptive stigmas. On any given day c, the proportion of flowers shedding pollen that are from position j can be represented as f^{m}_{cj}, or

$$f^{\mathrm{m}}_{cj} = N^{\mathrm{m}}_{cj}/\sum_{j} N^{\mathrm{m}}_{cj},$$

where the summation is taken over all positions. N^{m}_{cj} is the number of flowers shedding pollen from position j on day c. The sum $\Sigma_j N^{\mathrm{m}}_{cj}$ takes into account the total number of flowers shedding pollen on day c (from all positions). If the number of seeds set by each flower that was in the female phase on day c has been recorded, from each floral position, the number of seeds set by flowers open on day c can be estimated. This value is $S^{\mathrm{f}}_{c} = \Sigma_j S^{\mathrm{f}}_{cj}$, where S^{f}_{cj} is the number of seeds set by flowers at position j on day c.

Assuming that, on any given day, all flowers shedding pollen have the same probability of fertilizing the available ovules, male reproductive success for flowers at position j can be calculated as, $M_j = \Sigma_c f^{\mathrm{m}}_{cj} S^{\mathrm{f}}_{c}$, where the summation is taken over all days. To put this on a per flower basis, M_j is divided by the total number of flowers at the relevant donor position, $\Sigma_c N^{\mathrm{m}}_{cj}$, and the average number of seeds sired by pollen of a flower at position j becomes:

$$a_j = M_j/\sum_{c} N^{\mathrm{m}}_{cj}. \qquad (1)$$

In many cases, the number of seeds set in each flower may not be known. If the number of flowers with receptive stigmas, from different floral positions, opened on day c has been recorded (N^{f}_{ci}), the probability that pollen from position j would reach ovules at position $i(K_{ij})$ can be calculated, which is equivalent to ($\Sigma_c f^{\mathrm{m}}_{cj} N^{\mathrm{f}}_{ci}/\Sigma_c N^{\mathrm{m}}_{cj}$) (Brunet and Charlesworth 1995). If the average number of seeds set by flowers at position i (F_i) is known, male reproductive success at each floral position can be calculated as:

$$a_j = \sum_{j} K_{ij} F_j. \qquad (2)$$

This represents the average number of seeds sired by pollen from a flower at position j.

Female reproductive success and functional gender.—The average number of seeds set by flowers at each position, F_j, is a measure of female reproductive success for flowers at that position. The proportion of reproductive success of a flower at position j, which has been achieved through male relative to female function, A_j, then becomes:

$$A_j = a_j/(a_j + F_j). \qquad (3)$$

The variable A_j is a measure of functional maleness (one way of representing functional gender, the other being functional femaleness [Lloyd 1980]).

Data collection.—Flowering phenology data were collected from 60 plants at the BRM population. The day a flower opened as male was scored together with its floral position. In this study the sex of each protandrous flower was not recorded each day, but the average number of days elapsed between expression of male and female function within a flower was used to construct the female flowering curve of *A. caerulea* (Brunet and Charlesworth 1995). The raw data consisted of the number of flowers from different positions open as male and female for each day of the flowering season, N^{m}_{cj} and N^{f}_{cj}, respectively. The matrix of pollen transfer probabilities or K_{ij} was constructed from these data. Male reproductive success and functional maleness were then calculated for each floral position using Eqs. 2 and 3, respectively. No statistical tests were performed on the data, as only a single estimate of reproductive success and functional maleness can be obtained per position using the method described here.

Protandry.—In *A. caerulea,* protandry creates, on average, a 5-d time delay between expression of male and female function within each flower. Consequently, the female flowering curve is offset by 5 d relative to the male flowering curve (Brunet and Charlesworth 1995: Fig. 1). This is expected to reduce the probability of pollen transfer from first-position flowers but to increase it for last-position flowers (compared to an adichogamous species). To illustrate how protandry may influence the matrix of pollen transfer probabilities, male reproductive success, and functional maleness of different flowers, these quantities were calculated, as described above, using the flowering phenology data one would expect in the absence of protandry (or any form of dichogamy). Without protandry, each flower in the population expresses both male and female function simultaneously, and the male and female flowering curves are similar (there is no time offset between the two curves). This modifies the raw data or the number of male and female flowers open at each position on each day of the flowering season.

Interfloral competition for resources

To determine whether interfloral competition for resources occurs in *A. caerulea,* and is responsible for the low fruit and seed set of late flowers, the following treatments were performed on 40 plants at Bellview in the summer of 1987. These were a different set of plants from those used to measure fruit and seed set patterns. Treatment 1: Outcross pollen was added to all flowers on an inflorescence (control). Treatment 2: Pollination was prevented (by covering the stigmas with a straw) in all but the *first* flower to open on an inflorescence. The first flower was hand-pollinated with outcross pollen. Treatment 3: Pollination was prevented in all but the *last* flower to open on the inflorescence. The last flower was hand-pollinated with outcross pollen.

Comparing fruit and seed set of first flowers between treatments 1 and 2 will determine whether interfloral competition for resources can influence fruit and seed set of early flowers. Comparing fruit and seed set of last flowers between treatments 1 and 3 will indicate whether interfloral competition for resources can explain the low fruit and seed set of late flowers. The above design assumes that inflorescences are independent from each other. As early-opening flowers on all inflorescences of *A. caerulea* have a greater probability of setting fruits and seeds, regardless of among-inflorescence differences in flowering phenology (J. Brunet, personal observation), this suggested that each inflorescence can be viewed as an independent subunit with respect to allocation of resources to reproduction (Guitian 1994).

In all three treatments, outcross pollen was added to open-pollinated flowers (pollen augmentation). Each selected plant had at least three inflorescences with three flowers per inflorescence. On plants with fewer than six inflorescences, the three treatments were randomly applied to the first three inflorescences with open flowers. On plants with greater than five inflorescences, each treatment was duplicated on a plant. All flowers on extra inflorescences were hand-pollinated with outcross pollen in addition to open pollination (treatment 1). For outcross pollination, one freshly dehisced anther was collected from each of three outcross donors, located within 3 m of the pollen receiver. Pollen from the three donors were mixed in a multiwell tissue culture plate and applied to all stigmas within a flower using fine forceps. The forceps were cleaned in alcohol between each pollination. Pollen viability was checked in a random sample of pollinations. Leftover pollen from a pollination was placed in a vial, kept on ice, brought back to the laboratory at the end of the day, and checked for viability upon arrival, using the fluorochromatic procedure (FCR) (Heslop-Harrison and Y. Heslop-Harrison 1970, Kearns and Inouye 1993). The number of carpels per flower was recorded for each treated flower, and, at the end of the season, mature and aborted fruits were collected and the number of seeds counted in each fruit (all follicles combined).

Treatments were compared with analyses of variance using the GLM procedure of SAS (Type III sums of squares) (SAS 1985). Fruit and seed set were first examined for all flowers (including flowers that set no fruits) and then only for flowers that set at least one seed. Some plants or inflorescences within plants were lost to deer. As extra inflorescences on a plant received treatment 1, more inflorescences existed for the control group than for the other treatments. Consequently, when comparing treatments 1 and 2, in one case all inflorescences of treatment 1 were included, in another, only one inflorescence of treatment 1 was selected for each inflorescence of treatment 2. The inflorescence of treatment 1 with the most similar blooming sequence to the treatment 2 inflorescence was chosen. Data are presented in detail for this second case for which significance levels were lower than when all inflorescences of treatment 1 were included in the analyses.

Pollen limitation

Pollen quantity.—If low pollen availability is responsible for the low fruit and seed set of late flowers, there should be no differences in fruit and seed set among flowers when all flowers receive abundant pollen. The pattern of fruit and seed set among flowers was examined on inflorescences where all flowers received extra pollen in addition to open pollination (pollen augmentation) (treatment 1 of the interfloral resource competition experiment described above).

To determine whether pollen was limiting in the population as a whole, or for any position, fruit and seed sets were compared between open-pollinated flowers and flowers from the pollen augmentation treatment. The open-pollinated plants were described in the section on pattern of fruit and seed set, from Bellview. Fruit and seed sets were first compared for all floral positions, then separately for first-, second-, and third-position flowers. An increase in seed set in flowers which received extra pollen would suggest that pollen is limiting. As multiple tests were performed to test for pollinator limitation, the significance level was corrected for the number of tests (Bonferroni procedure) (Sokal and Rolhf 1987). Each hypothesis was tested against a probability level of 0.0125.

Self vs. outcross pollen.—Differences between self and outcross pollen are one measure of pollen quality, likely to occur in *A. caerulea* with its mixed breeding system (J. Brunet, *unpublished data*) and significant level of inbreeding depression (Montalvo 1994). If lower pollen quality is responsible for the low fruit and seed set of late flowers, there should be no pattern of variation in fruit and seed set among flowers when all flowers receive similar quality pollen. To test whether pollen quality influenced the pattern of fruit and seed maturation among flowers, the pattern of fruit and seed set were examined along selfed and outcrossed inflorescences on 17 plants at the BRM population in the summer of 1986. In this experiment pollen quality was controlled in that all flowers on an inflorescence received only self or outcross pollen and the same number of pollen donors were used in all flowers. Although

TABLE 1. Analyses of variance for fruit and seed production among plants and floral positions at the BRM and Bellview populations of *A. caerulea*. Plant and the interaction term (Plant × Flower) are random effects, Flower or floral position is a fixed effect. "Fruits" and "Seeds" include fruits that set no seed, while "Fruit" and "Seed" include only fruits with at least one seed. An angular transformation was performed on the percentages prior to analyses.

	BRM				Bellview			
Source	df	MS	*F*	*P*	df	MS	*F*	*P*
Fruits								
Plant	40	1.55	4.55	0.0001	38	0.49	1.69	0.013
Flower	4	4.06	11.95	0.0001	4	9.80	33.70	0.0001
Plant × Flower	111	0.34	0.99	0.51	111	0.28	0.87	0.79
Fruit								
Plant	38	0.07	2.13	0.0017	37	0.18	1.07	0.412
Flower	4	0.10	2.85	0.026	4	0.78	4.76	0.0019
Plant × Flower	81	0.03	0.88	0.74	38	0.17	1.10	0.349
Seeds								
Plant	40	23004.6	5.81	0.0001	38	1915.23	1.13	0.291
Flower	4	146528.8	35.13	0.0001	4	37320.17	21.90	0.0001
Plant × Flower	113	3745.5	0.69	0.99	111	1663.73	0.92	0.686
Seed								
Plant	38	10928.98	3.56	0.0001	37	3790.92	1.00	0.48
Flower	4	92647.70	27.47	0.0001	4	19050.47	5.03	0.001
Plant × Flower	79	3005.91	0.65	0.98	38	1900.10	0.50	0.99
Seed mass (g)								
Plant	38	2.2×10^{-7}	3.53	0.0001				
Flower	4	2.8×10^{-7}	4.59	0.0018				
Plant × Flower	76	6.0×10^{-7}	1.10	0.32				

environmental factors have been shown to influence pollen quality (Young and Stanton 1990) and can vary through time, variation in flowering phenology among plants will tend to randomize the effects of environmental factors among positions. Indeed second- and third-position flowers opened on some plants while first-position flowers were opening on other plants in the experiment (J. Brunet, *personal observation*).

Before anthesis of any flower, each inflorescence on a plant was bagged with bridal veil to prevent pollinator visits. Inflorescences and floral positions were marked with adhesive labels. Each flower was emasculated before any anther had dehisced. Within each flower, stigmas were counted and hand-pollinated with either self or outcross pollen when receptive. On each plant, all flowers on one inflorescence received outcross pollen, flowers on the other were selfed; when present, the third inflorescence was bagged but flowers were not hand-pollinated. Fourteen plants had two inflorescences, the other three plants had three. Freshly dehisced anthers from a distinct flower on the same plant were used for self-pollination (flowers are protandrous); for outcross pollination, one freshly dehisced anther was collected from three distinct pollen donors (located within three meters of the receiver), as described in the section on interfloral competition for resources. Bags were removed when the stigmas of the last-position flower were no longer receptive. Mature and aborted fruits were collected at the end of the season and the number of seeds per fruit counted (all follicles combined).

A fixed model analysis of variance (using the GLM procedure of SAS, Type III sums of squares) with pollination treatment and floral position as main factors, and number of mature seeds, or average mass of a seed as dependent variables, was used to detect significant differences among floral positions, pollination treatments, and any interaction between the main factors.

RESULTS

Pattern of fruit and seed set

At both the BRM and Bellview populations, fruit and seed set decreased significantly between early and late flowers (Table 1, Fig. 1). Swelling of separate carpels within a flower is not a reliable indicator of whether a follicle set a seed, at least for the BRM population, as most follicles tend to develop when at least one seed is set in the fruit (Fig. 1A). The decrease in fruit set from early to late flowers resulted mostly from an increase in the number of flowers setting no fruit in late positions (Fig. 1C, F). The number of mature seeds per fruit declined significantly even when fruits setting no seeds were excluded from the analyses (Fig. 1B, E). While the average mass of a seed differed significantly among positions (Table 1), Tukey tests indicated that only the first and fifth position flowers were significantly different from each other.

Pattern of pollen and ovule production

At both BRM and Bellview, the number of ovules per flower decreased significantly between first- and

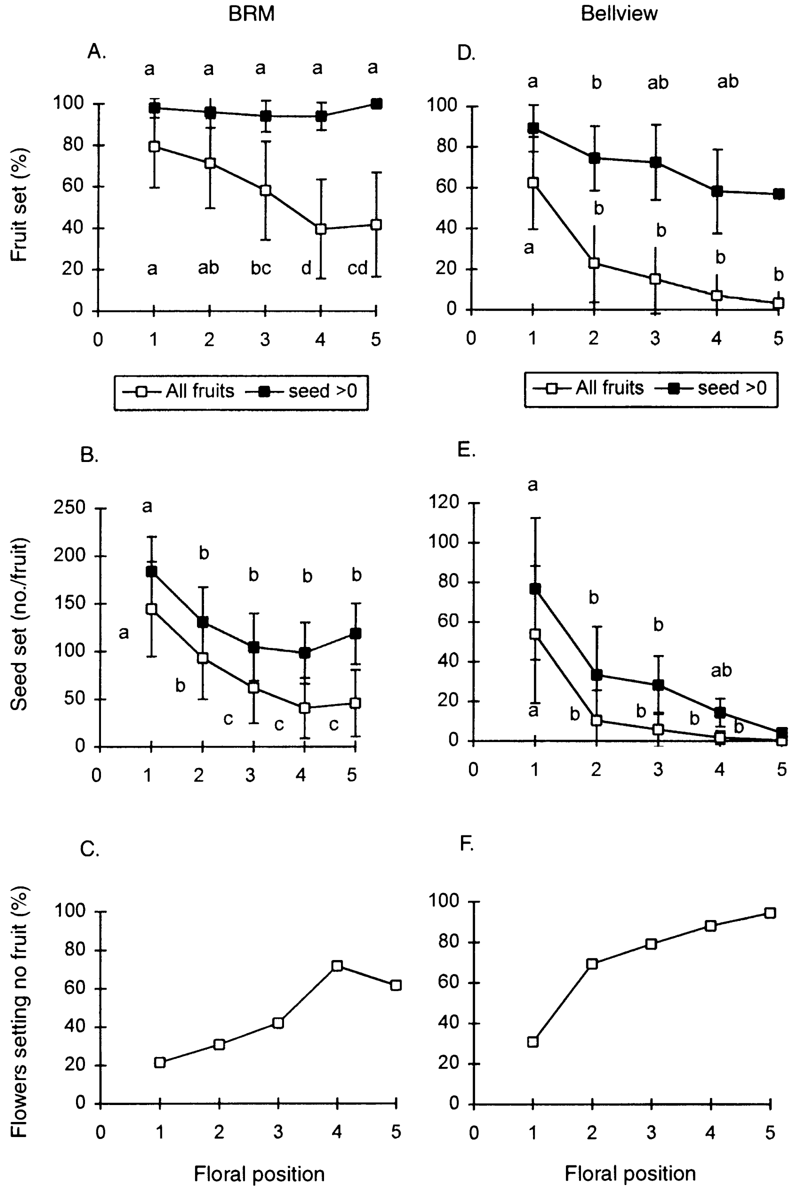

FIG. 1. Pattern of fruit and seed set among floral positions for the BRM and Bellview populations of *A. caerulea*. Fruit set is the percentage of carpels developing into mature follicles. Seed set is the number of mature seeds per fruit (all follicles combined). Position 1 flowers are the first flowers to open on inflorescences and are distal flowers. Tukey tests determined significant pairwise differences among positions. Positions sharing the same letter are not significantly different from one another. (A and D) Fruit set per position when fruits setting no seed are included (all fruits) or excluded (seed > 0) from the analyses for the BRM and Bellview populations, respectively. (B and E) Seed set per position when fruits setting no seed are included (all fruits) or excluded (seed > 0) from the analyses for the BRM and Bellview populations, respectively. (C and F) The percentage of aborted fruits (flowers setting no fruits) per floral position. The vertical bars indicate ±1 SD.

later-position flowers (Table 2, Fig. 2A, D). The number of anthers per flower did not vary among positions at Bellview and the significance level was borderline at BRM, with second-position flowers having fewer anthers than other-position flowers (Table 2, Fig. 2B, E). Pollen production did not vary significantly among flowers at BRM (Fig. 2C). Overall, there was no decrease in anther number or pollen production between early and late flowers in this species.

Protandry and estimates of reproductive success

Protandry increases the probability that pollen from a given position will fertilize stigmas of earlier position flowers (Table 3A, B). For example, while, on average, 24% (0.24/1.0) of flowers pollinated by position 4 pollen will be from position 3 when protandry is absent, this percentage increases to 38% (0.65/1.72) with protandry (a 5-d time delay, on average, between expres-

TABLE 2. Analyses of variance for pollen and ovule production among plants and floral positions at the BRM and Bellview populations of *A. caerulea*. Plant and the interaction term (Plant × Flower) are random effects, Flower or floral position is a fixed effect.

	BRM				Bellview			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Ovules								
Plant	21	5995.12	2.95	0.0015	34	3153.89	1.1	0.044
Flower	3	49742.35	24.61	0.0001	3	54516.96	18.9	0.0001
Plant × Flower	36	2039.11	1.05	0.41	39	1776.61	0.6	0.85
Anthers								
Plant	30	984.07	7.20	0.0001	39	520.86	4.44	0.0001
Flower	3	384.73	2.86	0.045	3	162.77	1.39	0.25
Plant × Flower	43	137.52	1.18	0.27	65	117.58	1.16	0.40
Pollen count								
Plant	25	1.92×10^{-11}	2.21	0.0087				
Flower	3	1.46×10^{-11}	1.80	0.157				
Plant × Flower	49	8.67×10^{-10}	1.70	0.0128				

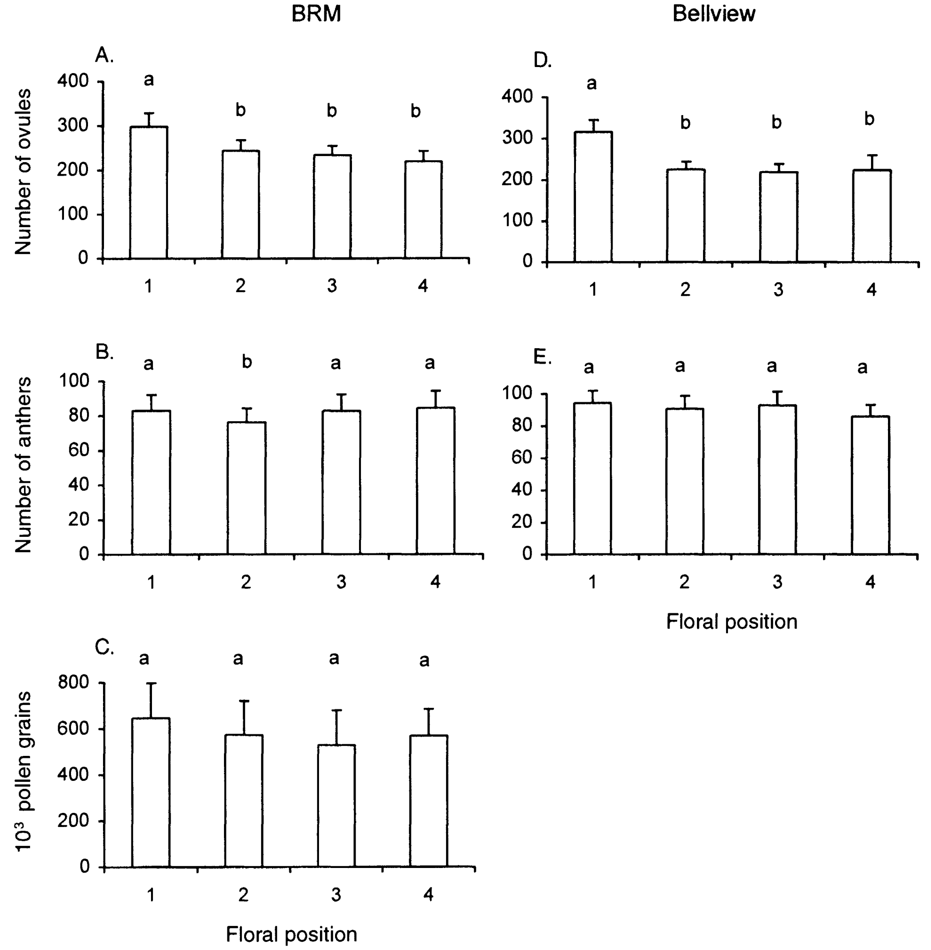

FIG. 2. Floral variation in ovule number, anther number, and pollen production for *A. caerulea* at the BRM and Bellview populations. (A and D) The number of ovules per floral position at the BRM and Bellview populations, respectively. (B and E) The number of anthers per flower at the BRM and Bellview populations, respectively. (C) Pollen production per flower at the BRM population. Tukey tests determined significant pairwise differences among positions. Positions sharing the same letter are not significantly different from one another. The vertical bars indicate ±1 SD.

TABLE 3. The matrix of pollen transfer probabilities (K_{ij}), the probability that pollen from position *j* will reach receptive stigmas of flowers at position *i*, in the (A) presence, or (B) absence of protandry. Protandry creates on average a 5-d time delay between expression of male and female function within a flower of *A. caerulea.* Under protandry, position 1 flowers are 44% of the flowers pollinated by position 2 pollen (0.36/0.81), but without protandry position 1 flowers are 25% of the flowers pollinated by position 2 pollen.

Position of female flower		Position of male flower				
		1	2	3	4	5
A) Protandry	1	0.49	0.36	0.26	0.30	0.26
	2	0.10	0.29	0.31	0.56	0.69
	3	0.07	0.12	0.13	0.65	0.67
	4	0.02	0.04	0.06	0.17	0.29
	5	0.00	0.00	0.00	0.04	0.10
Total		0.68	0.81	0.76	1.72	2.01
B) No protandry	1	0.73	0.25	0.18	0.13	0.10
	2	0.16	0.44	0.36	0.35	0.34
	3	0.08	0.21	0.33	0.24	0.23
	4	0.02	0.09	0.10	0.21	0.23
	5	0.00	0.02	0.02	0.06	0.10
Total		1.00	1.00	1.00	1.00	1.00

TABLE 4. Estimates of male and female reproductive success (RS), and functional maleness for flowers at different positions in *A. caerulea,* in the (A) presence or (B) absence of protandry. Protandry creates on average a 5-d time delay between expression of male and female function within a flower of *A. caerulea.* Male reproductive success represents the average number of seeds sired by pollen from a position *j* flower and was calculated using Eq. 2. Female reproductive success is the average number of seeds produced in flowers at position *j,* estimated from field data, while functional maleness represents the proportion of reproductive success accomplished through male function (relative to female function) and was calculated using Eq. 3.

Flower position		Male RS	Female RS	Functional maleness
A) Protandry	1	85.2	144.3	0.37
	2	88.3	92.8	0.49
	3	76.7	61.8	0.55
	4	144.6	40.5	0.78
	5	158.4	45.5	0.78
B) No protandry	1	125.8	144.3	0.47
	2	94.1	92.8	0.50
	3	84.1	61.8	0.58
	4	77.2	40.5	0.65
	5	74.1	45.5	0.62

sion of male and female functions). Protandry modifies the overall probability of pollen transfer for flowers at different positions, favoring later position flowers (Table 3A, B). While in the absence of protandry pollen from any position has the same probability of reaching stigmas, with protandry, late flowers have a greater probability than early flowers of reaching stigmas. In the absence of protandry, a flower in the male stage will reach, on average, one flower in the female stage (Table 3B), while with protandry a position 1 flower in the male stage will reach, on average, 0.68 flower in the female stage, while a position 5 flower in the male stage will reach, on average, 2.01 flowers with receptive stigmas (Table 3A).

When protandry is present, male reproductive success (RS) increases between early and late flowers, while the opposite is true in the absence of protandry (Table 4A, B). This reflects the fact that with protandry, pollen of late flowers has a greater probability of reaching stigmas than pollen of early flowers (Table 3A). To reconstruct total male reproductive success of all flowers in the population in the presence and absence of protandry, one must multiply the values for male reproductive success presented in Table 4 by the number of flowers at each position, in our case 177 position 1 flowers, 126 position 2, 74 position 3, 30 position 4 and 7 position 5. In the population, the overall reproductive success of male flowers will tend to be greater in the absence of protandry as with protandry the first male flowers to open in the population will not find any receptive stigmas. Plenty of pollen will, however, be available from later opening flowers when these early flowers have receptive stigmas (Brunet and Charlesworth 1995).

The fact that functional maleness increases between early and late flowers in both the presence or absence of protandry, reflects the fact that female seed set decreases between early and late flowers, and that pollen of late flowers pollinates early-position flowers whether protandry is present or not (Table 3A, B). With protandry however, early flowers propagate a greater proportion of their genes through female function, late flowers through male function (Table 4A).

Interfloral competition for resources

Preventing pollination, hence fruit and seed development in late flowers, increased both fruit and seed set of first flowers (Fig. 3). A greater proportion of flowers set seeds (85.2 vs. 60.7% in treated inflorescences vs. controls, respectively) ($\chi^2 = 4.23$, df = 1, $P < 0.05$), and more follicles developed ($F = 2.98$, df = 1, 54, $P = 0.09$) when pollination was prevented in later flowers. Similarly, seed set of first flowers increased significantly whether or not fruits setting no seeds were included in the analyses (Fig. 3; $F = 10.3$, df = 1, 47, $P < 0.01$ [all fruits], and $F = 3.8$, df = 1, 32, $P = 0.06$ for fruits that set at least one seed). If a flower produced a fruit, most of the follicles matured (97.6% for the controls ($n = 17$), and 92.3% for the treated inflorescences ($n = 23$)). All significance levels increased to $P < 0.01$ when all inflorescences from the controls were included in the analyses (see *Materials and methods*). In contrast, preventing fruit and seed development of early flowers did not increase fruit or seed set of late flowers. In the control, a single late fruit matured one of seven follicles and set four seeds, while no fruits were matured when pollination was prevented in all but the last flower to open on the inflo-

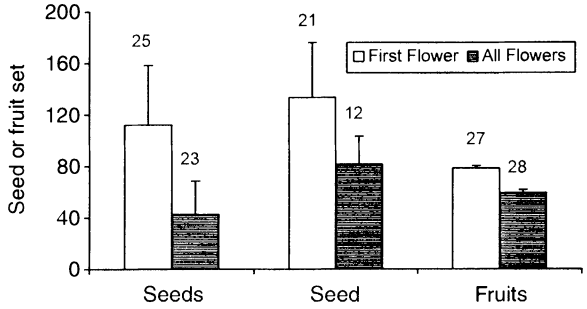

FIG. 3. Interfloral competition for resources. Fruit and seed set (see Fig. 1 for definitions) of first flowers in inflorescences where pollination was prevented in all but the first flowers (first flower), and inflorescences where all flowers received additional pollen (all flowers). "Seeds" represents the number of mature seeds in all fruits (including the fruits that set no seed), while "Seed" includes only the fruits that set at least one seed; the variable "Fruits" is the percentage of carpels developing into mature follicles for all fruits. The significance level for Seeds is $P < 0.01$, for Seed is $P = 0.06$, and for Fruits is $P = 0.09$. The sample size or the number of inflorescences per treatment is indicated above each bar. The vertical bars indicate ±1 SD.

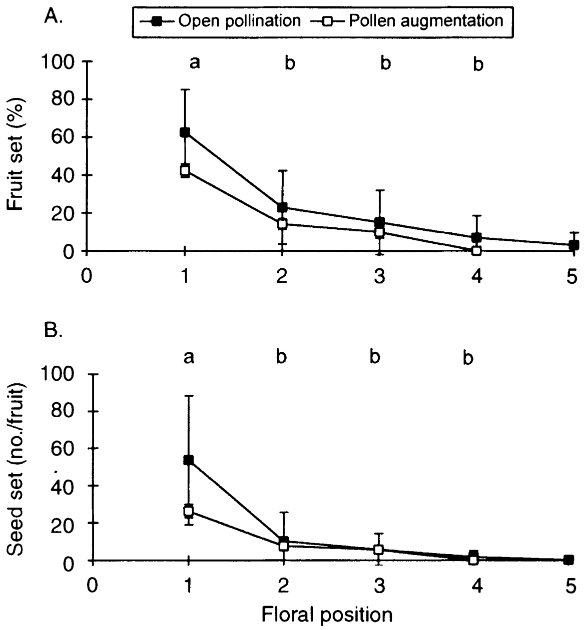

FIG. 4. Pollen quantity and fruit and seed set (see Fig. 1 for definitions) in successive flowers of *A. caerulea*. (A) Fruit set per floral position, for inflorescences that received additional pollen (pollen augmentation), and for open-pollinated flowers (open pollination). (B) Seed set per flower for the same treatments. Tukey tests determined significant pairwise differences among positions. Positions sharing the same letter are not significantly different from one another. As the pattern of variation was the same in both treatments, one set of letters is presented on each graph. The vertical bars indicate ±1 SD.

rescence. Pollen viability was high in all pollinations examined.

Pollen limitation

Pollen quantity.—The declines in fruit and seed set between early and late flowers remained significant even when all flowers on inflorescences received outcross pollen in addition to open pollination (Fig. 4A, B; $F = 5.44$, df = 3, 110, $P < 0.01$, for fruit set, and $F = 2.68$, df = 3, 117, $P = 0.01$ for seed set). This suggests that receipt of outcrossed pollen does not limit fruit and seed set of late flowers in *A. caerulea.*

Flowers from the pollen augmentation treatment did not set significantly more seeds than open-pollinated flowers, when flowers from all positions (22.3 vs. 12.6 seeds) $F = 4.01$, df = 1, 548, $P = 0.045$), or when first- (53.7 vs. 26.3 seeds) $F = 5.6$, df = 1, 182, $P = 0.02$), second- (10.2 vs. 7.6 seeds) $F = 0.22$, df = 1, 164, $P = 0.63$) and third-position flowers (5.8 vs. 5.6 seeds) $F = 0.002$, df = 1, 119, $P = 0.96$) were compared (Fig. 4B). Similar patterns were obtained for fruit set (the percentage of carpels developing into mature follicles) (Fig. 4A).

Self vs. outcross pollen.—The pollination treatment (self or outcross) did not influence the pattern of variation along the inflorescence in the number of mature seeds, seed mass, or percentage of flowers setting no seed (Table 5). No significant interaction between pollination treatment and floral position was observed for any of the dependent variables examined ($P > 0.50$ for all cases). Seed set decreased significantly between ear-

TABLE 5. Seed set, percent aborted fruits, and seed mass per floral position, for inflorescences whose flowers received self or outcross pollen. The sample size N is the number of inflorescences, and SE is the standard error of the mean.

Variable	Position	Self			Outcross		
		N	Mean	SE	N	Mean	SE
Seed set	1	17	110.5	21.1	12	151.5	33.3
	2	12	69.5	19.2	12	71.7	21.3
	3	6	40.1	20.6	6	36.8	39.3
% aborted fruits	1	17	11.8		12	8.3	
	2	12	25.0		12	33.3	
	3	6	33.3		6	50.0	
Seed mass (g)	1	15	0.00096	0.00040	11	0.00096	0.00010
	2	9	0.00086	0.00030	8	0.00086	0.00010
	3	4	0.00107	0.00050	2	0.00098	0.00020

ly and late flowers ($F = 6.72$, df = 2, 64, $P < 0.01$), while seed mass remained constant $F = 0.52$, df = 2, 48, $P > 0.50$). The percentage of flowers setting no seeds increased between early and late flowers, as was observed in the open-pollinated plants. Outcrossed flowers did not produce significantly more seeds than selfed flowers $F = 0.38$, df = 1, 64, $P = 0.54$) (96.6 seeds for outcross and 84.4 for self). Twenty and 27% of the flowers failed to set seed in the self and outcross treatment, respectively, and for both treatments, when a flower set seed, 97% of the carpels developed into mature follicles, on average.

DISCUSSION

In both populations of *A. caerulea,* fruit and seed set declined significantly between early and late flowers. The decrease in fruit set between early and late flowers reflects a greater percentage of flowers setting no seed in late flowers. Seed set also declined significantly even in the flowers that set fruits. The low seed set in late flowers is caused partly by a decrease in the number of ovules in these flowers. The experimental evidence suggests that neither resource nor pollen limitation is responsible for the low fruit and seed set of late flowers in *A. caerulea.* I will further argue that neither herbivory nor plant architecture can explain the low fruit and seed set of late flowers in this species. The fact that the presence of protandry increases the probability of pollen transfer for late relative to early flowers (compared to an adichogamous species) and that late flowers specialize as males morphologically and functionally is consistent with the notion that the low fruit and seed set of late flowers are related to the male specialization of late flowers. The evidence in support of these various hypotheses follows.

Interfloral competition for resources

The finding that preventing fruit and seed development of late flowers significantly increased fruit and seed sets of early flowers suggests that interfloral competition for resources occurs in *A. caerulea.* However, when fruit and seed set were prevented in early flowers, neither fruit nor seed set increased in late flowers, indicating that competition for resources is not a sufficient explanation for the low fruit and seed set of late flowers in this species. In a separate study, neither the number of anthers nor ovules increased in late flowers when resources were added in the form of N-P-K fertilizer (J. Brunet, *personal observation*). In contrast with *A. caerulea,* preventing pollination of late flowers did not increase seed set of early flowers in *Erythronium grandiflorum* nor in *Clintonia borealis,* while preventing pollination in early flowers increased seed set of late flowers in both species (Thomson 1989). Removing floral buds of early flowers, increased the probability of fruit set of later flowers in *Calochortus leichtlinii* (Holtsford 1985), increased ovary size of late flowers in *Solanum carolinense* (Solomon 1988) and increased ovary diameter of last-position flowers without modifying the number of anthers in *Muntingia calabura* (Bawa and Webb 1983). Adding resources in the form of N-P-K fertilizer did not modify the pattern of fruit abortion on inflorescences of *Lotus corniculatus* (Stephenson 1984). If resources were limiting fruit and seed set of late flowers, one would expect the pattern of fruit abortion to disappear when ample resources are made available. While *C. leichtlinii, S. carolinense,* and *M. calabura* have indeterminate inflorescences, the other species studied have determinate inflorescences.

Discrepancies among the above studies were not related to whether species had determinant or indeterminant inflorescences, whether experiments were performed at the level of the inflorescence or the whole plant, nor to whether resource augmentation was obtained through removing flower buds, preventing pollination or addition of nutrients. Although the presence of a strong sink from early flowers may be necessary before fruits can develop in late flowers (Lee 1988), this factor cannot explain the differences observed among the different studies. Interestingly, in all the studies mentioned above where ovule number or fruit and seed set increased in later flowers, it never reached the level found in early-position flowers. While further studies may elucidate why the response to resource limitation differs among species, it is clear from the cases examined that resource limitation alone cannot explain the pattern of floral variation in fruit, seed or ovule number observed in many hermaphroditic plants.

Pollen limitation

Pollen quantity.—As fruit and seed set of late flowers remained low when all flowers received abundant pollen, variation in pollen availability is not responsible for the low fruit and seed set of these flowers. Pollen was not limiting for the population as a whole, nor for any single floral position. Overall, fruit or seed set tended to be greater in open-pollinated compared to flowers of the pollen augmentation treatment. Reduction in fruit and seed set when extra pollen is added to flowers has been observed previously in many hermaphroditic plants (Young and Young 1992). Pollen addition did not increase fruit and seed set of late flowers in *Calochortus leichtlinii* (Holtsford 1985) nor in *Lupinus nannus* where the pattern of fruit maturation remained unchanged when additional pollen was added to all flowers (Karoly 1992). In the species examined so far, variation in the quantity of pollen reaching stigmas at different floral positions does not explain the lower fruit and seed set of late flowers.

Pollen quality.—If pollen quality is responsible for the low fruit and seed set of late flowers, the pattern of variation in fruit and seed set should disappear when all flowers receive similar quality pollen. In the self and outcross treatments, all trends among flowers remained in the same direction as for open-pollinated inflorescences, for all dependent variables examined.

In this experiment, some (application of self and outcross pollen, the number of pollen donors per flower, and to some degree the influence of environmental factors on pollen quality) but not all aspects of pollen quality (age of flower, status of recipient plant) were controlled for. We can only conclude that, to the degree to which pollen quality was controlled for, variation in pollen quality among flowers cannot explain the low fruit and seed set in late flowers of *A. caerulea.* In *C. leichtlinii,* only the first flower to open set fruit whether self or outcross pollen was used to hand-pollinate the first and second flowers (Holtsford 1985). In *Lupinus nannus,* pollen augmentation increased the probability that a seed would be outcrossed, but did not modify the pattern of fruit and seed set among flowers (Karoly 1992).

Flowers of *A. caerulea* are strongly protandrous, with distal flowers opening first within and mostly among inflorescences of a plant (J. Brunet, *personal observation*). Consequently, when first-position flowers are in the male phase, there are few later position flowers in the female phase on the same plant, but plenty of first-position flowers are in the female phase when later position flowers shed pollen (J. Brunet, personal observation). If the level of geitonogamous selfing were to vary among flowers of *A. caerulea,* based on the flowering phenology and strong protandry alone, one would expect it to be greater in early flowers. No clear pattern of pollinator movement (up or down inflorescences) has been observed in *A. caerulea* (J. Brunet, *personal observation*), so neither variation in geitonogamy level nor pollinator movement can explain the low fruit and seed set in late flowers of *A. caerulea.*

Furthermore, fruit and seed set did not differ significantly between selfed and outcrossed flowers, although outcrossed flowers produced slightly more seeds than selfed flowers (96.6 vs. 84.4 seeds per flower). The difference between the self and outcross treatments was largest for first-position flowers (Table 5). In this same species, Montalvo (1992) reported significantly fewer seeds following self pollination, when self or outcross pollen were applied to different stigmas within a flower. The difference between studies could reflect the fact that the diallel designs used in Montalvo's experiments tend to favor early flowers, which are more likely to set seeds, and show the largest difference between self and outcross treatments.

Herbivory and dispersal

It has been suggested that high level of herbivory in late flowers could select for low fruit and seed set in these flowers (Stephenson 1980, Hendrix and Trapp 1981, Hendrix 1984, Lee 1988). In the populations of *A. caerulea* examined, herbivory was caused by deer and marmot eating flowers on inflorescences. Left unprotected, plants in the population could suffer serious damage. If a plant was attacked, all flowers on the inflorescence tended to disappear at the same time, and only late-opening buds were at times protected (J. Brunet, personal observation). The level of herbivory was not greater in later flowers (J. Brunet, *personal observation*), and variation in the level of herbivory among flowers does not explain the low fruit and seed set of late flowers in *A. caerulea.*

Plant architecture

As the factors previously suggested as possible adaptive explanations for the low fruit and seed set of late flowers in many hermaphroditic plants have failed to explain the pattern in *A. caerulea,* one wonders if the pattern requires any adaptive explanation or if it may be a mere consequence of inflorescence architecture. As late-opening flowers of *A. caerulea* are proximal rather than distal flowers, the low fruit and seed set in these flowers are unlikely to result from fluctuations in meristem size during shoot ontogeny where later produced reproductive structures contain less vascular tissue (Nicholls 1987, Diggle 1992, Wolfe 1992). This explanation applies to cases where late flowers are also distal flowers. Furthermore if the pattern of floral variation observed in *A. caerulea* were a consequence of plant architecture one would expect late flowers to be simply smaller or larger versions of early flowers. But while ovule number decreases between early and late flowers of *A. caerulea,* the number of anthers and pollen grains per flower remain constant. Early flowers have a lower pollen–ovule ratio relative to later flowers. These observations suggest that the pattern of floral variation in *A. caerulea* is not the mere result of plant architecture.

Resource allocation

All adaptive hypotheses presented so far to explain the low fruit and seed set of late flowers have emphasized a positive aspect to female reproductive success, but have not considered male reproductive success. None of these hypotheses could satisfactorily explain the low fruit and seed set of late flowers in *A. caerulea.* An alternative possibility is that late flowers in this species specialize as males, which in turn may explain the low fruit and seed set in these flowers.

It is interesting that, morphologically, the relative allocation to male function increases between early and late flowers in *A. caerulea* due to a decline in the number of ovules between early and late flowers, while the numbers of anthers or pollen grains produced per flower remain constant. Functionally, male reproductive success increases between early and late flowers, and early flowers propagate a greater proportion of their genes through female function, late flowers through male function. Constraint on the pollination mechanism may help explain why anther number is not lower in early flowers. Bumble bees actively collect pollen in this species.

Compared to an adichogamous species, protandry increases the probability that pollen from a given position will fertilize stigmas of earlier position flowers, and modifies the overall pattern of pollen transfer for pollen at different positions, with a greater probability of pollen transfer for late compared to early flowers. This in turn influences male reproductive success of the different flowers, with greater male reproductive success of late compared to early flowers, in contrast to the reverse pattern when protandry is absent.

The findings in *A. caerulea* suggest that the low fruit and seed set of late flowers are consistent with male specialization of late flowers. Such patterns of sexual specialization among flowers are expected to have been selected by the strong protandry present in this species. By modifying the probability of pollen transfer among positions, such that late flowers are more likely to transfer pollen to stigmas relative to early flowers, protandry can select for male specialization of late flowers (Brunet and Charlesworth 1995). These observations indicate that, to properly understand patterns of fruit and seed set among flowers, inflorescences, or umbels in hermaphroditic plants, one should consider both male and female functions and determine whether any pattern of sexual specialization of flowers exist in the species under consideration. Factors such as dichogamy and pollinator directionality can select for specific patterns of relative male allocation among flowers (inflorescences or umbels) (Brunet and Charlesworth 1995). Such patterns of floral allocation have an adaptive explanation and may help explain patterns of ovule, fruit, and seed set among flowers in many hermaphroditic plants.

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LITERATURE CITED

Bawa, K. S., and C. J. Webb. 1983. Floral variation and sexual differentiation in *Muntingia calabura* (Elaeocarpaceae), a species with hermaphrodite flowers. Evolution **37**: 1271–1282.

Bawa, K. S., and C. J. Webb. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. American Journal of Botany **71**:736–751.

Bertin, R. I. 1982. Paternity and fruit production in trumpet creeper (*Campsis radicans*). American Naturalist **119**:694–709.

———. 1990. Effects of pollination intensity in *Campsis radicans*. American Journal of Botany **77**:178–187.

Bertin, I., and P. J. Peters. 1992. Paternal effects on offspring quality in *Campsis radicans*. American Naturalist **140**:166–178.

Bookman, S. 1983. Costs and benefits of flower abscission and fruit abortion in *Asclepias speciosa*. Ecology **64**:264–273.

———. 1984. Evidence for selective fruit production in *Asclepias*. Evolution **38**:72–86.

Brunet, J., and D. Charlesworth. 1995. Floral sex allocation in sequentially blooming plants. Evolution **49**:70–79.

Byrne, M., and S. J. Mazer. 1990. The effect of position on fruit characteristics and relationships among components of yield in *Phytolaca revinoides* (Phytolaccaceae). Biotropica **22**:353–365.

Cruzan, M. B. 1990. Pollen–pollen and pollen–style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). American Journal of Botany **77**:116–122.

Delesalle, V. A. 1989. Year-to-year changes in phenotypic gender in a monoecious cucurbit, *Apodanthera undulata.* American Journal of Botany **76**:30–39.

Devlin, B., and A. G. Stephenson. 1987. Sexual variations among plants of a perfect-flowered species. American Naturalist **130**:199–218.

Diggle, P. K. 1991. Labile sex expression in andromonoecious *Solanum hirtum*: pattern of variation in floral structure. Canadian Journal of Botany **69**:2033–2043.

———. 1992. Development and the evolution of plant reproductive characters. Pages 326–335 *in* R. Wyatt, editor. Ecology and evolution of plant reproduction: new approaches. Chapman and Hall, New York, New York, USA.

Ellstrand, N. C., and D. L. Marshall. 1986. Patterns of multiple paternity in populations of *Raphanus sativus.* Evolution **40**:837–842.

Guitian, J. 1994. Selective fruit abortion in *Prunus Mahaleb* (Rosaceae). American Journal of Botany **81**:1555–1558.

Hendrix, S. D. 1984. Reactions of *Heracleum lanatum* to floral herbivory by *Depressaria pastinacella.* Ecology **65**: 191–197.

Hendrix, S. D., and E. J. Trapp. 1981. Plant–herbivore interactions: insect induced changes in host plant sex expression and fecundity. Oecologia **49**:119–122.

Herrera, J. 1991. Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Lamiaceae). American Journal of Botany **78**:789–794.

Heslop-Harrison, J., and Y. Heslop-Harrison. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intra-cellular hydrolysis of fluorescein diacetate. Stain Technology **45**:115–122.

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

Kang, H., and R. B. Primack. 1991. Temporal variation of flower and fruit size in relation to seed yield in celandine poppy (*Chelidonium majus*; Papaveraceae). American Journal of Botany **78**:711–722.

Karoly, K. 1992. Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). American Journal of Botany **79**:49–56.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

Lee, T. D. 1988. Patterns of fruit and seed production. Pages 179–202 *in* J. Lovett Doust and L. Lovett Doust, editors. Plant reproductive ecology. Oxford University Press, New York, New York, USA.

Lloyd, D. G. 1980. Sexual strategies in plants. III. A quantitative method for describing gender of plants. New Zealand Journal of Botany **18**:103–108.

Marshall, D. L. 1988. Postpollination effects on seed paternity: mechanisms in addition to microgametophyte competition operate in wild radish. Evolution **42**:1256–1266.

———. 1991. Nonrandom mating in wild radish: variation in pollen donor success and effects of multiple paternity among one- to six-donor pollinations. American Journal of Botany **78**:1404–1418.

Marshall, D. L., and N. C. Ellstrand. 1986. Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice and consequences of multiple paternity. American Naturalist **127**:446–461.

Marshall, D. L., and N. C. Ellstrand. 1988. Effective mate choice in wild radish: evidence for selective seed abortion and its mechanism. American Naturalist **131**:739–756.

Mazer, S. J., R. R. Nakamura, and M. L. Stanton. 1989. Seasonal changes in components of male and female reproductive success in *Raphanus sativus* L. (Brassicaceae). Oecologia **81**:345–353.

Mazer, S. J., A. A. Snow, and M. L. Stanton. 1986. Fertilization dynamics and parental effects upon fruit development in *Raphanus raphanistrum*: consequences for seed size variation. American Journal of Botany **73**:500–511.

McDade, L. A., and P. Davidar. 1984. Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). Oecologia (Berlin) **64**:61–67.

McKone, M. J. 1985. Reproductive biology of several bromegrasses (*Bromus*): breeding system, pattern of fruit maturation, and seed set. American Journal of Botany **72**: 1334–1339.

Miller, R. B. 1978. The pollination ecology of *Aquilegia elegantula* and *caerulea* (Ranunculaceae) in Colorado. American Journal of Botany **65**:406–414.

———. 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea.* Evolution **35**:763–774.

Montalvo, A. M. 1992. Relative success of self and outcross pollen comparing mixed- and single-donor pollinations in *Aquilegia caerulea.* Evolution **46**:1181–1198.

———. 1994. Inbreeding depression and maternal effects in *Aquilegia caerulea.* Evolution **41**:760–771.

Nakamura, R. R. 1986. Maternal investment and fruit abortion in *Phaseolus vulgaris.* American Journal of Botany **73**: 1049–1057.

Nicholls, M. S. 1987. Spatial pattern of ovule maturation in the inflorescence of *Echium vulgare*: demography, resource allocation and the constraints of architecture. Biological Journal of the Linnean Society **31**:247–256.

Obeso, R. 1993. Selective fruit and seed maturation in *Asphodelus albus* Miller (Liliaceae). Oecologia (Berlin) **93**: 564–570.

Primack, R. B., and D. G. Lloyd. 1980. Sexual strategies in plants. IV. The distribution of gender in two monomorphic shrub populations. New Zealand Journal of Botany **18**:109–114.

Rigney, L. P., J. D. Thomson, M. B. Cruzan, and J. Brunet. 1993. Differential success of pollen donors in a self-compatible lily. Evolution **47**:915–924.

SAS. 1985. SAS user's guide: statistics. Version 5 edition. SAS Institute, Cary, North Carolina, USA.

Schemske, D. W., and L. P. Pautler. 1984. The effect of pollen composition on fitness components in a Neotropical herb. Oecologia (Berlin) **62**:31–36.

Sokal, R. R., and F. J. Rohlf. 1987. Introduction to biostatistics. Second edition. W. M. Freeman, New York, New York, USA.

Solomon, B. P. 1985. Environmentally influenced changes in sex expression in an andromonoecious plant. Ecology **66**:1321–1332.

———. 1988. Patterns of pre- and postfertilization resource allocation within an inflorescence: evidence for interovary competition. American Journal of Botany **75**:1074–1079.

Stephenson, A. G. 1980. Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). Ecology **61**:57–64.

———. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253–279.

———. 1984. The regulation of maternal investment in an indeterminate flowering plant (*Lotus corniculatus*). Ecology **65**:113–121.

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

Thomson, J. D., and C. H. Barrett. 1981. Temporal variation of gender in *Aralia hispida* Vent. (Araliaceae). Evolution **35**:1094–1107.

Waller, D. M. 1988. Plant morphology and reproduction. Pages 203–227 *in* J. Lovett Doust and L. Lovett Doust, eds. Plant reproductive ecology. Oxford University Press, New York, New York, USA.

Waser, N. M. 1983. Population structure, optimal outbreeding, and assortative mating in angiosperms. Pages 1–13 *in* N. W. Thornhill, editor. The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. University of Chicago Press, Chicago, Illinois, USA.

Waser, N. M., and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution **43**:1097–1109.

Watson, M. A., and B. B. Casper. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Annual Review of Ecology and Systematics **15**:233–258.

Willson, M. F., and N. Burley. 1983. Mate choice in plants: tactics, mechanisms and consequences. Princeton University Press, Princeton, New Jersey, USA.

Winsor, J. A., L. E. Davis, and A. G. Stephenson. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo.* American Naturalist **129**:643–656.

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.

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. Influence of environmental quality on pollen competitive ability in wild radish. Science **248**:1631–1633.

Young, H. J., and T. P. Young. 1992. Alternative outcomes of natural and experimental high pollen loads. Ecology **73**: 639–647.