Reproductive investment within inflorescences of *Stylidium armeria* varies with the strength of early resource commitment

Rowan H. Brookes1,*, Linley K. Jesson2 and Martin Burd1

1School of Biological Sciences, Monash University, Melbourne, Victoria 3800, Australia and 2Department of Biology, University of New Brunswick, 10 Bailey Drive, Fredericton, Canada E3B 5A3

* For correspondence. E-mail rowan.brookes@gmail.com

INTRODUCTION

Floral and fruit traits often vary temporally and spatially within the inflorescences of flowering plants. Neighbouring flowers may differ in their ovule number (female reproductive potential), and nearby fruits may differ in seed number and size (realised reproductive success). Sometimes variation is imposed haphazardly by environmental factors, as when disparity in seed set is produced by stochastic pollinator service to individual flowers (Burd, 1994; Engel and Irwin, 2003; Herrera, 2004). But often a consistent, regular pattern of reproductive variation occurs within inflorescences (Diggle and Miller, 2004). A common pattern is for early flowers in inflorescences with sequential blooming to have larger corollas, more ovules, and greater seed number or mass than later flowers. A temporal sequence of reproductive investment may correspond to a spatial sequence. For instance, plants with acropetal flowering often display a basal-to-distal decline in allocation to flowers and fruits (Stephenson, 1981; Thompson, 1989; Wolfe, 1992; Ashman and Hitchens, 2000; Vallius, 2000; Zhao et al., 2008), although the opposite pattern also occurs (see Diggle, 2003).

Intra-inflorescence variation in floral and fruit investment may provide a window into the selective factors affecting sex allocation, monoeony, and other forms of sexual diversification in plants (Barrett, 2002; Ortiz et al., 2009). For example, the typical decline in female allocation in distal hermaphroditic flowers (Mazer and Dawson, 2001; Ishii and Sakai, 2002) has a parallel, but more extreme expression, in andromonoecious inflorescences with distal stamine flowers (Diggle and Miller, 2004). But it is not known if similar selective forces are behind these parallel patterns of sexual investment. Despite a good deal of attention to intra-inflorescence variation, the question of what causes temporally and distally declining investment in reproduction is not settled.

Two mechanisms that might explain variation in floral traits like corolla size and ovule number are resource pre-emption and architectural effects. These might also apply to post-fertilization traits like seed number and fruit investment, but a third mechanism, pollen limitation, could also play a role. A difficulty in assessing these factors is that they need not be mutually exclusive.

The resource pre-emption hypothesis suggests that the strength of internal constraints is contingent upon investment in basal structures, which then act as sinks for available resources and limit a plant’s ability to invest in more distal structures (Stephenson, 1981; Lee, 1988). Resource pre-emption should create a trade-off between early and late
allocation that is modulated by the resource status of the plant and the strength of basal resource sinks. For example, stronger resource sinks in structures produced early should directly reduce allocation in later structures, while increases in resource availability, or decreases in the resource demands of basal flowers, will reduce the trade-off between allocations to early and later structures (Medrano et al., 2000).

If declining investment in distal structures still occurs when resources are adequate or when maturation of basal fruits is prevented, this can be attributable to architectural effects (Wolfe, 1992; Diggle, 1995; Ashman and Hitchens, 2000; Buide, 2008; Ortiz et al., 2009). Architectural effects represent a constraint on the translocation of resources to flowers, fruits and seeds due to inherent structural features of an inflorescence, such as variation in the diameter of supporting structures (e.g. Corner’s rules; see Corner, 1949; Midgley and Bond, 1989) or the waning of the vasculature in distal structures (Diggle, 1995; Wolfe and Denton, 2001). However, there seems to be no universal mechanistic explanation of architectural effects (Diggle, 2003).

Non-uniform pollination within the inflorescence could also result in reduced allocation to distal fruits and seeds, including a declining probability of fruit set (Tremblay, 2006). For instance, pollen deposition and pollen quality can differ among stigmas depending on their position on the inflorescence (Brunet and Charlesworth, 1995; Price et al., 2005; Wiggam and Ferguson, 2005). If declining investment in distal flowers affects their ability to attract pollinator visits, then internal resource constraints may interact with pollen limitation to accelerate the drop in investment toward the tip of a raceme. But if visitation is not closely related to investment in attraction, a highly variable pattern of ovule fertilization along an inflorescence could produce a seemingly random pattern of investment in seeds and pre-fertilization traits of subsequent flowers. If post-fertilization patterns of investment are due to limitations imposed by pollinators, supplemental pollination should remove these patterns. In contrast, a steeper decline in allocation as a result of pollen addition would suggest competition between developing fruits for resources.

These within-plant variations in reproductive investment can be studied especially well in plants with indeterminate inflorescences, such as racemes. The study plant, Stylidium armeria, produces up to about seven racemes with acropetal anthesis. The sequential flowering allows us to examine how flowers and fruits at different positions within the inflorescence respond to pollen and resource manipulations. In a previous study, Brookes et al. (2008) showed that neither pollen nor resource availability alone limited seed set in S. armeria at the level of the whole plant, but that simultaneous addition of both pollen and resources increased seed set by an average of 47.7% over control plants. Measures of whole-plant fitness may, however, disguise within-plant variation in the dynamics of pollen and resource limitation (Wesselingh, 2007). In this study, the allocation of floral and fruit biomass within inflorescences is examined by using pollen and resource manipulations in combination with natural variation among plants in their resource commitment to early (basal) reproductive structures.

Resource pre-emption should produce a trade-off between early and later investment in reproductive structures: plants that invested more heavily in basal structures would face stronger constraints on later investment. But the strength of such a trade-off should change with resource status, and could disappear with sufficient resources or few initial sinks. In contrast, if architectural effects alone limit allocation to reproductive structures, there would be no change in relative investment between basal and distal structures regardless of the plant’s resource status or strength of the sinks. However, architectural effects might interact with resource pre-emption. In this case, it could be predicted that a general decline in allocation to subsequent structures would be ameliorated but not eliminated by resource supplementation. Finally, if patterns of allocations to post-fertilization traits vary greatly between plants receiving supplemental or natural pollination, this would suggest that the external pollen environment strongly influences resource partitioning.

MATERIALS AND METHODS

Study species and site description

A population of Stylidium armeria Labill. (Stylidiaceae) was studied at Lake Mountain, Victoria, Australia (37°31’S, 145°52’E; 1433 m a.s.l.), a subalpine ecosystem dominated by snow gum (Eucalyptus pauciflora) with a mixture of understorey shrubs. At the time of this study, the forest had been undisturbed since fires that occurred in 1939 (Ashton and Hargreaves, 1983). The mean daily temperature at Lake Mountain was 13.3 °C during the 2006 season and 16.6 °C during the 2007 season (data provided by Lake Mountain Alpine Resort). Stylidium armeria is a perennial, rhizomatous herb occurring in habitats ranging from lowland woods to subalpine snowfields throughout south-eastern Australia. Individual plants at Lake Mountain usually produce two or three (occasionally up to seven) racemes between December and February. The pinkish, zygomorphic flowers are presented acropetally on a raceme with an average of 56 sequentially blooming flowers per plant (standard deviation = 49, range = 6–330, n = 150 plants). Flowers are protandrous: the anthers are presented for an average of 4.3 d, followed by an average stigmatic phase of 4.8 d (s.d. = 2.0 and 2.8, respectively, n = 1478 flowers). Bees, butterflies and flies visit the flowers (Raulings and Ladiges, 2001). Seeds are gravity dispersed from dry, dehiscent capsules. Stylidium armeria at Lake Mountain is not autonomously selfing, as bagged plants did not produce seed (R. H. Brookes, pers. obs.). However, during this study, plants were not artificially self-pollinated to test compatibility. A voucher specimen of S. armeria from this study has been placed at the Victorian National Herbarium, Australia (MEL).

Pollen and resource manipulations

A full description of the pollen and resource manipulation methods and sample sizes is detailed in Brookes et al. (2008). Briefly, during October and November 2005, before plants bolted, 35 replicates were selected for each of six treatments groups formed by crossing three resource levels (reduction, control and addition) with two pollination levels (control and addition). All treatment combinations were...
applied consistently to the same plants for two flowering seasons, December 2005 until February 2006 (2006 season) and December 2006 until February 2007 (2007 season). Resource reduction was imposed by removing 50–60% of leaves from a plant prior to flowering. Plants in the control resource treatment were unmanipulated. Resource addition was accomplished by applying a liquid N-P-K (9-2-6) fertilizer around the base of plants once a month during the flowering season. Plants in the control pollination treatment were unmanipulated. Plants in the pollen-addition treatment received supplemental pollen taken from at least five plants at least 5 m away and transferred to receptive stigmas using a small paintbrush. Supplemental pollination occurred once a week during the 2006 season and 2 or 3 times a week during the 2007 season. Not all plants flowered and so final numbers varied across treatments. Measurements of reproductive traits were taken in the second season.

Unmanipulated plants

In order to supplement the information gathered from the pollen and resource manipulations, floral and fruiting biomass were measured on 31 unmanipulated plants. One flower was removed from the bottom, middle and top (early, middle and late season) of each raceme once the flower had completely opened, but prior to dehiscence of its anthers. Only three flowers were removed from each raceme to minimize alteration of the resource status in the remaining flowers. Each flower was stored in 70% ethanol, then oven-dried at 40 °C, and separated into the ovary, gynostemium, calyx and corolla for weighing. When fruits from these unmanipulated plants had matured, the racemes were collected and allowed to dry naturally. The position of each fruit was recorded, the fruit-to-flower ratio was determined for each plant, and the fruits at all positions were weighed. When there were multiple racemes per plant, a mean biomass for flowers and fruits was calculated across the racemes.

Measurement of reproductive traits

Following the treatments in the second season, fruits were allowed to mature and then the racemes collected from plants in the six pollen-resource treatment groups for measurement of reproductive traits in the laboratory. The floral position of each fruit on a raceme was numbered sequentially starting from 1 at the base. Flowering is acropetal, and so the floral position and Julian date of flower presentation were highly correlated ($r = 0.77$, $P < 0.0001$), with flowers blooming late in the season occurring at the distal end of the raceme. To determine the influence of pollen and resource manipulations on reproductive traits, the number of ovules, fertilized ovules (aborted ovules + seeds) and seeds was counted for every third fruit (which may be more than three floral positions if abortion occurred) on one haphazardly selected raceme per plant.

To determine if subsampling influenced the estimates of the investment pattern along an inflorescence, the 31 unmanipulated plants for which every position was sampled were compared with the same plants that had every third fruit sampled. Ovule number and seed number at each position were used as dependent variables in analyses of co-variance with position as an independent variable and subsampling vs. full sampling as a categorical factor. This test allows us to determine if subsampling resulted in a significant difference in either the estimated intercept (a significant sampling effect) or the slope (an interaction of position with sampling regime). There was no significant difference between the different sampling regimes for ovule or seed number (ovule number – subsampled plants: $\alpha = 6.8 \times 10^{-3}; \beta = -6.4 \times 10^{-3}$; all positions: $\alpha = 6.8 \times 10^{-4}; \beta = 6.5 \times 10^{-6}; P = 0.98$; seed number – subsampled plants: $\alpha = 40.99; \beta = -0.40$; all positions: $\alpha = 40.74; \beta = -0.39; P = 0.91$), suggesting that sampling only every third fruit was sufficient.

Statistical analysis

All statistical computations were conducted using R version 2.7 (R Foundation for Statistical Computing, Vienna, Austria). The data analysis had two parts. In the first, the overall effects of the resource and pollination treatments and of inflorescence position were considered. These analyses treat individual plants as random deviations from global treatment effects and, more importantly, as random deviations from a universal pattern of floral or fruit investment along the inflorescence. This is a traditional way to analyse a factorial experiment with a covariate.

To test for these overall effects, linear mixed models were used to analyse each of three dependent variables – the number of ovules per flower, the proportion of ovules that were fertilized, and the number of seeds per fruit – with respect to pollen treatments, resource treatments, fruit position on the inflorescence, and all their two-way interactions as fixed independent effects; and individual plant identity as a random independent effect. In these analyses, a pollination × position interaction or a resource treatment × position interaction would indicate whether an experimental manipulation altered the intra-inflorescence pattern of investment (the regression slope relating a dependent variable to inflorescence position) compared with other treatments. To normalize residuals for percentage fertilization and for seed number the Box–Cox power transformation $Y^\lambda = Y^{\lambda}$ (Kutner et al., 2005) was used. The maximum likelihood estimates of $\lambda$ were 0.40 for transformation of percentage fertilization, and 0.42 for transformation of seed number.

In separate tests, a supplementary sample of 31 unmanipulated plants was used to examine how inflorescence position affects flower mass (measured at three positions on each raceme), fruit mass (measured at all positions except the three used for flower measurements), and the probability of successful fruit set. For the flower and fruit mass data, repeated measures ANOVAs were conducted with the plant as the subject and flower position as the repeated measure. For the probability of fruit set, the fruiting proportion was calculated at each position across all plants in the sample and this proportion analysed with respect to position in a general linear model. In this analysis, proportion fruit set was arcsine transformed to improve normality, and fruit position was considered as a both a linear and a quadratic term to test for non-linearities (Barrett and Harder, 1992).
The second part of the analysis looked explicitly at differences among individual plants. First it was tested whether a model in which each plant has its own slope and intercept to describe the linear change in ovule or seed number along the inflorescence was statistically superior to models with a common slope or intercept for all plants. This procedure tests the extent to which architectural effects enforce a homogeneous pattern of investment along the inflorescence. Having confirmed that plants should be treated individually, their slopes were then compared against their intercepts. This procedure tests the resource pre-emption hypothesis, as explained below.

The test of homogenous vs. heterogeneous patterns of intra-inflorescence investment used four linear mixed models for each dependent variable: one with a common slope and intercept among all plants; one with a common slope but heterogeneous intercepts; one with a common intercept but heterogeneous slopes; and one with idiosyncratic slopes and intercepts for each plant. Likelihood ratio tests were used to compare these models (Crawley, 2007).

When it emerged that plants had heterogeneous slopes and intercepts, each plant’s slope (which represent an average rate of change in investment with each successive floral position) was plotted against its intercept (a plant’s average resource commitment to basal flowers and fruits) from the mixed model, i.e. every point on the plot contained information about a single plant’s initial and subsequent investment pattern. Then treatment groups were compared using ANCOVA: individual plant slopes were the dependent variable, the corresponding intercepts were the covariate, and the three resource and two pollen treatments were factor levels. Initially the three-way interaction between the intercept, pollen and resource treatments was tested. If this term was significant (as it was for both ovule and seed number), the effect of resources was analysed separately for each pollen treatment. Flower number had been initially included as an additional covariate, in order to examine whether intra-inflorescence investment depended on the total number of resource sinks. There was no significant effect of this term and it was subsequently excluded from the analysis.

**RESULTS**

**Overall patterns and main treatment effects**

The *S. armeria* plants in the sample showed a general trend of declining ovule number and seed number in the more distal flowers and fruits, as seen in Fig. 1, which summarizes the data by showing linear relationships for each plant. There were average reductions of 1.94 ovules per flower and 0.32 seeds per fruit with each successive position along the raceme, pooling across all plants in all treatments.

These overall patterns appear as highly significant main effects of inflorescence position on ovule number and seed number in the linear mixed models (Table 1).

There was also a significant main effect of the resource treatments on ovule number, and a significant resource × position interaction (Table 1). NPK addition tended to elevate the number of ovules per flower and ameliorate the decline in ovule number across successive positions relative to

unmanipulated controls, while resource reduction through leaf clipping tended to reduce overall allocation to ovules and create a steeper decline in ovule number across successive inflorescence positions (Fig. 1A, D). In contrast to the case for ovule production, resource manipulations had no significant effect on seed number (Table 1), although it appears that resource addition sometimes increased seed production relative to the other treatment levels (Fig. 1C, F).

The results for the fertilization rate differed substantially from the results for ovule and seed production. There was no significant main effect of position, although there was a significant pollination × position interaction (Table 1). Inspection of Fig. 1 reveals that, for many plants, the fraction of fertilized ovules tended to rise slightly in more distal flowers, and this was especially true in naturally pollinated plants. Both natural and supplemental pollination produced large and erratic variation in the rate of ovule fertilization among fruits. In many racemes, nearly full fertilization of ovules could occur in one fruit, but extremely low fertilization in the next sampled fruit, and back to near full fertilization in the next. The intra-inflorescence pattern of fertilization was not coherent among plants, as indicated by the assortment of fitted lines shown in Fig. 1B. There were no significant effects involving resource treatments (Table 1).

In unmanipulated plants, flowers (Table 2) and fruits at the top of the raceme tended to have smaller biomass than those in basal positions. The total weight of all floral parts (calyx, corolla, ovary, gynostemium) in the top flowers was less than that of the bottom flowers (all *P* < 0.0001; Table 2). Resource allocation to fruits declined towards the distal fruits (*t* = -15.9, *d.f.* = 1206, *r*² = 0.732, *P* < 0.0001). Dry fruit weights ranged from 47.19 mg at the distal end to 0.12 mg at the distal end (overall mean = 15.83 mg, s.d. = 8.08). Although fruit set decreased with position in unmanipulated plants, there was no statistical decline explained by either the linear or quadratic position term (both *P* > 0.05).

**Variation among individuals**

Inspection of Fig. 1 suggests that plants vary greatly in their patterns of intra-inflorescence reproductive investment and fertilization success. This impression was confirmed by the comparison of mixed models. Likelihood ratio tests showed that, for each of the three dependent variables, a model that estimated separate slopes and intercepts for each plant was significantly better supported than models with either a common slope or a common intercept, and these models in turn were superior to a model with both common slope and intercept for all plants. Likelihood ratios for these comparisons were always highly significant for all dependent variables (*P* < 0.0001), with the exception of one comparison involving seed number (model of heterogeneous slopes and intercepts compared with a model of uniform slopes with heterogeneous intercepts) for which *P* = 0.003.

Considering the plants individually shows the extent of variation in intra-inflorescence patterns. Slopes (the effect of inflorescence position) ranged from 2.4 down to −4.9 for ovule number; 0.04 to −0.03 for fertilization rate; and 1.8 to −6.1 (with a probable outlier at −19) for seed number, taken across all treatment groups (Fig. 1). Although the main
effect of inflorescence position on ovule and seed number (Table 1) is negative, there seems not to be a consistent, strong constraint on the investment pattern.

A plot of these slopes against the corresponding intercepts for each plant yields an important insight into the dynamic nature of resource allocation (Fig. 2). Lower intercepts (less investment in basal flowers and fruits) tend to be associated with higher slopes (slight increases or mild decreases in allocation to more distal structures), and higher intercepts with more strongly negative slopes (high basal investment with strong decline distally). The pattern in Fig. 2 can be summarized by what is called here a meta-slope, the slope of a relationship among data points that themselves represent (on the abscissa) the slope of a reproductive trait with respect to inflorescence position for an individual plant. The more negative the meta-slope, the stronger the trade-off is between early, basal investment and later investment, as predicted by resource pre-emption.

The homogeneity of meta-slopes in Fig. 2 among the pollen and resource treatment groups was tested via ANCOVA. The three-way interaction of pollen/C2 resource/C2 intercept (covariate value along the abscissa in Fig. 2) was significant for ovule

{**Table 1. Results of linear mixed models testing whether differences in ovule number, ovule fertilization rate and seed number in S. armeria are affected by the position of the fruit and the pollen and resource treatments**}

<table>
<thead>
<tr>
<th>Effect</th>
<th>Ovule number</th>
<th>Fertilization rate</th>
<th>Seed number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>d.f.</td>
<td>$P$</td>
</tr>
<tr>
<td>Position</td>
<td>403.920</td>
<td>1</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Resource</td>
<td>7.331</td>
<td>2</td>
<td>0.026</td>
</tr>
<tr>
<td>Pollen</td>
<td>0.440</td>
<td>1</td>
<td>0.507</td>
</tr>
<tr>
<td>Pollen × position</td>
<td>2.477</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td>Resource × position</td>
<td>26.698</td>
<td>2</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

Plant was considered as a random effect.
number \((P = 0.032)\) and seed number \((P = 0.002)\), but not fertilization rate \((P = 0.64)\). The resource × covariate terms were also significant for both these variables \((P = 0.0003\) for ovule number; \(P = 0.00002\) for seed number), and the pollen × covariate term was significant for seed number \((P = 0.02)\). Fertilization rate was influenced only by the effect of the covariate \((P < 0.001)\). However, even for fertilization rate the meta-slope was negative \((\beta = -0.048)\), with plants with low initial investment having increases in fertilization in subsequent positions across the inflorescence, while those with high initial fertilization rates tended to have declines in fertilization in more distal flowers on the inflorescence (results not shown).

Given the interaction effects for ovule and seed number, meta-slopes were calculated for each combination of pollen and resource treatment separately (Table 3). There is a consistent pattern for ovule number: whether plants receive supplemental pollen or not, resource addition raises the meta-slope (makes it less negative) while resource reduction lowers it, relative to the natural resource regime. A shallower, less negative meta-slope indicates an amelioration of resource pre-emption, while a steeper, more negative one reveals an exacerbation of resource pre-emption. Meta-slopes for seed number were more anomalous. Both the resource addition

**TABLE 2.** Mean dried weight (in milligrams) and ± standard deviation of dissected S. armeria flowers

<table>
<thead>
<tr>
<th>Character</th>
<th>Lower</th>
<th>Middle</th>
<th>Top</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calyx mass (mg)</td>
<td>1.58 ± 0.44</td>
<td>1.05 ± 0.24</td>
<td>0.66 ± 0.21</td>
</tr>
<tr>
<td>Corolla mass (mg)</td>
<td>1.0 ± 0.24</td>
<td>0.83 ± 0.16</td>
<td>0.63 ± 0.14</td>
</tr>
<tr>
<td>Ovary mass (mg)</td>
<td>0.25 ± 0.06</td>
<td>0.16 ± 0.04</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>Gynostemium (mg)</td>
<td>0.76 ± 0.18</td>
<td>0.72 ± 0.20</td>
<td>0.58 ± 0.14</td>
</tr>
<tr>
<td>Flower mass (mg)</td>
<td>3.59 ± 0.09</td>
<td>2.80 ± 0.06</td>
<td>1.94 ± 0.04</td>
</tr>
</tbody>
</table>

Gynostemium is the fused staminate and pistillate tissues. 

\(n = 31\) plants, three flowers from each plant. 
All \(P < 0.0001\), d.f.2,138.

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**FIG. 2.** Intra-inflorescence variation in ovule and seed number among individual plants of *Stylidium armeria*. Each point represents the intercept (horizontal axis) and slope (vertical axis) of the linear relationship between ovule number (A, C) or seed number (B, D) and inflorescence position for an individual plant (see Fig. 1). Resource treatments are indicated by filled diamonds (resource addition), open diamonds (natural resource regime), and open triangles (resource reduction). The natural pollination treatment groups are shown in (A) and (B); supplementary pollination treatment groups in (C) and (D). Regression lines are shown for each pollen-resource treatment combination; these are the meta-slopes referred to in the text. (ANCOVA showed significant non-heterogeneity of slopes among resource treatments for all panels in this figure; see text.) Two outliers in (B) (intercept = 114, slope = -19; and intercept = 94.5, slope = -6.1) that strongly influence the regression slope for the natural resources treatment are not shown on the graph, but the data were retained when calculating the regression line.
DISCUSSION

A majority of plants in each treatment group showed negative linear relationships of ovule number per flower, fertilization rate per flower, and seed number per fruit with respect to fruit position (Fig. 1). In this broad sense, *S. armeria* resembles many other species. But the quantitative details of this tendency varied from plant to plant, and even negative relationships were not universal. Thus, *S. armeria* shows not only intra-inflorescence variation, but differences among plants in the pattern of variation.

Although architectural effects and resource pre-emption are not mutually exclusive hypotheses for intra-inflorescence differences in flower and fruit allocation, there are some basic predictions that differ between the two: resource pre-emption can result in whole-plant resource manipulations altering the patterns of investment, if early flowers pre-empt resources that could have been allocated to later flowers; whereas, architectural effects will cause consistent reductions in allocation to subsequent flowers, regardless of the plant’s resource status. Investment and resource allocation to seeds may also be influenced by a plant’s pollination history, although no statistical evidence was found of an effect of supplemental pollination on the intra-inflorescence pattern of seed number per fruit (Table 1). The implications of these results for each of these three potential explanations is considered below.

Resource pre-emption

Plants were not uniform in their investment at basal positions (the intercepts of the fitted lines in Fig. 1), and these differences in early investment affected distal investment in the manner predicted by the resource pre-emption hypothesis: plants with the fewest ovules and seeds in their basal fruits tended to show the least pre-emption of distal investment, while those plants with larger basal investments showed sharper drops in ovule and seed number in distal fruits (Fig. 2). The pattern of variation among plants shown in Fig. 2 can be explained by an internal, within-plant resource trade-off between early and late fruits. The resource manipulations reinforce this conclusion: the trade-off between basal and distal investment was less severe among plants that had received NPK additions, and more severe among plants that had received partial defoliation (Fig. 2).

Interestingly, the fertilization of ovules did not respond to resource treatment, despite a trade-off in investment between early and late fruits. The greatest resource sink for a maternal plant is likely to be developing seeds to maturity, rather than fertilizing seeds initially. Any effect of resource pre-emption on the fertilization rate would probably have to act through flower size or the number of open flowers later in inflorescence development. The different effects of the resource treatments on the fertilization of ovules versus seed maturation suggests some degree of maternal choice may be occurring – the amount of abortion is strongly influenced by the maternal plant’s resource status.

Pollen supplementation tended to ameliorate the trade-offs between seed production in early and late fruits. This treatment effect can be seen in the flatter (less negative) meta-slopes for seed number under pollen addition than under natural pollination (Fig. 2 and Table 3). The benefit of extra pollen for seed output in distal fruits, even under the resource reduction treatment, suggests that, by restricting the size of distal flowers, resource pre-emption may be diminishing their ability to attract pollinators.

Architectural constraints in allocation to reproductive traits

*Stylidium armeria* at Lake Mountain showed a basal-to-distal decline in ovule number (Fig. 1A, D) and floral biomass (Table 2), as well as in seed number (Fig. 2) and fruiting biomass in the majority of plants. This tendency was not fully removed, even after 2 years of resource manipulations, a result that is consistent with some degree of architectural effect on investment in distal portions of the raceme (Diggles, 1995). This effect could be due to vascular constraints on the delivery of photosynthates and other resources to distal flowers, or a decline in photosynthetic rates or other resource harvesting ability as a growing season progresses (Matsumoto et al., 2008). But any effect of this kind must impose a rather broad tendency on allocation patterns rather than a strong constraint. Linear trends of increasing ovule number in distal flowers, rather than the decrease expected from architectural constraints, occurred in 4% of the sample

### Table 3. Meta-slopes (see text and Fig. 2 for explanation of this term) for each of six combinations of pollen and resource treatments

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Ovule number*</th>
<th>Seed number*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural pollination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resource addition</td>
<td>−0.013 (−0.023, −0.003)</td>
<td>−0.020 (−0.031, −0.009)</td>
</tr>
<tr>
<td>Natural resources</td>
<td>−0.029 (−0.043, −0.015)</td>
<td>−0.136 (−0.190, −0.082)</td>
</tr>
<tr>
<td>Resource reduction</td>
<td>−0.044 (−0.069, −0.019)</td>
<td>−0.052 (−0.084, −0.021)</td>
</tr>
<tr>
<td>Pollen addition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resource addition</td>
<td>−0.011 (−0.025, 0.003)</td>
<td>−0.028 (−0.039, −0.018)</td>
</tr>
<tr>
<td>Natural resources</td>
<td>−0.023 (−0.033, −0.012)</td>
<td>−0.042 (−0.077, −0.005)</td>
</tr>
<tr>
<td>Resource reduction</td>
<td>−0.036 (−0.047, −0.025)</td>
<td>−0.020 (−0.038, −0.002)</td>
</tr>
</tbody>
</table>

* The 95% confidence intervals are given in parenthesis.

and the reduction treatments produced shallower meta-slopes than did the natural resource treatment (Table 3). Supplemental pollination also tended to flatten the meta-slopes for seed number (Fig. 2 and Table 3) relative to the outcome under natural pollination.
plants (pooled across all experimental treatments; Fig. 1). The corresponding figure for fertilization rate was 54%, and for seed number, 20% (Fig. 1).

Pollen limitation effects on allocation to post-fertilization traits

For naturally pollinated plants of *S. armeria*, percentage fertilization, seed number (Table 1 and Fig. 1) and fruit set show a high degree of variability across all fruit positions on the inflorescence. Regardless of the fruit position, there is a roughly equal probability of high or low fertilization and seed set. This means that, in contrast to the typical pattern in other species (Diggle, 1995; Kudo and Molau, 1999; Kliber and Eckert, 2004), the distal fruits do not have a lower reproductive success, whether or not resource manipulations are made. When supplemental pollen is added to *S. armeria*, the variation among the plants decreases and the slopes become somewhat more homogeneous (compare C and F in Fig. 1). This pattern would be expected if supplemental pollination reduces the influence of stochastic pollination across fruit positions. Pollen supplementation increased the pollen quantity on the stigmas, yet the seed set in all positions remained similar to naturally pollinated plants (Table 1 and Fig. 1). The similarity in seed set suggests that the quality of the pollen restricts the number of seeds that can be produced. For example, if the quality of stigmatic pollen varies greatly among flowers, variable seed set could be due to genetic load in the population, if some embryos are lost from early-acting lethal mutations (Porch and Lande, 2005).

However, not all variation in post-fertilization traits could be attributed to the pollinating environment. Plants that had low rates of fertilization in basal flowers had high rates at distal positions, and the converse occurred for plants with high initial fertilization. This trade-off was not influenced by the plant’s resource or pollen status. This result appears counter-intuitive. While the resource cost of fertilizing ovules is likely to be low for the maternal plant, attraction to pollinators and trade-offs in floral display will influence fertilization, and so supplemental pollination should change the relationship between early and later investment. Ideally, supplemental pollination should result in a meta-slope of 0, i.e. consistent rates of fertilization across all flowers over all positions. One obvious explanation is that the uncertainty of the estimates of the slopes and intercepts obscures a pollination effect, although this would not explain the strongly negative meta-slope. Alternatively, differences in the size of floral structures (e.g. stigmatic area) or the length of floral display and stigmatic receptivity could influence number of pollen grains deposited on a stigma or the amount of pollen tube growth, and could act as a mechanism by the maternal plant to adjust fertilization once some threshold of seed fertilization has been achieved.

Selective consequences of variation among flowers

The high degree of variation in fertilization and seed number among individual flowers of *S. armeria* has implications for natural selection on reproductive traits. In the face of unpredictable success, the optimal number of ovules per flower is likely to be greater than would be the case with greater consistency of reproductive success among flowers (Burd, 1995). A strategy of overproduction allows a plant to capitalize on random large successes, and this could enhance total female fitness even though resources are necessarily invested in less-successful flowers (Burd, 1995). High ovule number per flower was associated with high inter-floral uncertainty in pollination and fertilization success in a taxonomically broad sample of angiosperms (Burd et al., 2009). *Stylidium armeria* fits well within this broader pattern: its mean ovule number per flower of 117.6 (Burd et al., 2008) is relatively high among angiosperms, and fertilization success is highly variable (Fig. 1B). It is surprising, however, that supplemental pollination did not erase that variability (Fig. 1E). Part of the uncertainty faced by individual flowers may be over the genetic quality of the pollen received, as well as the quantity (Porch and Lande, 2005).

Andromonoecious species often have smaller, staminate distal flowers (Emms, 1993; Diggle and Miller, 2004), giving them a resource investment pattern similar to that of *S. armeria*. It is interesting to consider whether the dynamic resource allocation we have seen in *S. armeria* could play a role in the evolution of andromonoecy or other forms of floral dimorphism. If basal flowers enjoy high mating success, then resource pre-emption would restrict the potential for female fitness in more distal flowers and leave them with a predominately male function. Since stamine flowers may be less costly, even controlling for the effect of inflorescence position (Vallejo-Marin and Rausher, 2007), selection could favour andromonoecy as a more economical use of resources. Thus, resource pre-emption might increase the probability of an evolutionary transition to andromonoecy, although testing this hypothesis would require much additional investigation because so many other factors, including trade-offs between male and female success or the potential for geitonogamy (Elle and Meagher, 2000), are likely to affect the evolution of floral dimorphism.

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