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Life-history trade-offs, seed predation and total offspring fitness

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Abstract. Optimal timing of reproduction within a season may be influenced by several abiotic and biotic factors. These factors sometimes affect different components of fitness, making assessments of net selection difficult. We used estimates of offspring fitness to examine how pre-dispersal seed predation influences selection on flowering schedule in an herb with a bimodal flowering pattern, *Actaea spicata*. Within individuals, seeds from flowers on early terminal inflorescences had a higher germination rate and produced larger seedlings than seeds from flowers on late basal inflorescences. Reproductive value, estimated using demographic integral projection models and accounting for size-dependent differences in future performance, was two times higher for intact seeds from early flowers than for seeds from late flowers. Fruits from late flowers were, however, much more likely to escape seed predation than fruits from early flowers. Reproductive values of early and late flowers balanced at a predation intensity of 63%. Across 15 natural populations, the strength of selection for allocation to late flowers was positively correlated with mean seed predation intensity. Our results suggest that the optimal shape of the flowering schedule, in terms of the allocation between early and late flowers, is determined by the trade-off between offspring number and quality, and that variation in antagonistic interactions among populations influences the balancing of this trade-off. At the same time they illustrate that phenotypic selection analyses that fail to account for differences in offspring fitness might be misleading.

Key words: biotic interactions; fitness components; flowering schedule; lifetime fitness; offspring quality; phenology; phenotypic selection; seed predation.

Introduction

Timing of reproduction is a key aspect of the life history of an organism. Organisms have evolved a range of different strategies to optimize the distribution of the reproductive events within a season, in response to selection mediated by the abiotic environment and by other organisms. Selection mediated by different agents might often act in opposite directions, yielding conflicting selective pressures (e.g., Evans et al. 1989, Brody 1997, Gomez 2004, Elzinga et al. 2007). In these cases, the optimal timing of reproduction will be a compromise, and the optimum will depend on the relative strength of selection mediated by the respective agents in a given environment. Moreover, some interactions influence only offspring number, while other interactions influence offspring quality as well. Because natural selection acts through differences in total offspring fitness and not through differences in offspring number, it is necessary to account for differences in offspring quality, in terms of their reproductive values (Fisher

1930, Taylor 1990, Grafen 2006, Engen et al. 2009, Kolb and Ehrlén 2010, Ehrlén 2015). Yet studies estimating natural selection based on estimates of offspring total fitness in iteroparous organisms are still rare.

In plants, the shape of the flowering schedule (i.e., the distribution of flowers over the season) might be regarded as a strategy to maximize offspring fitness in a given abiotic and biotic environment (Rathke and Lacey 1985, Primack 1987, Johnson 1993, Oberrath and Böhning-Gaese 2002, Elzinga et al. 2007, Kolb et al. 2007, Sandring and Ågren 2009, Fukano et al. 2013). Several recent papers have stressed the ecological and evolutionary importance of the shape of the flowering schedule of individuals (e.g., Malo 2002, Fox 2003). Yet few empirical studies have examined the fitness consequences of variation in individual flowering schedule shape in natural populations. Two important groups of agents of selection on flowering schedules are pollinators and pre-dispersal seed predators (e.g., Rathke and Lacey 1985, Elzinga et al. 2007). Selection mediated by these two types of agents is often conflicting (e.g., Elzinga et al. 2007, Ehrlén and Münzbergová 2009). In such cases, the optimal distribution of flowering times will be a compromise between attracting pollinators and avoiding seed predators.

One flowering schedule strategy, suggested to be associated with conflicting selection from pollinators and antagonists, is bimodal flowering, with early and late flowers within the same individual (Eriksson 1995). In such systems, selection may act on both the timing of early and late flowers and on the distribution of flowers between these two types. In the perennial herb *Actaea spicata*, the shape of the flowering schedule is clearly bimodal with flowers distributed between early terminal inflorescences and late basal inflorescences. It has been suggested that flowers in early inflorescences receive more pollinator visits, and therefore have a lower selfing rate and a higher seed mass than flowers in late inflorescences (Eriksson 1995). On balance, fruits in late inflorescences largely escape predation by the specialist moth *Eupithecia immundata*, while early inflorescences often experience high levels of predation (Eriksson 1995). Thus, in this system optimal allocation to early vs. late flowers might depend on a trade-off between the number and quality of offspring, the balance of this trade-off depending on the intensity of pre-dispersal seed predation. We examined this hypothesis and asked four specific questions: (1) Do seeds from early flowers result in offspring with a higher mean fitness than seeds from late flowers? (2) Do fruits from late flowers more often escape predation than fruits from early flowers, consistent with previous observations? (3) Is there a level of seed predation where differences in offspring quality are balanced? and (4) Is a higher intensity of seed predation within populations associated with a stronger selection for allocation to late flowers across natural populations? To address these questions, we recorded traits, intensity of seed predation, and seed number in 1118 plants in 15 natural populations. We also recorded the performance of the offspring from early and late flowers in a common-garden experiment and combined this information with demographic information in integral projection models to estimate reproductive values for seeds from early and late flowers. Lastly, we examined phenotypic selection gradients for allocation to late flowers based both on estimated total offspring fitness and on a single fitness component (number of seeds).

METHODS

Study system

Actaea spicata (Ranunculaceae) is long-lived understory herb of nutrient-rich deciduous forest distributed throughout most of Europe (Hultén and Fries 1986). In the study area, it occurs in shady, well-drained, rich deciduous and coniferous forests, often on limestone (Pellmyr 1984). Shoots emerge in the beginning of May. The plant produces determinate inflorescences at two distinct points in time, with terminal inflorescences flowering in late May–early June, and basal inflorescences flowering in late June–early July (hereafter referred to as early and late flowers, respectively; Appendix A). The main pollinator is the beetle *Byturus*

ochraceus (Byturidae) (Pellmyr 1984). Flowers are self-compatible and fruit set is often 100% (Pellmyr 1984). The fruit is a black berry, ripening in August. Flowers that are outcrossed produce seeds that are slightly heavier than seed from self-fertilized flowers, while seed numbers are similar between outcrossed and selfed flowers (Eriksson 1995), suggesting that pollinator availability is important for the size and quality, although not for the number of seeds. Removal of early inflorescences has no effect on seed mass in late inflorescences, suggesting that resource availability is not important for differences in seed size between inflorescence types (Eriksson 1995). In some areas, seeds of *A. spicata* are preyed upon by the specialist seed predator *Eupithecia immundata* (Geometridae). There is considerable variation in seed predation intensity among populations in the study area (von Zeipel et al. 2006). *Eupithecia immundata* oviposition takes place from mid to late June, such that fruits in late inflorescences often escape seed predation (Eriksson 1995, von Zeipel et al. 2006). Despite extensive collections and analyses of fruits, we have not found evidence that any other pre-dispersal seed predators attack the seeds of the study species in the study area.

Data collection

We collected field data on the number of early and late flowers, seed number per fruit, seed mass and pre-dispersal seed predation in 2008, and used seeds from this year to conduct a sowing experiment in 2009–2010 to quantify offspring fitness. These new data were combined with demographic data from a previous study to calculate differences in total offspring fitness across individuals. In 2008, we selected 15 populations of *A. spicata* in the Tullgarn natural reserve, 45 km south-southwest of Stockholm, Sweden (58°6′ N, 17°4′ E; Table 1) for the study. Distances between populations ranged from 0.4 to 2 km. In the beginning of June 2008, we marked 36–112 flowering individuals per population (in total 1118 individuals) with a plastic stick stuck into the ground and with a numbered ring attached to the plant stem. We counted the number of early and late flowers in all individuals and used the proportion of the total number of flowers that occurred in late inflorescences to describe the flowering schedule of individuals. We revisited the plants in August, after seed predator larval development was completed and larvae had left the fruit through an exit hole. We counted the number of fruits in each inflorescence in the following categories: intact fruits, fruits attacked by seed predators (with exit holes), and aborted flowers or immature fruits (seemingly undeveloped fruits). We recorded almost no signs of aborted flowers or fruits in this study. In the following, we therefore assume that the numbers of flowers initially present was equal to the observed number of fruits, and that the reproductive values of early and late flowers equal the reproductive values of early and late fruits, respectively. In individuals with up

TABLE 1. Properties of the 15 *Actaea spicata* populations included in this study.

Population number	Total population size	Number of measured individuals	Percentage of early fruits attacked, mean (1st quartile, 3rd quartile) [%]	Percentage of late fruits attacked, mean (1st quartile, 3rd quartile) [%]	Allocation to late flowers, mean, (1st quartile, 3rd quartile) [%]
1	162	91	45 (14, 74)	20 (0, 38)	15 (0, 24)
2	144	91	38 (17, 55)	30 (0, 100)	16 (0, 26)
3	185	89	61 (38, 100)	17 (0, 25)	15 (0, 28)
4	100	83	52 (11, 85)	11 (0, 0)	16 (0, 28)
5	110	85	43 (0, 78)	8 (0, 0)	8 (0, 13)
6	214	86	51 (0, 100)	10 (0, 0)	13 (0, 23)
7	120	87	35 (0, 50)	14 (0, 0)	13 (0, 25)
8	149	95	63 (40, 100)	11 (0, 0)	18 (0, 30)
9	3370	91	37 (0, 73)	5 (0, 0)	4 (0, 0)
10	214	86	49 (0, 92)	76 (50, 100)	14 (0, 26)
11	59	51	47 (0, 100)	33 (0, 60)	12 (0, 20)
12	44	43	51 (0, 92)	7 (0, 0)	13 (0, 24)
13	73	61	58 (33, 91)	20 (0, 23)	17 (0, 26)
14	36	23	45 (0, 84)	34 (0, 68)	8 (0, 10)
15	62	56	26 (0, 31)	3 (0, 0)	17 (0, 29)

Notes: Parameters include the percentage of fruits in early and late inflorescences, respectively, for individuals that were attacked by the pre-dispersal seed predator *Eupithecia immundata*, and allocation to late flowers (number of flowers in late inflorescences)/ (number of flowers in early inflorescences + number of flowers in late inflorescences).

to five intact and five attacked fruits in both early and late inflorescences, we collected all intact and attacked fruits from each individual. In individuals with more fruits, we collected five intact and five attacked fruits from both early and late inflorescences. For each fruit, the number of seeds was counted in the laboratory.

To estimate fitness of offspring from early and late flowers, we sowed seeds from 5 to 31 plants with both types of flowers from each of four populations in October 2008 (1, 4, 5, and 12, Table 1). For each individual, we sowed seeds from fruits of four early and four late flowers. We sowed all the available seeds from one fruit into one 10×10 cm pot filled with garden soil. The number of seeds sown per pot ranged from 1 to 13 and did not differ between early and late flowers (early, 6.62 ± 0.13 seeds [mean $\pm$ SE]; late, 6.63 ± 0.15 seeds). In total 4376 seeds were sown. Pots were placed in a randomized design in an unheated greenhouse in the experimental garden of the Institute of Botany of the Academy of Sciences in Pruhonice, Czech Republic (49°59′30″ N; 14°34′00″ E) and watered daily. In April 2009, we transferred the pots into a common garden and covered them with a green shading net. Cotyledons of *Actaea* seedlings appear aboveground only in the second year after seed dispersal (Ehrlén and Eriksson 2000). Germination was thus recorded in May 2010, when we also measured the length and width of the longest leaf of the seedlings on two occasions (7 and 17 May).

Differences between early and late seeds

Offspring number and offspring quality were estimated separately for early and late flowers. Offspring number was measured for each individual and flower type by the number of seeds per fruit. Offspring quality estimates were based both on the proportion of sown seeds within a fruit that germinated and on the size of the largest seedling from each fruit. Seed predation

intensity was estimated by the proportion of fruits damaged within each inflorescence. Differences in the number of seeds per fruit, germination rate, seedling size, and predation intensity between early and late flowers were modeled using the lmer R function in the lme4 package for linear and generalized linear mixed models (GLMMs). In all analyses, the identity of the mother plant was included as a grouping (random) variable to account for repeated measures. We specified models including seed type (from early vs. late flowers), population and their interaction as predictor variables. Population was included as a fixed factor because populations were chosen to represent a broad spectrum of predation intensities and we were interested in quantifying selection in these particular populations. In the analyses of germination and seedling sizes of seeds from a subset of the populations we treated population as a fixed factor because the number of populations (four) was too low to accurately estimate variances. Mean seed number and seedling size models were linear. Size of seedlings was estimated by the width of the longest leaf on 17 May (analyses based on leaf foliage height or width 10 days earlier yielded very similar results). Germination and predation intensity models were logistic regressions, with binomial error distributions and logit link functions. Germination was estimated as the proportion of seeds that had emerged as seedlings by 7 and 17 May. The effects of seed type and population were determined by comparing "full" models with models without seed type and population, respectively. If there was a significant effect of population, we compared models with and without the interaction term to see if the effect of population included a difference in the effect of seed type. Statistical significances of parameters or groups of parameters (when "main effects" and interactions were tested together) in the

GLMMs were calculated with likelikood ratio (LR) chi-square tests.

Calculation of reproductive values using integral projection modeling

Offspring quality was estimated as the reproductive values of seeds and fruits from early and late flowers, respectively. Our estimate of reproductive value measures the contribution of an individual to future population growth (cf. Caswell 2001). Calculations were based on information on the average number of seeds per fruit in intact and attacked fruits, the proportion of sown seeds germinating, and size of seedlings collected in this study, as well as on available demographic information. A previous study has shown that differences in seedling size influence future probabilities of both survival and reproduction (Dahlgren and Ehrlén 2009). As an integral measure of offspring fitness, we calculated reproductive values of the two seed and fruit types using demographic integral projection models (IPM). We based the IPM on a model for *A. spicata* parameterized with data from censuses of 1543 individuals in four populations in the same area in 2004–2007 (Dahlgren and Ehrlén 2011). We included information from the present study in terms of the relative differences in germination probability and the seedling size probability distribution between seeds from early and late flowers in the IPM. Reproductive values for early and late seeds were calculated by obtaining the left eigenvector associated with the dominant eigenvalue of a matrix representing the transition kernel and extracting the values corresponding to the respective seed type, which were separated into two different classes in the model (which is the standard method for matrix models as well as IPMs [Caswell 2001, Ellner and Rees 2006]). Our estimates of fitness and reproductive values thus reflect only the outcome of one episode of selection, i.e., one reproductive bout in an iteroparous species. Moreover, our estimates are based on female fitness, while we did not explicitly consider male fitness. However, given that early and late flowers are separated in time, female and male fitness should on average be equal for early and late flowers. Possibly, seeds from late flowers in *Actaea* are selfed to a larger extent than seeds from early flowers. Early and late flowers might thus differ in that the fitness gains through the male function in early flowers are incurred to a larger extent through fertilizing seeds on other individuals, while late flowers on average gain more through self-fertilization.

To examine if there is a level of seed predation intensity where benefits and costs of allocating resources to late flowers balance, we also calculated reproductive values for early and late flowers (equivalent to the sum of the reproductive values of all seeds within one fruit) at different predation levels, and determined the level of predation where reproductive values of early and late flowers were equal. In these calculations we assumed that the relation between predation intensity of early and late flowers was constant and equal to the observed mean relationship in this study (see *Results*). The number of seeds in intact and predated fruits was set to the observed average of the respective type. Under conditions with no predation, relative reproductive values of early and late flowers correspond to those of early and late seeds (as seed number per fruit was assumed to be the same in accordance with our observations; see *Results*). With increasing intensity of seed predation, the reproductive value of fruits from early flowers decreases more rapidly than the value for fruits from late flowers, and a point may be reached where reproductive values are equal.

Selection analyses

Analyses of selection were designed to account for the fact that natural selection acts through differences among individuals in offspring total fitness, and that differences in allocation to early vs. late flowers can influence multiple components of fitness. To quantify the strength of selection acting on flowering schedule in terms of the relative allocation to late flowers ((number of flowers in late inflorescences)/(number of flowers in early inflorescences + number of flowers in late inflorescences)), we used the regression procedure of Lande and Arnold (1983). Selection differentials estimating both direct and indirect selection on a trait, and selection gradients estimating direct selection on a focal trait independently of other measured traits, were calculated as the regression coefficients from models where relative fitness was regressed on standardized values of single and multiple traits, respectively. Fitness was calculated as the sum of the number of seeds in each category (early and late), weighted by their respective reproductive values. Fitness was relativized within each population by dividing by the mean value. The two traits, number of flowers and relative allocation to late flowers, were standardized within populations by subtracting mean values and dividing by the standard deviation. Selection differentials and selection gradients for relative allocation to late flowers were calculated independently for each population. Relationships between selection gradients and mean predation intensity across populations were examined using generalized least squares models to account for nonconstant variances (using the gls function in the nlme R package to fit the variance parameter, and assuming an exponential relationship between mean and variance).

To assess how estimates of selection on relative allocation to late flowers based on a single fitness component differed from estimates based on offspring total fitness, i.e., how differences in the expected future reproductive output of offspring originating from the two seed types influenced our estimates of selection, we also calculated selection estimates based on unweighted seed number as the measure of fitness. We then compared selection estimates from these models using a paired *t* test, counting all seeds as equal, with estimates
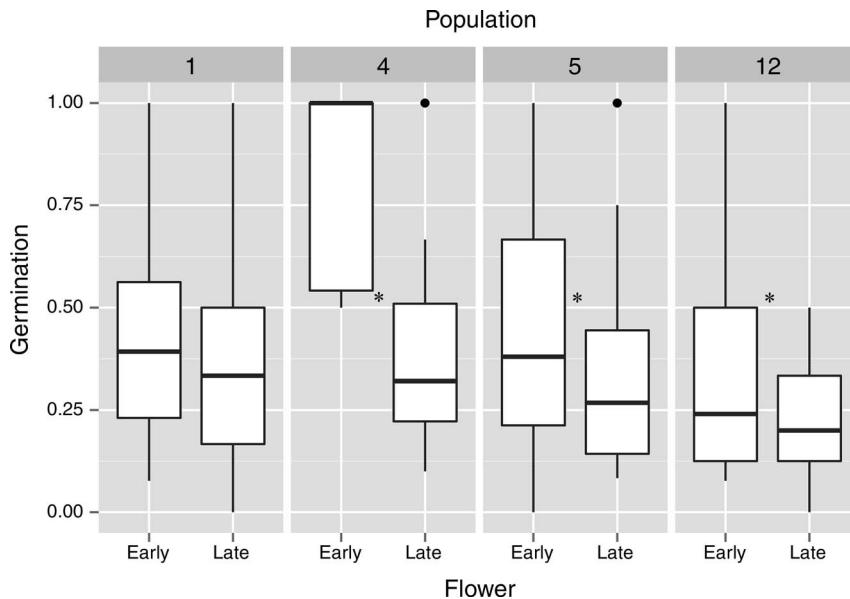
Fɪɢ. 1. Box-and-whisker plots of germination probability for seeds from early and late flowers from four populations of the herb *Actaea spicata* (Populations 1, 4, 5, and 12) (seed type, LR chi-square = 30.5, df = 4, $P < 0.001$; population, LR = 29.8, df = 6, $P < 0.001$; seed type × population, LR = 15.2, df = 3, $P = 0.002$). The box endpoints represent the first and third quartiles, and the line within the box is the median. Black circles represent outliers (50% lower or higher than the first and third quartiles, respectively), and the whiskers extend to the last data point not defined as an outlier. Asterisk symbols between bars indicate significant differences ($\alpha < 0.05$) between seeds from early and late flowers. Population-wise tests of differences in (logit) means for early and late seeds (b): Population 1 ($b = 0.07$, LR = 0.26, $P = 0.61$); Population 4 ($b = -1.47$, LR = 9.90, $P = 0.002$); Population 5 ($b = -0.44$, LR = 15.4, $P < 0.001$); and Population 12 ($b = -0.55$, LR = 4.31, $P = 0.038$).

from the models based on fitness values accounting for differences in reproductive values between seed types.

RESULTS

Seeds from early flowers had higher germination rates and produced larger seedlings than seeds from late flowers. Of the 1118 individuals observed during this study, 594 (53.1%) produced both early and late flowers, and other individuals produced only early flowers. All 15 study populations included individuals with this mixed strategy. The average proportion of late flowers in individuals with both flower types was 0.253 ± 0.140 (mean ± SD). Mean seed number per intact fruit did not differ significantly between early and late flowers (6.44 vs. 6.34 seeds (mean ± SD), LR = 0.41, df = 1, $P = 0.52$), but differed among populations (LR = 56, df = 14, $P < 0.001$). The proportion of seeds that germinated was significantly higher for seeds from early flowers than for seeds from late flowers in three of four populations, and the magnitude of the difference varied significantly among populations (Fig. 1). Moreover, seedlings from early seeds were larger than those from late seeds (mean leaf width on 17 May was 14.2 ± 5.61 mm (mean ± SD) for seedlings from early seeds and 11.7 ± 5.16 mm for seedlings from late seeds, $P < 0.001$), and these differences were consistent between populations (no effect of population or the interaction between seed type and population; LR = 7.54, df = 6, $P = 0.27$). As a result of these differences, the calculated reproductive value,

accounting for modeled future differences in survival and reproduction, was 2.03 times higher for intact seeds from early flowers than for seeds from late flowers.

Seed predation reduced the mean number of intact seeds in fruits from both early and late flowers, from an average 6.36 ± 3.1 seeds (mean ± SD), $N = 1733$ seeds in intact fruits to 0.90 ± 1.5 seeds, $N = 1049$ seeds) in attacked fruits. The proportion of damaged fruits was much higher for early flowers (0.46 ± 0.42, $N = 1433$ fruits), than for late (0.07 ± 0.39, $N = 770$ fruits; LR = 1931, df = 1, $P < 0.001$). Mean intensity of predation differed also between populations (range = 0.24–0.59, LR = 973, df = 28, $P < 0.001$), and there was a significant effect of the interaction between flower type and population (LR = 902, df = 14, $P < 0.001$). The relative allocation to late flowers was not related to seed predation in early inflorescences (mixed effects linear model including population as a random factor: LR = 0.25, $P = 0.62$).

Integral projection modeling showed that reproductive values of early and late flowers were similar at a predation intensity corresponding to 63% of seeds lost in early flowers. At lower predation intensities, early flowers had a higher fitness and at higher intensities late flowers had higher fitness.

Selection differentials within populations were on average positive (0.234 ± 0.178 [mean ± SD], $N = 15$; Fig. 2; Appendix B), indicating a net selection for higher allocation to late flowers. However, this relationship was
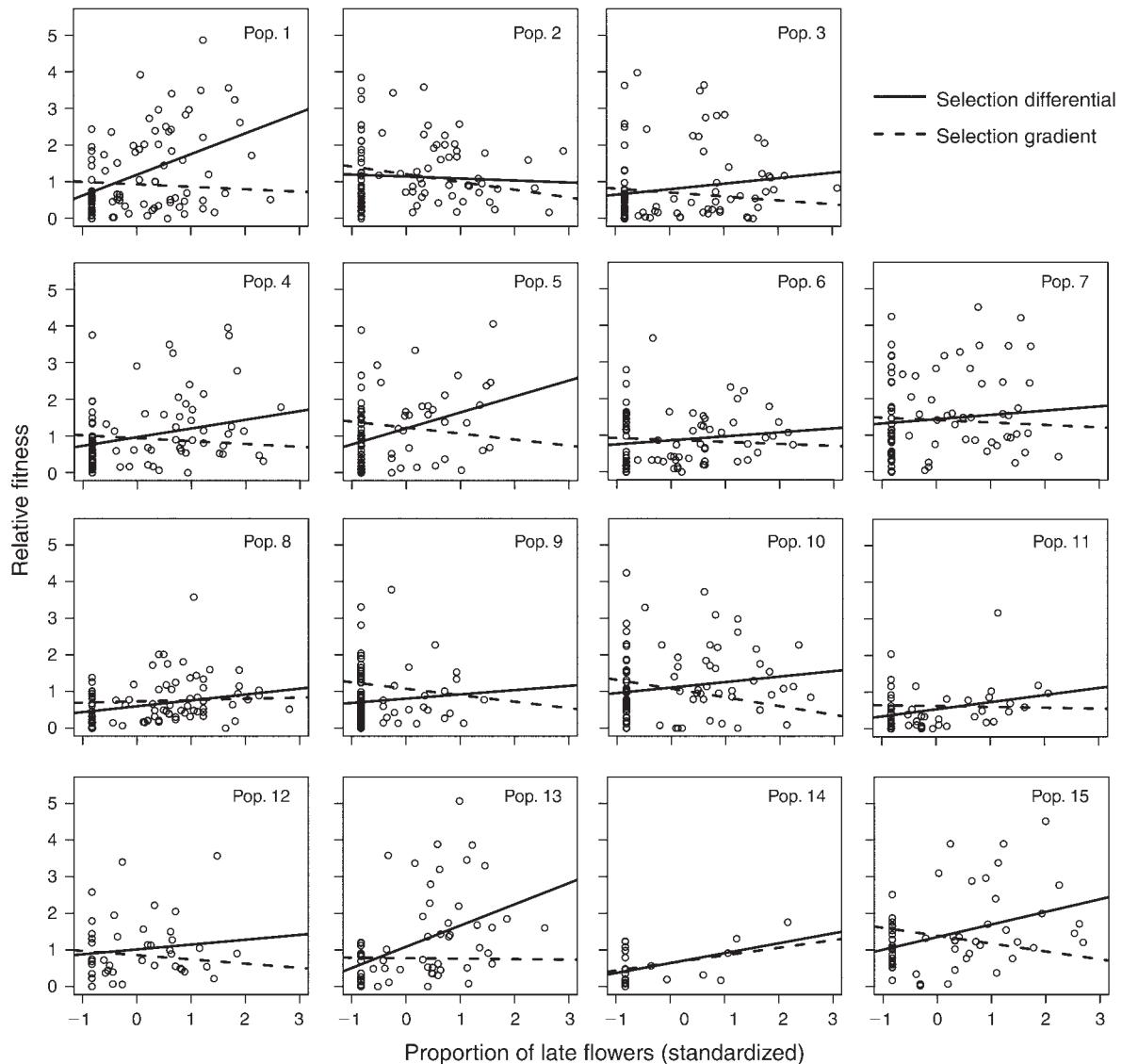
Fig. 2. Selection differentials and selection gradients for relative allocation to late flowers in 15 populations of *Actaea spicata*. Analyses were based on RV-weighted seed number. Selection gradient analyses included also total flower number. Points represent the relationship between the observed values of fitness and the proportion of flowers in late inflorescences for all individuals in the population. Points thus correspond to the selection differential fits only, while lines represent differentials and gradients.

driven by a positive correlation between the total number of flowers and allocation to late flowers (Pearson's product-moment correlation coefficient = 0.54, $P < 0.001$). Selection gradients, estimating direct selection on relative allocation to late flowers, were on average negative (-0.086 ± 0.114 [mean $\pm$ SD], $N = 15$; Fig. 2; Appendix B). Nonlinear effects of allocation to late flowers on fitness were generally weak and not significant (pooled data, second-degree polynomial term = 0.014 ± 0.016 [mean $\pm$ SE], $t = 0.86$, $P = 0.39$).

As hypothesized, among-population variation in selection gradients for relative allocation to late flowers was significantly positively related to the mean intensity of seed predation (Fig. 3). The fitted relationship

indicated that in populations with predation intensities corresponding to >61% of early fruits attacked, selection will favor increased allocation to late flowers.

In contrast to estimates based on total fitness of offspring, selection estimates not accounting for differences in reproductive value between seeds from the two flower types suggested that direct selection should favor increased allocation to late rather than early flowers, selection gradients for allocation to late flowers being on average positive (0.032). These estimates were in opposite direction and differed significantly from the estimates derived from models accounting for differences in reproductive value (paired t test: $t = 9.65$, df = 14, $P < 0.001$).
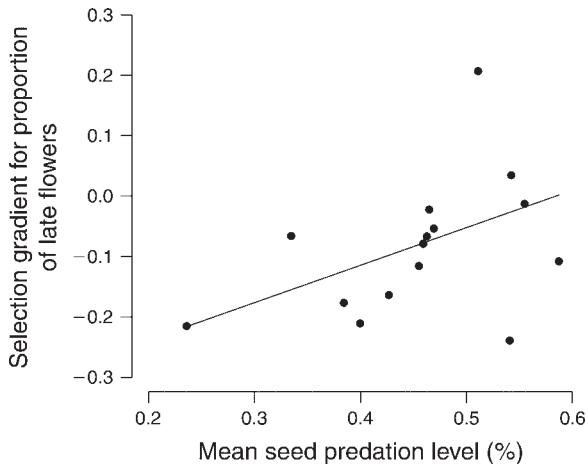
Fig. 3. The relationship between mean seed predation and selection for relative allocation to late flowers in 15 populations of *Actaea spicata* (generalized least squares (GLS): $b = 0.56$; $t = 2.74$; $P = 0.017$).

DISCUSSION

Our results demonstrate that selection on the shape of the flowering schedule in *A. spicata*, in terms of the relative allocation to late basal flowers, involves a trade-off between offspring number and quality, and that the intensity of antagonistic interaction influences the balancing of this trade-off. While intact seeds from early flowers had twice as high reproductive value as seeds from late flowers, fruits from late flowers much more often escaped attacks from seed predators. In agreement, selection gradients for allocation to late flowers were significantly positively related to the average intensity of seed predation across natural populations. Importantly, this pattern was only evident when selection gradients were based on estimated offspring reproductive values. Gradients based on the number of intact seeds, and not accounting for differences in reproductive values, suggested selection for increased investments in late flowers at all intensities of predation. This illustrates that phenotypic selection analyses that fail to account for differences in offspring fitness can be misleading.

This study showed that intact seeds from early flowers produced offspring that had twice as high reproductive values as seeds from late flowers, as a result of higher germination rates and larger seedlings. Several other studies that have examined the effects of within-plant variation in the phenology of flowers have also found that seed set is higher in early flowers, and have attributed this to limited resource availability for seeds from later flowers (e.g., Stephenson 1981, Ehrlén 1992, Diggle 1999, Ashman et al. 2001, Kliber and Eckert 2004). However, differences in seed set between early and late flowers might also be the result of temporal variability in pollinator availability. Differences in germination rates among seeds from flowers with different dates of anthesis have previously been shown also for *Campanula americana* (Galloway 2002). In this study, we did not experimentally examine the causes of differences between seeds from early vs. late flowers, but earlier studies with this species suggest that differences in seed size are associated with differences in out-crossing rates rather than with differences in resource availability (Eriksson 1995). Taken together, the results of this and previous studies clearly show that early and late flowers within an individual can differ in the quality of offspring they produce. This implies that selection analyses should be based on the summed reproductive values of the offspring rather than on the number of offspring only.

Within individuals, fruits from early flowers were more often attacked by seed predators than fruits from late flowers. There are at least two possible explanations for this pattern. First, the activity of seed predators might be better synchronized with the development of early than late flowers. Second, the larger size of fruits and seeds from early flowers might constitute a more attractive food source than the smaller fruits and seeds from late flowers. Relatively few other studies have investigated differences in predation rates among flowers with different opening dates within individuals (but see Albrectsen 2000, Kliber and Eckert 2004, Östergård et al. 2007). Contrary to the results in our study, early flowers were less prone to herbivore attack than late flowers in the monocarpic plant *Tripolium vulgare* (Albrectsen 2000) and in the perennial herb *Aquilegia canadensis* (Kliber and Eckert 2004). Kliber and Eckert (2004) suggested that temporal variation in herbivory had selected for disproportionate investments in early flowers. If differences in pollinator availability over the season contributed to the observed differences in fitness between early and late intact seeds in *A. spicata*, then the results of this study suggest that pollinators and seed predators constitute opposing selective pressures (sensu Brody 1997) on allocation to late flowers. As a corollary, the balancing of trade-offs and the direction of selection acting on the proportion of resources allocated to production of the respective type of offspring should depend on the relative intensity of interactions with pollinators and seed predators.

In our study, reproductive values of early and late flowers accounting for differences in predation rates balanced at a predation intensity corresponding to 63% of fruits from early flowers attacked. This implies that at higher predation intensities we should expect selection for increased allocation to late flowers while at lower intensities we should expect selection for decreased allocation to late flowers. We indeed found that selection gradients for relative allocation to late flowers across 15 investigated natural populations were positively related to intensity of predation. The fitted relationship in Fig. 3 suggests that in populations with predation intensities corresponding to >61% of fruits from early flowers attacked, selection will favor increased allocation to late flowers. This agrees well with the finding that reproduc-

tive values of early and late flowers balanced at a predation intensity corresponding to 63% of fruits lost in early inflorescences.

In the 15 natural populations included in this study, we observed large differences in intensity of seed predation among populations. This is in agreement with a previous study with the same system examining a larger set of populations (von Zeipel et al. 2006). This pattern suggests that spatial variation in intensity of pre-dispersal seed predation constitutes an important source of variation in selection on the shape of the flowering schedule and resource allocation to early vs. late flowers in this system. Moreover, a relationship between intensity of predation and selection also implies that the factors that influence intensity of seed predation will influence selection on the shape of the flowering schedule in this system. We do not yet have information about the heritability of flowering schedule and are thus not able to predict the likelihood of a response to selection. However, the fact that the seed predator is patchily distributed also at larger spatial scales (J. Ehrlén and J. P. Dahlgren, *personal observation*), and that populations often are spatially isolated suggest that divergent responses to selection should be possible if there is some genetically based variation in flowering schedule.

The mean level of predation across populations in this study (46% of fruits from early flowers) was lower than the level (63%) where early and late flowers contributed similarly to fitness, and only in one population was predation intensity high enough to expect selection for allocation to late flowers (population 8, Table 1). There are several possible reasons why a mixed phenological strategy with two flower types is still present in all investigated populations. First, averaged over a longer time period, predation, and thus the relative fitness of late flowers, might be higher than during the study year. In fact, the observed mean level of predation across populations in the same study system between 2001 and 2004 was markedly higher than during the current study (67, 63, 67 and 50% of fruits in primary inflorescences attacked, respectively; H. von Zeipel and J. Ehrlén, *unpublished data*). Second, our selection estimates are based on the assumption that the distribution of flowers among inflorescences within individuals does not affect predation rates, and that conditions are static. A mixed strategy, producing both early and late flowers, should be more advantageous if allocating resources to late flowers also reduces predation rates in early flowers, e.g., by providing a smaller resource or a less recognizable cue. However, in this study we found no evidence that the relative allocation to late flowers was related to seed predation in early flowers. Lastly, a mixed strategy might be more beneficial than suggested by our analyses if it reduces differences in seed production between low and high seed predation years, and thus increases geometric mean fitness.

This study illustrates that fitness components, such as the number of seeds, are not always useful surrogates for offspring total fitness. Selection gradients not accounting for differences in reproductive values among offspring suggested that selection mostly favored increased allocation to late flowers, while analyses accounting for differences in offspring reproductive values instead suggested overall selection for decreased allocation. This underscores the importance of basing estimates of selection on integrated estimates of fitness rather than solely on single fitness components whenever multiple fitness components are involved. To appropriately examine selection on reproductive schedules in iteroparous organisms, we therefore need to combine selection analyses examining relationships between traits and offspring number, with demographic analyses examining differences in reproductive values of offspring (e.g., van Tienderen 2000, Caswell 2001, Coulson et al. 2010, Childs et al. 2011, Ehrlén 2015). This type of analysis has so far been carried out only rarely, but should be instrumental to achieve a better knowledge about the direction and strength of selection in natural systems. This is true for selection on reproductive schedules as well as for selection on any other trait whenever multiple fitness components are involved.

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Supplemental Material

Ecological Archives

Appendices A and B are available online: http://dx.doi.org/10.1890/14-1860.1.sm