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Within-plant variation in reproductive investment: consequences for selection on flowering time

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Abstract

Variation among the leaves, flowers or fruit produced by a plant is often regarded as a nuisance to the experimenter and an impediment to selection. Here, we suggest that within-plant variation can drive selection on other plant-level traits. We examine within-plant variation in floral sex allocation and in fruit set and predict that such variation generates variation in male success among plants, thereby driving selection on flowering time. We tested this prediction in a simulation model estimating selection on flowering time through male fitness when floral sex allocation and/or fruit set vary directionally among flowers on plants. We parameterized the model through a quantitative literature survey of within-plant change in sex allocation. As predicted, within-plant variation in floral sex allocation and in fruit set probability can generate selection on flowering time through male fitness. Declining fruit set from first to last flowers on plants, as occurs in many species, selected for early flowering onset through male fitness. This result was robust to self-incompatibility and to varying returns on male versus female investment. Selection caused by declining fruit set was strong enough to reverse the selection for late flowering that can be caused by intrafloral protandry. Our model provides testable predictions regarding selection on flowering time through male fitness. The model also establishes the intriguing possibility that within-plant variation may influence selection on other traits, regardless of whether that variation is under selection itself.

Introduction

As modular organisms, plants typically produce multiple leaves, flowers and other organs during the growing season and/or along their branches. These repeated structures are rarely perfect copies of one another (Herrera, 2009). Traits such as flower size, pollen production per flower and fruit set frequently vary within individuals (Diggle, 2003), and within-plant variation in such traits can be comparable to or greater than among-plant variation (Campbell, 1992; Obeso & Herrera, 1994; Williams & Conner, 2001; Herrera, 2009; Zhao *et al.*, 2010). Variation within plants can be random, following no apparent pattern; patchy, as when structures produced on one branch differ from those produced on another; or can follow a regular, predictable trend. Where the latter occurs, spatial and temporal patterns are often linked, as when the size of sequentially produced leaves varies along the length of a branch.

Within-plant variation is of evolutionary importance for at least two reasons. First, if most variation in a modular trait, such as fruit size (Obeso & Herrera, 1994) or flower shape (Williams & Conner, 2001), occurs within rather than among plants, estimates of selection on that trait that do not account for within-plant variation may predict a response where none is possible (Herrera, 2009). Second, within-plant variation can affect a plant's interactions with mutualists and/or antagonists and may therefore itself be a target of selection. For example, within-plant variation in the quantity or quality of floral rewards may discourage variance-averse pollinators from visiting several flowers on the same individual (Biernaskie *et al.*, 2002), or

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variation in the production of chemical defences among the leaves of a plant may help hosts constrain pest outbreaks (Whitham, 1983; Anderson & Agrell, 2005). In cases like these, the particular pattern of within-plant variation could affect plant survival or reproduction. If so, selection may act on ‘function-valued traits’, such as variance and skew (Herrera, 2009), or intercept and slope (Stinchcombe & Kirkpatrick, 2012), that describe the pattern of within-plant variation expressed. Genetic (reviewed in Herrera, 2009 Chapter 7) or epigenetic (e.g. Herrera *et al.*, 2014) control of within-plant variation enables response to such selection.

A considerable amount of within-plant variation may be environmental in origin. Micro-environmental differences in temperature, light availability, or wind velocity can cause small deviations in leaf or floral development among the different parts of a plant (Herrera, 2009). Environmental variation internal to the plant, such as a resource gradient created by a proximal-to-distal decline in vasculature size (Byrne & Mazer, 1990; Wolfe, 1992), can similarly generate within-plant variation. An internal resource gradient can also result if the first fruit to develop on a plant pre-empt resources and thereby alter the internal environment experienced by later-developing flowers and fruit (Diggle, 1997). The direction of a gradient caused by resource pre-emption may depend on whether flowering is acropetal (i.e. flowers are deployed sequentially from the bottom to the top of inflorescences) or basipetal (i.e. flowers are deployed sequentially from the top down). Given the prevalence of decreasing diameter along shoots, and of inflorescences that deploy flowers sequentially over a number of days, it is reasonable to expect some form of within-plant variation in most species, even if this variation is of no adaptive significance.

Adaptive or not, a third evolutionary outcome of within-plant variation may be the generation of selection on other traits, particularly when variation among repeated structures influences interactions between individuals. For example, temporal trends in floral sex allocation (i.e. relative investment in female vs. male function) may induce selection on flowering time. If individuals invest relatively more in female function in the first flowers they produce, then these first flowers present a more valuable siring opportunity to neighbours than the last flowers produced (Brunet, 1996). Individuals that start flowering relatively early should have a greater probability of siring seed on the valuable first flowers of others, simply because they are more likely to temporally coincide with those flowers. The population may therefore experience selection for early flowering owing to temporal variance in male reproductive success (Weis & Kossler, 2004; Austen & Weis, 2014). This selection may partially explain the tendency of males of dioecious species to flower earlier than females (Forrest, 2014). Implications have not been examined for hermaphroditic species, but we hypothesize that within-plant variation in floral sex allocation may lead to stronger selection for early flowering onset than would be predicted if selection were estimated through female fitness alone.

This hypothesis is, in a sense, the inverse of a prediction put forth by Brunet & Charlesworth (1995). These authors demonstrated that variation in pollen transfer probability among the flowers on a plant will drive selection for variation in sex allocation among flowers. Dichogamy (i.e. temporal offset of male and female function within flowers) can cause variation in pollen transfer probability, leading Brunet & Charlesworth (1995) to predict that protogyny (‘first-female’, flowers whose stigmas are receptive before dispersing pollen) selects for increasingly female sex allocation from first to last flowers on plants, and protandry (‘first-male’, flowers that disperse pollen before their stigmas are receptive) selects for the reverse. This prediction has motivated several studies of within-plant variation in allocation (e.g. Brunet, 1996; Huang *et al.*, 2004; Zhao *et al.*, 2008; Brookes & Jesson, 2010). Our hypothesis suggests that in addition to being a *response* to variation in siring opportunity, within-plant variation in sex allocation might be a *cause* of variation in realized siring at the among-plant level.

Within-plant variation in sex allocation may be apparent at flowering (prefertilization) or at fruiting (post-fertilization) or both. In perfect-flowered hermaphrodites, subtle prefertilization variation occurs when flowers differ in their relative investment in male structures (stamens) and female structures (pistils). Post-fertilization variation occurs when flower positions differ in resources invested in fruit and seed maturation (female investment). All else being equal, flowers that set fruit are necessarily more female in their post-fertilization allocation than those that do not. Several studies have quantified within-plant variation in male and female investment, and a few reviews have catalogued the direction of variation in these traits (Lee, 1988; Thomson, 1989; Diggle, 1995, 1997, 2003; Delesalle *et al.*, 2008), but findings have not been compiled in a quantitative comparative analysis.

Our goal was to determine whether within-plant variation in floral sex allocation and/or in fruit set can drive selection on flowering time through male fitness in the manner proposed. We start by asking, (1) By how much do floral sex allocation and fruit set vary from flower to flower within plants? In addressing this question, we also test a key prediction of Brunet & Charlesworth (1995), asking (2) Is floral dichogamy associated with the direction of within-plant variation in floral sex allocation? We next parameterize a simulation model using findings from question (1) to ask, (3) Does within-plant variation in floral sex allocation and/or fruit set lead to male fitness variation among plants initiating flowering at different times? Finally, we consider interactions between prefertilization sex allocation, fruit set and dichogamy, asking (4) Does dichogamy alter the male fitness effects of within-plant variation in sex allocation and fruit set? This work is a first demonstration of the principle that within-plant variation can drive selection on other traits. The model generates testable predictions regarding the nature of selection on flowering time through male fitness.

Materials and methods

Comparative analysis of prefertilization male and female investment

To survey documented within-plant variation in prefertilization male and female investment, we searched the ISI Web of Science database in March 2013 using combinations of the keywords hermaphrodite, sex allocation, flower-, flower position, ontogeny, pollen and ovule to identify papers reporting the necessary data. We added relevant studies citing/cited by papers identified in our query and papers cited in previous descriptive reviews of within-plant variation. We limited our search to studies of perfect-flowered species providing data on prefertilization male and female investment in early and late flowers within individuals. Because investment in corolla, calyx and other structures is not always reported, and because partitioning of these structures to male and female function varies among studies, we restricted male and female investment to floral structures directly involved in gametogenesis (i.e. stamens and carpels) or to production of male gametes (pollen) and female gametes (ovules) themselves. Pollen and ovules are not gametes, but the production of egg and sperm is directly tied to the production of these gametophytes (pollen) or gametophyte-bearing structures (ovules).

For each species, we extracted the mean and standard error of male and female investment in first and last flowers, using GraphClick v. 3.0 (Arizona Software, 2008) where data were presented graphically. Investment can be measured by the biomass of male and female structures or by their nitrogen, carbon or phosphorous content or can be approximated by counting. Where multiple types of data were available within a single study, we preferred pollen and ovule counts to stamen and pistil counts, and either of these measures to androecium and gynoecium biomass. We did not include chemical composition data in our analysis, as these were infrequent. Pollen and ovule counts do not directly measure investment, but they likely correlate with actual investment because within species, reproductive units are expected to vary more in number than in size (Lloyd, 1987). Where available, we collected data on mean flowering duration, dichogamy and the sequence of flower deployment (acropetal or basipetal) as reported in the studies in our data set, or elsewhere in the literature, or through personal communication with study authors.

Several studies reported data for *Clarkia* taxa. From these, we chose to include in our analysis a paper in which sex allocation was examined in natural field populations (Delesalle *et al.*, 2008). We note, however, that for some of these *Clarkia* taxa, the direction of within-plant variation reported in this paper differed from the direction of variation reported in a paper describing a glasshouse experiment (Delesalle *et al.*, 2008; Mazer *et al.*, 2009). In two instances (Guitián *et al.*, 2004; Delesalle *et al.*, 2008), data were available for multiple populations of a species within a single study, but populations did not differ in direction or relative magnitude of within-plant trends in male and female investment. In these cases, we included in our analysis the population with the larger sample size, or randomly selected one population for inclusion where samples were equal.

To facilitate comparisons of pollen and ovule production within species, and comparisons across species, we converted male investment to units of female equivalents using a formula based on the equivalency factor (E) developed by Lloyd (1980). For each species,

$$E = \frac{v_f + v_l}{p_f + p_l},$$

where v_f and v_l are the mean female investment (e.g. number of ovules) of first and last flowers, respectively, and p_f and p_l are the mean male investment (e.g. pollen content) of first and last flowers. Multiplying male investment by E converts male effort to units of female equivalents (‘adjusted male investment’). We implicitly assume that male and female investment both vary linearly in the unmeasured flowers between the first and the last. Because many studies examine just two to four flower positions per inflorescence, it is difficult to test this assumption directly. It is supported, however, by the findings of one study examining several flowers per plant (Austen & Weis, 2014), and data from several other studies also suggest linearity (e.g. Vogler *et al.*, 1999; Kudo *et al.*, 2001; Guitián, 2006).

We calculated the total reproductive investment of first and of last flowers within each species by summing female and adjusted male investment and calculated flower femaleness as the ratio of female investment to total reproductive investment (Lloyd, 1980; Lloyd & Bawa, 1984). Flower femaleness is not a direct measure of sex allocation because it is not based on a direct measure of resources invested. It does, however, convey the emphasis that first flowers place on male versus female function as compared to last flowers. We calculated the change in total investment from first to last flowers as a percentage of first flower investment, and the magnitude and direction of change in flower femaleness. Where flowering-duration data were available, we also calculated per-day rates of change in investment and femaleness, again assuming linearity.

The compiled prefertilization sex allocation data set provided us an opportunity to test a key prediction of Brunet & Charlesworth (1995): that the direction of within-plant variation in allocation varies according to floral dichogamy. To this end, we examined associations between dichogamy (protandry, protogyny, adichogamy [i.e. intrafloral male–female synchrony] or unknown) and the direction of within-plant trends. Very uneven sample sizes across these categories precluded a statistical test across dichogamy categories. Instead, we used single sample one-sided t-tests to determine whether the within-plant change in flower femaleness was significantly less than zero in protandrous species and significantly greater than zero in protogynous species.

Comparative analysis of within-plant variation in fruit set probability

Several components of post-fertilization female investment commonly decrease from first to last flowers, including seed set (e.g. Forrest & Thomson, 2010), seed number (e.g. Itagaki & Sakai, 2006), fruit mass (e.g. Brookes *et al.*, 2010) and seed mass (e.g. Winn, 1991). We focus on fruit set because this is the first order of post-fertilization female investment: seed set, seed number, fruit mass and seed mass cannot be measured in fruits that do not mature.

The tendency towards declining fruit set among the flowers on a plant is well documented (e.g. Lee, 1988; Thomson, 1989; Diggle, 1995, 2003), and we do not attempt an exhaustive review. Our goal was to quantify biologically reasonable magnitudes of variation in fruit set probability with which to parameterize a simulation model. We identified studies reporting fruit set probability of first and last flowers from our own records and by searching the ISI Web of Science database in May 2013. From these studies, we extracted the mean fruit set probability of first flowers and of last flowers, or, if these data were unavailable, the predicted fruit set of first and last flowers based on the author's analysis. If authors had examined enough flower positions to allow characterization of the nature of fruit set variation, we also recorded the overall function form (e.g. S-shaped, linear). Where experimental treatments were imposed on plants, we used only results from the control group (i.e. open-pollinated, with no defoliation or other resource manipulation).

Model of fitness effects of within-plant variation

We modelled the intensity of natural selection on flowering time through male fitness as caused by within-plant change in flower femaleness and fruit set probability. All code was written in R (R Development Core Team, 2012) and is presented in Appendix S1. The model estimated selection within a single generation; it did not seek to find an evolutionary optimal flowering time. Within the model realizations presented here, we assumed that plants varied in date of flowering onset, but were otherwise identical. We developed additional model parameterizations that allowed flower deployment schedules, total investment per flower (i_{j0}, see below), and/or the strength of within-plant decline in fruit set to vary among plants, but these modifications did not qualitatively alter results (Appendix S2). We therefore present only the simple model here. All parameterizations of the model are summarized in the Table S2.1 (Appendix S2).

The model examined a population of 250 plants, in which day of flowering onset, o_j, followed a unimodal symmetric distribution over 30 days, which is a reasonable flowering onset range for temperate herbaceous plants (Weis *et al.*, 2014). We denote a plant j's flower production on day d by f_{jd}. Plants produced four flowers on each of 12 days beginning on their day of flowering onset (48 flowers total). Symmetric unimodal, left-skewed or right-skewed flower deployment distributions did not qualitatively alter results (Fig. S2.1).

For each day of its flowering, a plant's per-flower prefertilization total reproductive investment, i_{jd}, is a linear function of the per-flower investment of its first-day flowers, i_{j0}, and a rate of change in investment, β_i:

$$i_{jd} = i_{j0} + \beta_i \times (d - o_j)$$

The linear function form for investment is a simplification: if male and female investments are made from a finite resource pool, one would expect that investment per day follows a saturating (e.g. logistic) function form. However, as noted above, available data are insufficient to parameterize a more complex function form. We set $i_{j0} = 100$ for all plants (see Fig. S2.3 for cases where i_{j0} varies). Based on results of our review, we set $\beta_i = -1$, that is total investment per flower declines 1% per day. The term $(d - o_j)$ is the number of days that have passed since flowering onset of plant j.

The partitioning of investment into female (a_{jd}, i.e. flower femaleness) and male ($1 - a_{jd}$) effort is a linear function with an intercept equal to the plant's allocation on its day of flowering onset and a rate of change in allocation β_a:

$$a_{jd} = a_{j0} + \beta_a \times (d - o_j)$$

Based on the results of our review, we examined β_a ranging from −0.01 (increasingly male) to + 0.01 (increasingly female). With a 12-day flowering duration, the maximum rates of change amounted to a 0.12 magnitude of difference in flower femaleness from first to last flowers. Intercept a_{j0} was constant among plants, but necessarily varied with β_a across model cases because population mean flower femaleness must equal

0.5. This necessity arises because our model was parameterized by findings of our comparative analysis, and in this analysis, male investment was converted to female equivalents using the conversion factor *E*, which enforces equal male and female investment across the population.

From these data, we calculated each plant's daily per-flower ovule production (v_{jd}) and pollen production in units of ovule equivalents (p_{jd}) as the product of the per-flower investment and flower femaleness and maleness, respectively:

$$v_{jd} = i_{jd} \times a_{jd}$$

$$p_{jd} = i_{jd} \times (1 - a_{jd})$$

To determine the number of seeds available for siring on a given day, we first determined the number of flowers per plant maturing into fruit per day, which in turn was a function of the probability of fruit set for plant *j* on day *d* (r_{jd}). Because fruit set probability is bounded by (0, 1), we assumed a reverse S-shape function form:

$$r_{jd} = \frac{ce^{g(d-o_j-h)}}{ce^{g(d-o_j-h)} + (1-c)}$$

Parameter *c* is the value of r_{jd} when $(d{-}o_j{-}h) = 0$, *g* sets the steepness of the S-shape, and *h* shifts the function horizontally allowing us to centre the desired portion of the S-shape over the 12-day flowering duration (Otto & Day, 2007). With small *g*, the function accommodates a linear relationship. We tested moderate and strong declines in fruit set probability (Fig. 1), with parameters of the S-shape chosen to achieve fruit set probabilities of first and last flowers consistent with the findings of our comparative analysis. The number of fruit maturing from flowers produced by individual *j* on day *d* (u_{jd}) was determined by f_{jd} draws from a binomial distribution with probability of success equal to r_{jd}. The number of seeds available for siring on individual *j* on day *d* was the product of fruit number and ovules per flower:

$$s_{jd} = u_{jd} \times v_{jd}.$$

We assumed all ovules mature within successful fruits. The sum of s_{jd} across days is the female fitness of plant *j*.

To calculate male fitness, we first calculated each individual's daily proportional contribution to the pollen pool, c_{jd}:

$$c_{jd} = \frac{p_{jd}f_{jd}}{\sum_{J=1}^{N} p_{Jd}f_{Jd}}$$

Here, plant *j*'s pollen production on day *d* is divided by the total pollen production of all *N* plants. We then sampled a father for each seed produced on day *d*, with the probability of selecting each father *j* equal to c_{jd}. The total male fitness of plant *j* is equal to the sum of its seeds sired.

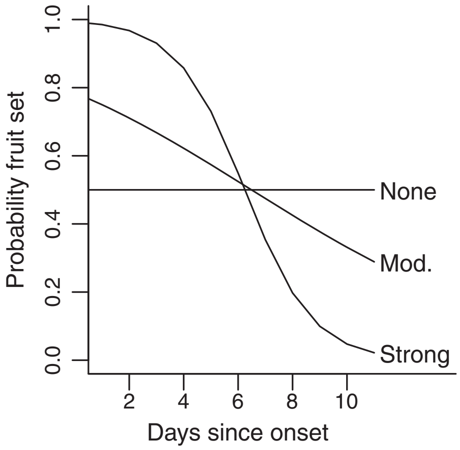

Fig. 1 Three within-plant trends in fruit set probability adopted in numerical model. Fruit set probability ranges from 0.99 to 0.02 from first to last flowers under a strong decline (parameters of reverse S-shape function: $c = 0.9$, $g = -0.8$, $h = 3.5$) and from 0.78 to 0.28 under a moderate decline ($c = 0.75$, $g = -0.2$, $h = 1$).

We investigated the effects of self-incompatibility (Fig. S2.4) and of diminishing returns on pollen and seed production (Fig. S2.5). These modifications did not qualitatively alter results.

Because Brunet & Charlesworth (1995) predicted an association between flower dichogamy and the direction of within-plant change in flower femaleness, we investigated effects of a 1-day offset in male and female function of flowers. When testing effects of protogyny, a flower's fruits were sired on day *d*, the first day of that flower's opening, and its pollen was dispersed on day *d* + 1. When testing effects of protandry, a flower's pollen was dispersed on day *d* and its fruit sired on day *d* + 1. Calculations were otherwise unaltered.

We repeated the model using all combinations of five rates of within-plant change in sex allocation chosen based on values obtained from our literature survey (−0.010, −0.005, 0, +0.005, +0.010), three levels of decline in fruit set probability (none, moderate and strong, Fig. 1) and three levels of dichogamy (adichogamy, protandry and protogyny). The processes of determining fruit production and seed siring are stochastic. We therefore ran each model parameterization 30 times to estimate average outcomes. Following each run, we standardized flowering onset to a mean of zero and standard deviation of one, mean relativized male fitness, and calculated linear (i.e. directional) and quadratic (i.e. stabilizing or disruptive) selection differentials through multiple regression of relativized male fitness on standardized flowering onset and its square

(Lande & Arnold, 1983). We calculated the mean coefficients of this regression across the 30 repeated runs to characterize the average relationship between relative male fitness and flowering onset under a given parameterization. The mean coefficient for the linear term provides an estimate of the strength of directional selection for a parameterization, and the doubled quadratic coefficient estimates the strength of quadratic selection (Stinchcombe *et al.*, 2008).

Results

Position-dependent prefertilization male and female investment

We found 18 studies reporting male and female investment by first and last flowers in 21 species from 8 families (Table 1). With one exception (*Helleborus foetidus*), total prefertilization reproductive investment (i.e. female investment + adjusted male investment) declined from first to last flowers within plants (Fig. 2). The rate of this decline was remarkably constant among species, with most exhibiting a decline of approximately 1% of the investment in first flowers per day (Table 1). Patterns of within-plant variation in sex allocation were more variable (Fig. 2), with some species tending towards increasingly female allocation, others towards increasingly male allocation and still others showing no change. The magnitude of change in flower femaleness from first to last flowers ranged from −0.135 (decreasingly female allocation) to +0.172 (increasingly female), with rates of change ranging from −0.030 to +0.009 per day.

As predicted by Brunet & Charlesworth (1995), the direction of change in flower femaleness seems to be associated with dichogamy (Fig. 3). Six of seven species reported to be protandrous exhibited a decrease in femaleness from first to last flowers (one-sided *t*-test, $t_6 = -4.40$, $P = 0.003$). Two of three species reported to be protogynous exhibited increasing floral femaleness from first to last flowers. The mean change in flower femaleness across these protogynous species was not, however, statistically greater than zero, likely due to low sample size (one-sided *t*-test, $t_2 = 1.23$, $P = 0.172$).

Position-dependent variation in fruit set

Fruit set probability declined from first to last flowers on plants in nearly all studies reviewed (Table 2). In the one exception, Pei *et al.* (2011) examined fruit set probability in compound umbels and found the highest probability of fruit set in second-order umbels, generating an inverted-V pattern of fruit set probability; fruit set declined strongly from second- to third-order umbels. In some species (e.g. *Brassica rapa*, *Lobelia sessiflora*), fruit set declined from nearly 1 in first flowers to nearly 0 in last flowers, whereas the decline was more moderate in others [e.g. *Hosta rectifolia* (0.71–0.40), *Polygonatum odoratum* (0.89–0.39)]. Fruit set probability most often followed an S-shaped decline (Table 2). Eight of the 13 studies reviewed reported resource pre-emption by first flowers as the likely cause of lower fruit set probability of later flowers. Of the remaining five, one proposed that later flowers suffer pollen limitation as pollinators learn to avoid nonrewarding flowers in a deceit-pollinated orchid (Jersáková & Kindlmann, 1998); one interpreted declining fruit set as an outcome of selection for increasingly male allocation between first and last flowers (Brunet, 1996; also proposed as an ultimate cause by Guitián *et al.*, 2001); and one excluded pollen limitation as a causal mechanism but did not propose an alternative (Hiraga & Sakai, 2007). The final two (Weis & Kossler, 2004; Cao *et al.*, 2007) did not experimentally examine or discuss the cause of declining fruit set.

Model of fitness effects of within-plant variation

As predicted, the model revealed that both within-plant variation in flower femaleness (Fig. 4a) and a within-plant decline in the probability of fruit set (Fig. 4b) influence selection on flowering time through male fitness. The effect of prefertilization femaleness was, however, minor compared to that of declining fruit set probability. When flower femaleness changed by 0.12 from first to last flowers ($\beta_a = +0.010$ or -0.010), the absolute value of the directional selection differential on flowering time through male fitness was just 0.06 ± 0.01 (mean ± SD, $n = 30$ runs with $\beta_a = +0.010$ and 30 runs with $\beta_a = -0.010$) (Fig. 4a). By comparison, a moderate decline in fruit set probability induced a directional selection differential of −0.13 ± 0.01, and a strong decline in fruit set probability yielded a directional selection differential of −0.29 ± 0.004 (Fig. 4b). Within-plant variation in sex allocation can reinforce ($\beta_a = -0.010$, Fig. 4c) or alleviate ($\beta_a = +0.010$, Fig. 4c) the effects of declining fruit set probability. Quadratic selection differentials were weak in all cases presented in Fig. 4, the strongest being −0.02 ± 0.01 (mean ± SD, $n = 30$ runs with strong fruit set decline, Fig. 4b).

Within-plant temporal variation in fruit set probability interacted with floral dichogamy. Selection does not act on flowering time in adichogamous plants when flower femaleness ($\beta_a = 0$, Fig. 4a) and fruit set probability (Fig. 4c, fruit set decline = 'none') do not vary among flowers. In contrast, protandry concentrates siring opportunities in the late part of the season leading to selection for later flowering onset through male fitness in the absence of within-plant variation in sex allocation (Fig. 5a, fruit set decline = 'none'). Depending on the schedule of flower deployment within plants, a strong or moderate decline in fruit set probability can lessen, neutralize or reverse the selection induced by protandry (Fig. 5a). Protogyny had the opposite effect (Fig. 5b, fruit set decline = 'none'). Moderate and strong within-plant

Table 1 Magnitude and direction of change in total reproductive investment and floral femaleness from first-measured flowers to last-measured flowers in 21 species.

Species	Seq	Dich	*N*	Curr	Dur	ΔInv (%)	ΔInv/day	ΔGen	ΔGen/day	References‡
Brassicaceae										
Brassica rapa	A	AD	70	PO number	34	−0.372 ± 0.055	−0.011 ± 0.002	0.113 ± 0.043	0.003 ± 0.001	1
Raphanus sativus	A	NA	4	PO number	36	−0.214 ± 0.503	−0.006 ± 0.014	0.066 ± 0.148	0.002 ± 0.004	2
Campanulaceae										
Campanula rapunculoides	A	PA	96	PO number	NA	−0.03 ± NA	NA	−0.116 ± NA	NA	3
Lobelia sessiliflora	A	PA	73	PO number	NA	−0.202 ± 0.164	NA	−0.046 ± 0.066	NA	4
Cistaceae										
Tuberaria guttata	NA	NA	20	SO number	25	−0.19 ± 0.264	−0.008 ± 0.011	−0.08 ± 0.098	−0.003 ± 0.004	5
Fumariaceae										
Corydalis ambigua	A	NA	49	SP biomass	2	−0.131 ± 0.32	−0.066 ± 0.16	−0.059 ± 0.064	−0.030 ± 0.032	6
Liliaceae										
Hosta ventricosa	A	AD	40	PO number	11.38	−0.158 ± 0.265	−0.014 ± 0.023	0.044 ± 0.101	0.004 ± 0.009	7
Hosta rectifolia	A	AD	98	PO number	NA	−0.222 ± NA	NA	0.012 ± NA	NA	8
Narthecium asiaticum	A	AD	25	PO number	7	−0.198 ± 0.1	−0.028 ± 0.014	−0.064 ± 0.051	−0.009 ± 0.007	9
Polygonatum odoratum	A	NA	20	PO number	NA	−0.296 ± 0.231	NA	−0.135 ± 0.161	NA	10
Onagraceae										
Clarkia exilis	A	NA	81	PO number	13.21	−0.126 ± 0.285	−0.01 ± 0.022	0.033 ± 0.043	0.002 ± 0.003	11
Clarkia unguiculata	A	PA	87	PO number	7.35	−0.105 ± 0.144	−0.014 ± 0.02	−0.059 ± 0.029	−0.008 ± 0.004	11
Clarkia xantiana ssp. parviflora	A	AD	87	PO number	5.2	−0.122 ± 0.146	−0.024 ± 0.028	−0.039 ± 0.024	−0.007 ± 0.005	11
Clarkia xantiana ssp. xantiana	A	PA	91	PO number	4.7	−0.162 ± 0.113	−0.034 ± 0.024	0.006 ± 0.032	0.001 ± 0.007	11
Poaceae										
Pennisetum typhoides	A	PG	22	PO number*	10	−0.097 ± NA	−0.01 ± NA	−0.04 ± NA	−0.004 ± NA	12
Ranunculaceae										
Aconitum gymnandrum	A	PA	25	SP number	NA	−0.101 ± 0.166	NA	−0.071 ± 0.037	NA	13
Aquilegia caerulea	B	PA	26	PO number	NA	−0.185 ± 0.168	NA	−0.044 ± 0.045	NA	14
Aquilegia canadensis	B	AD	40	PO number	9	−0.137 ± 0.219	−0.015 ± 0.024	0.028 ± 0.046	0.003 ± 0.005	15
Aquilegia yabeana	B	PG	20	PO number	20	−0.36 ± 0.07	−0.018 ± 0.004	0.172 ± 0.051	0.009 ± 0.003	16
Delphinium glaucum	A	PA	34	SO number†	7.6	−0.081 ± 0.303	−0.011 ± 0.04	−0.057 ± 0.052	−0.007 ± 0.007	17
Helleborus foetidus	NA	PG	14	SO number	23	−0.019 ± 1.847	−0.001 ± 0.08	0.098 ± 0.067	0.004 ± 0.003	18

Seq = sequence of flower deployment on inflorescence acropetal (bottom up, A), basipetal (top down, B), or not reported (NA); Dich = flowers protandrous (PA), protogynous (PG), adichogamous (AD) or dichogamy not reported (NA); *N* = number of plants; Curr = currency of investment: Pollen and ovule number (PO number), Stamen and ovule number (SO number), Stamen and pistil number (SP number), Stamen and pistil biomass (SP biomass); Dur = mean flowering duration (days) of individuals in sample; ΔInv (%) = change in mean reproductive investment (ovules + adjusted pollen (ovule equivalents) from first to last flowers, as percentage of reproductive investment of first flowers; ΔInv/day = rate of change in mean reproductive investment (ΔInv (%)/Dur), NA where duration not reported; ΔGen = change in mean flower femaleness (ovules/(ovules + adjusted pollen)) from first to last flowers on plants; ΔGen/day = rate of change in mean flower femaleness (ΔGen/Dur), NA where duration not reported.

*Comparison across early and late spikes on plants (not individual flowers).

†Stamen and ovule counts per bud after correcting for variation attributable to first flowering day.

‡References: 1. Austen & Weis, 2014; 2. Young & Stanton, 1990; 3. Vogler *et al.*, 1999; 4. Hiraga & Sakai, 2007; 5. Herrera, 2004; 6. Kudo *et al.*, 2001; 7. Cao *et al.*, 2011; 8. Cao *et al.*, 2007; 9. Ishii & Sakai, 2002; 10. Guitián *et al.*, 2004 (site Santiago); 11. Delesalle *et al.*, 2008 (one site chosen per species: sites DC, PH, LV01, & CFR01, respectively); 12. Sandmeier & Delph, 1997; 13. Zhao *et al.*, 2008; 14. Brunet, 1996 (site BRM); 15. Kliber & Eckert, 2004; 16. Huang *et al.*, 2004; 17. Ishii & Harder, 2012; 18. Guitián, 2006.

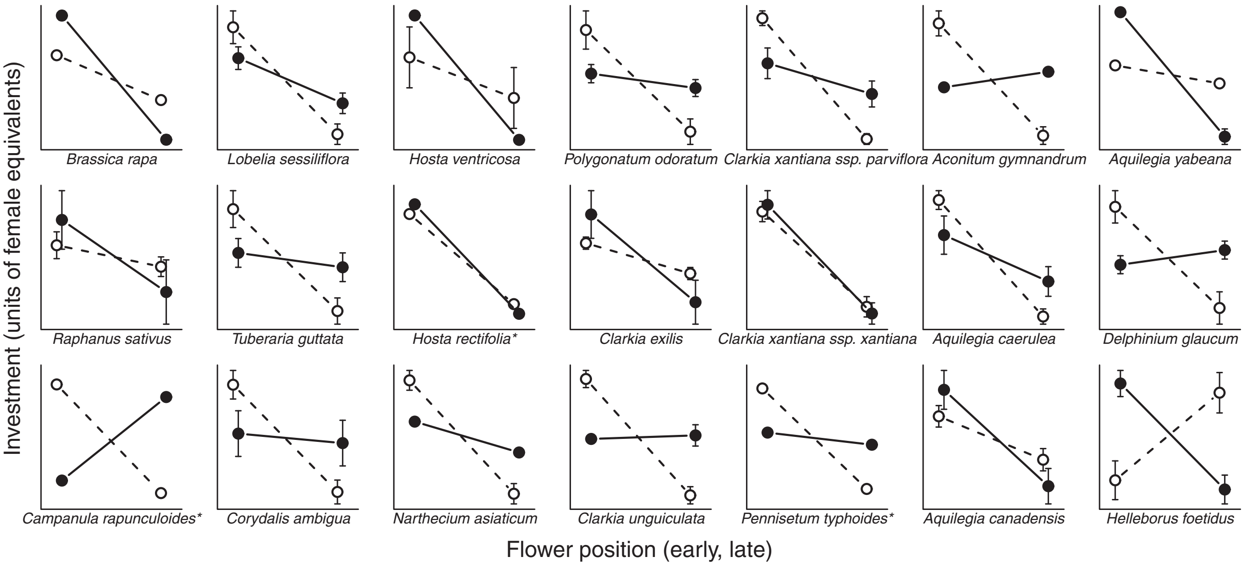

Fig. 2 Female (open symbol) and adjusted male (closed symbol) prefertilization investment in first (leftmost point) and last (rightmost point) flowers of perfect-flowered hermaphroditic taxa (mean ± SE). See Table 1 for *n* within taxa and currency of male and female investment. *indicates SE not reported. Taxa ordered by family, filling columns left to right.

declines in fruit set probability amplified the negative selection on flowering time induced by protogyny, regardless of the schedule of flower deployment within plants (Fig. 5b). Within-plant variation in flower femaleness similarly weakened or strengthened the effects of dichogamy, although it never entirely removed selection induced by dichogamy (Fig. S2.6).

Discussion

The first and last flowers on plants often vary markedly in their total reproductive investment (measured as ovule and adjusted pollen production), their flower femaleness and their fruit set probability (Tables 1, and 2). Total reproductive investment and fruit set probability decreased almost universally from first to last flowers, whereas the direction of change in flower femaleness was highly variable and seemed to be associated with dichogamy (Fig. 3). We developed a model to test whether the observed magnitudes and rates of within-plant change in flower femaleness and/or in fruit set probability could drive selection on flowering time through male fitness. The model indicated that the observed rates of change in flower femaleness were unlikely to cause appreciable selection on flowering time through male fitness (Fig. 4a), but the observed declines in fruit set probability can induce strong selection (Fig. 4b). Increasing flower femaleness from first to last

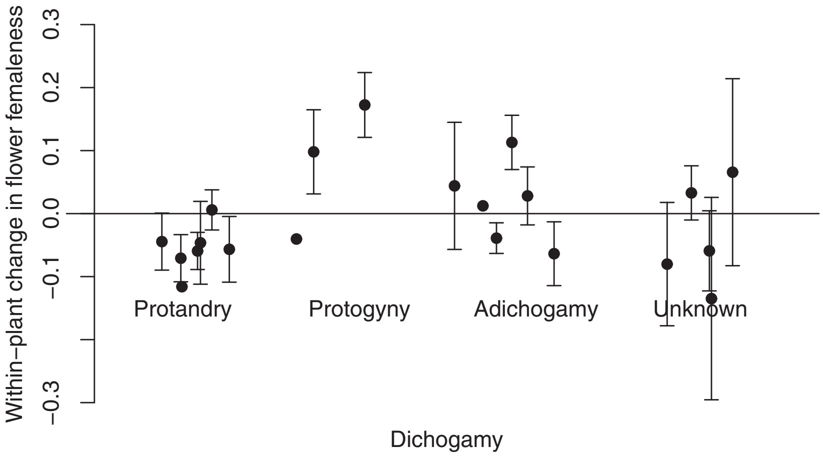

Fig. 3 Within-plant change in flower femaleness from first to last flowers (mean ± SE) by type of floral dichogamy. *n* within taxon ranges from 4 to 98 individuals (mean = 49, see Table 1).

Table 2 Fruit set probability of first and last flowers produced for 13 perfect-flowered hermaphroditic species.

Species	Habit	LH	Scale	*N*	FS first	FS last	Trend	Shape	References
Aquilegia canadensis (Ranunculaceae)	H	P	plant	26	1	0.76	D	S*	1
Aquilegia caerulea (Ranunculaceae)	H	P	plant	40	0.69	0.05	D	lin*	2
Brassica rapa (Brassicaceae)	H	A	plant	17	0.9	0	D	S	3
Geranium maculatum (Geraniaceae)	H	P	plant	30	0.6	0.16	D	NA	4
Hosta rectifolia (Liliaceae)	H	P	plant	41	0.71	0.40	D	S*	5
Lathyrus vernus (Fabaceae)	H	P	plant	45	0.16	0.05	D	NA	6
Lavandula stoechas (Lamiaceae)	S	P	plant	8	0.38	0.24	D	NA	7
Lobelia sessiflora (Campanulaceae)	H	P	plant	39	0.95	0.16	D	lin*	8
Orchis morio (Orchidaceae)	H	P	plant	103	0.41	0	D	S	9
Polygonatum odoratum (Liliaceae)	H	P	plant	20	0.89	0.39	D	NA	10
Prunus mahaleb (Rosaceae)	S	P	infl	5	0.6	0	D	NA	11
Schefflera heptaphylla (Araliaceae)	S	P	infl	5	0.04	0.1	Q	inv-V*	12
Zigandenus paniculatus (Liliaceae)	H	P	plant	40	0.77	0.17	D	NA	13

Habit = herbaceous (H) or shrub/tree (S); life history (LH) = annual (A) or perennial (P); Scale = change in fruit set from first to last flowers on a plant (plant) or change in fruit set from first to last flowers within inflorescences (infl); *N* = number of plants included in study; FS first = predicted fruit set probability of first flowers; FS last = predicted fruit set probability of last flowers; trend = fruit set declines from first to last (D), or fruit set rises and declines (Q); Shape (sh) = general shape of trend in fruit set probability from first to last flowers, linear (lin), S-shaped (S) or inverted-V (inv-V).

*Shape determined based on visual inspection of plotted data; NA indicates insufficient data points within plants to describe a shape of fruit set decline.

References: 1. Kliber & Eckert, 2004; 2. Brunet, 1996 (population Bellview); 3. Weis & Kossler, 2004; 4. Ågren & Willson, 1992; 5. Cao *et al*., 2007; 6. Ehrlén, 1992; 7. Herrera, 1993; 8. Hiraga & Sakai, 2007; 9; Jersáková & Kindlmann, 1998; 10. Guitián *et al*., 2001; 11. Guitian, 1994; 12. Pei *et al*., 2011; 13. Emms, 1996.

flowers partially alleviated selection driven by declining fruit set probability, but did not remove it entirely (Fig. 4c). We also found that declining fruit set can neutralize the selection for later male flowering induced by protandry and can strengthen the selection for earlier flowering induced by protogyny (Fig. 5). Given the prevalence of declining fruit set from first to last flowers on plants, we suggest that selection for early flowering through male fitness could be common in species that deploy flowers over an extended period of time.

Causes of within-plant variation

We observed several instances in our review of prefertilization trends in investment where members of the same family differed in the direction of change in flower femaleness (Liliacaeae, Onagraceae, Ranunculaceae, Table 1). The apparent lability of within-plant change in flower femaleness within families suggests either plastic response to the local environment, or rapid response to selection, or both. Our analysis does not permit us to disentangle these possibilities. We note, however, that dichogamous species generally adhere to the predictions of Brunet & Charlesworth (1995): protandrous species usually exhibit a proximal-to-distal increase in flower femaleness, and protogynous species, a decrease (Fig. 3). *Aquilegia* species differing in dichogamy provide a striking example (Table 1). This implies that the change in mating environment induced by floral dichogamy exerts strong selection on the direction of within-plant change in flower femaleness (or possibly *vice versa*) and that populations can rapidly respond to this selection.

On the other hand, in some *Clarkia* taxa, the directions of within-plant change in pollen production and in pollen-to-ovule ratios measured in the field (Delesalle *et al*., 2008) disagreed with those measured in the glasshouse (Mazer *et al*., 2009). Because several populations were shared across these two studies, this result implies large environmental effects on trends in prefertilization investment (Mazer *et al*., 2009). Mazer *et al*. (2009) suggested that these differences may be attributable to differing water availability across the two environments, with increased ovule production and suppressed pollen production in the water-rich glasshouse. This hypothesis awaits testing in an experiment. Further insight into the relative importance of environmental plasticity versus adaptation in setting the direction of within-plant variation in floral sex allocation may be gained through reciprocal transplants across populations.

The studies we identified generally did not report among-plant variation in within-plant rates of change in floral sex allocation, but such information may shed light on the control, and ultimately, the heritability, of within-plant gradients. Our previous work suggests that glasshouse-grown *Brassica rapa* plants are surprisingly uniform in their expression of within-plant variation in sex allocation (Austen & Weis, 2014). In contrast, Brookes & Jesson (2010) reported high variability in within-plant change in pollen-to-ovule ratios among individuals of gynodioecious *Stylidium armeria* (Stylidiaceae).

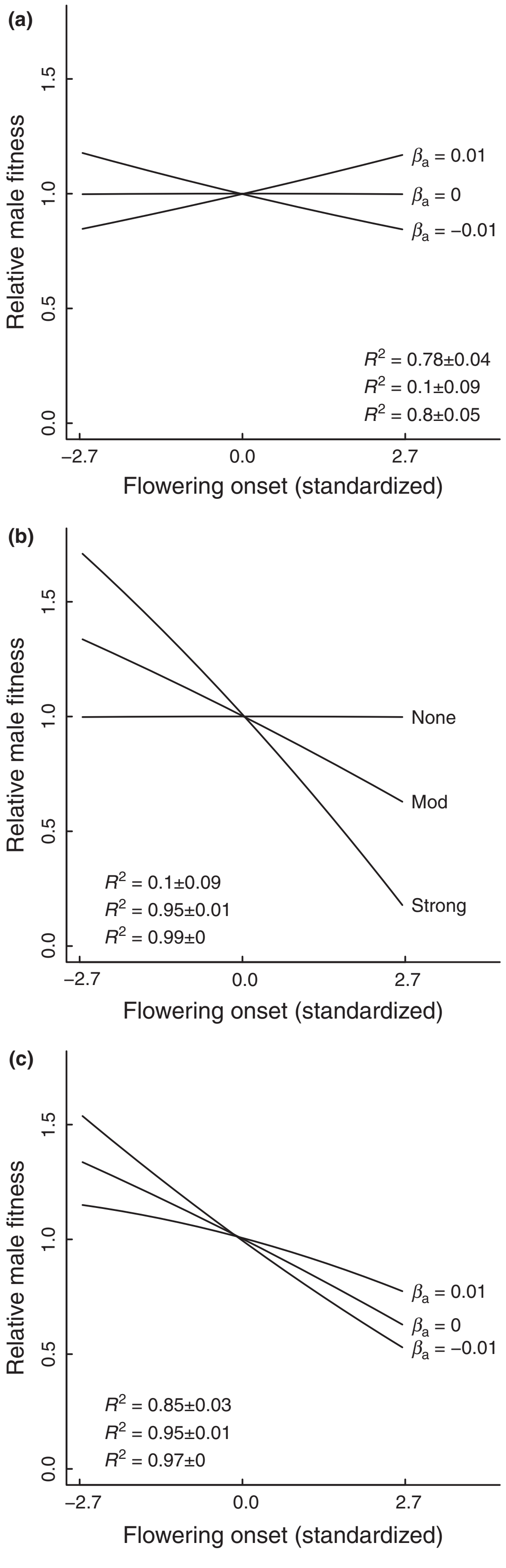

Fig. 4 Average associations between relative male fitness and standardized flowering time for 30 replicate runs of each of eight parameterizations of a numerical model (β_a = 0.000 in (a) and fruit set decline = none in (b) are the same parameterization). Lines for each parameterization depict the mean coefficients of the multiple regression of relative fitness against standardized flowering onset and its square (n = 30 estimates per coefficient per parameterization). Average R^2 (mean ± SD) across replicate runs are in the same top-to-bottom order as line labels within each panel. (a) Three parameterizations varying in the strength and direction of within-plant trends in prefertilization flower femaleness, with no within-plant decline in fruit set probability. For clarity, results for β_a = ±0.005 are omitted (these were intermediate to those for β_a = 0.000 and β_a = ±0.010). (b) Three parameterizations varying in the strength of the within-plant decline in fruit set probability, with no variation in prefertilization flower femaleness among the flowers on plants. (c) Three parameterizations varying in the direction of within-plant variation in flower femaleness, with fruit set probability following a moderate decline from first to last flowers on plants. Flowers are adichogamous throughout.

Additional studies reporting among-plant variation in within-plant trends in flower femaleness will be an important step towards determining the proximate and ultimate causes of such variation.

As expected, fruit set probability predictably declined from first to last flowers on plants with few exceptions. We encountered one system in which fruit set probability instead increased from first to last flowers (Goldingay & Whelan, 1993), but omitted this study from Table 2 because data were not reported in a manner comparable to other studies reviewed. Study of this system was motivated by its ‘unusual’ pattern of fruit set (Goldingay & Whelan, 1993, p. 502), underscoring the prevalence of a proximal-to-distal decline. Diggle (1995) identified additional cases where fruit set does not follow a decline, but these are again presented as exceptions to an otherwise widespread pattern.

Implications for the evolution of flowering time

Ollerton & Lack (1993) remarked on our collective ignorance of the nature of selection on flowering time through male fitness; twenty years later, studies examining male fitness in relation to flowering time remain scarce (Munguia-Rosas *et al.*, 2011). The model we have presented suggests that male fitness may decrease with later flowering onset in species that deploy flowers sequentially over a number of days if fruit set probability decreases within plants (Figs 4b,c and 5). Field studies estimating selection on flowering time through the male component of fitness are required to test this prediction. Genetic paternity analysis on carefully structured offspring samples may be particularly informative: if the model is correct, selection for early flowering through male function should be stronger

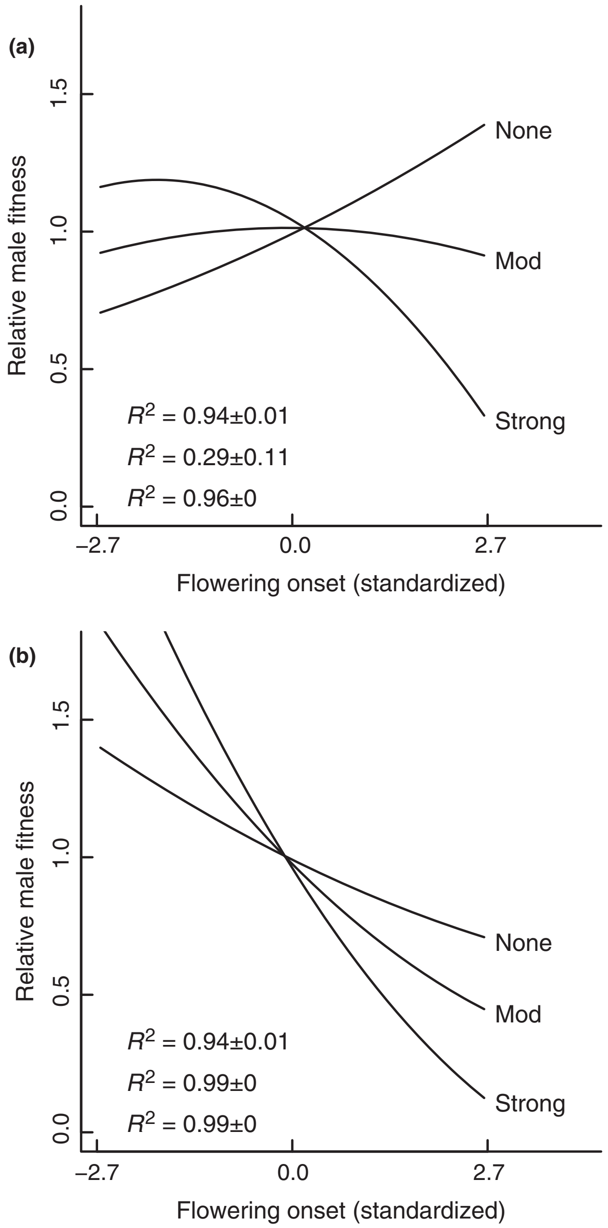

Fig. 5 Average association between relative male fitness and standardized flowering time in a numerical model when flowers are (a) protandrous or (b) protogynous. Lines for each parameterization depict the mean coefficients of the multiple regression of relative fitness against standardized flowering onset and its square (n = 30 estimates per coefficient per parameterization). Line labels provide strength of within-plant fruit set decline for given parameterization; average R^2 (mean ± SD) across replicate runs are in order of line labels.

when estimated from an offspring sample that includes seeds from the first few fruit produced by each individual than when estimated from an offspring sample that excludes the first fruit.

Austen & Weis (2014) proposed that declining fruit set probability might induce selection on the within-plant trend in prefertilization allocation, that is that the rate and direction of within-plant change in floral sex allocation may not be independent of the strength of fruit set decline. Even if fruit set probability and prefertilization sex allocation are nonindependent, the model here found that no biologically plausible rate of change in allocation fully alleviates the selection on flowering time resulting from even a moderate decline in fruit set probability (Fig. 4c). The prediction of selection for early flowering through male fitness was also robust to assumptions about the uniformity of plants in the population, the distribution of flowering onset, self-compatibility and decreasing returns on male and female investment (Appendix S2).

The prediction rests, however, on the assumption that female fitness is resource-limited. Pollen limitation is detected in approximately 60% of pollen supplementation experiments (Burd, 1994; Ashman *et al.*, 2004; Knight *et al.*, 2005), although this frequency probably overestimates its true incidence (Aizen & Harder, 2007). Whether occurring for part or all of a season, pollen limitation is likely to weaken or disrupt the pattern of declining fruit set probability within plants (Diggle, 2003) and thus weaken or disrupt selection for early flowering through male fitness. Pollen limitation is associated with perennial life history, and specialization on one or a few pollinators (Knight *et al.*, 2005). Monocarpic species visited by several pollinators are therefore most likely to meet the model assumptions.

Further, the model assumed flowering duration did not vary among plants, but timing of flowering onset and flowering duration are sometimes negatively correlated (Hendry & Day, 2005; Weis *et al.*, 2014). Such a correlation does not preclude the possibility of direct selection for early flowering onset through male fitness (assuming declining fruit set probability within plants). In fact, in such cases, total selection on flowering onset will likely be enhanced by indirect selection favouring longer flowering duration.

Finally, the model assumed the absence of selection through female fitness (all plants produced the same number of fruit), but selection through female fitness usually favours early flowering (Munguia-Rosas *et al.*, 2011). In dioecious species, greater fecundity of early-flowering females can lead to the evolution of earlier flowering onset in males (Forrest, 2014). We expect that in hermaphrodites, greater female fecundity of early-flowering plants would similarly strengthen selection for early flowering caused by a within-plant decline in fruit set. Congruent selection through male and female fitness should result in the evolution of progressively earlier flowering, but this response cannot be sustained indefinitely. Plants face a developmental, and therefore, temporal, threshold to flowering: an annual, for example, must grow vegetatively and produce flower buds before flowering (Primack, 1987). As population mean flowering approaches this threshold, flowering time variance is expected to decrease (Colautti *et al.*, 2010). Persistence of flowering time variation in the wild, and evidence for its heritability (Hendry & Day, 2005), suggests that some additional selective agent [e.g. temporally varying seed predation (Pilson, 2000), or occasional early season frost killing first buds (Inouye, 2008)] nudges the flowering time optimum away from the threshold.

Consequences of within-plant variation

Within-plant variation has been viewed as a nuisance when comparing plant phenotypes and as an impediment to the action of selection. Our model suggests that within-plant variability can ultimately drive selection on whole-plant features. The example explored here concerned a decline in post-fertilization female investment in successively produced flowers within individuals. Projected over the population, this led to decreasing mean quality of 'pollen recipients' as the season progressed, reducing the opportunity for successful pollen donation by late-flowering plants and thus driving selection for early flowering through male function. The essential conditions are as follows: within-individual change in the modular trait causes a temporal change in individual quality, and the fitness of individuals depends on the quality of neighbours with which they interact.

The possibility that within-plant variation can influence performance at the individual level was discussed at length by Herrera (2009). We have further developed this idea by providing a mechanistic example wherein within-plant variation may lead to selection on flowering time. Are there other potential examples where systematic within-plant variation could alter selection on a plant-level trait? One might be variation in leaf size and selection on germination time. The first true leaves produced by a seedling are typically smaller than those produced further up the stem (e.g. Merrill, 1986). A focal individual may occupy canopy space at an accelerating rate as it produces more leaves, intensifying the competitive disadvantage experienced by late-germinating neighbours relative to that which they would experience if leaf size were uniform, possibly driving selection for early germination. As a second example, a decline in nectar production in successively produced flowers (e.g. Devlin *et al.*, 1987) could alter selection on flowering time when seed production is limited by pollinator visitation. A late-flowering individual may produce rewarding proximal flowers, but if these are surrounded by the last feeble flowers of early-flowering neighbours, pollinators may seek resources elsewhere, potentially leading to selection for early flowering through both male and female fitness. Other possibilities can be imagined.

Within-plant variation in modular traits is widespread and, as such, merits greater research attention than it currently receives (Herrera, 2009). We have developed a testable prediction regarding selection on flowering time through male fitness by considering the implications of within-plant variation for mating opportunities. This, and other potential examples, supports the exciting possibility that within-plant variation may influence selection on other traits, regardless of whether this variation is under selection itself.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 R coding and sample 'initiation' data for one parameterization of the model (zip folder).

Appendix S2 Document including six figures depicting model outcomes when assumptions about distribution of flowering onset, schedule of flower deployment, uniformity of plants, self-compatibility, and the rate of return on male and female investment are relaxed.

Table S2.1 Summary of scenarios examined in the numerical model.

Figure S2.1 Effect of flowering onset (standardized) on relative male fitness when the distribution of flowering onset is (a) uniform, (b) left-skewed (skewness = 0.998), and (c) right-skewed (skewness = 0.998) (contrast to normal distribution, Fig. 4), as estimated in a numerical model.

Figure S2.2 Effect of timing of flowering onset (standardized) on relative male fitness when schedules of flower deployment within individuals follows a normal (solid line), left-skewed (dashed line), or right-skewed (dotted line) distribution (contrast to even distribution, Fig. 4), as estimated in a numerical model.

Figure S2.3 Effect of timing of flowering onset (standardized) on relative male fitness when plants vary in their schedule of flower deployment and in their magnitude of reproductive investment.

Figure S2.4 Effect of timing of flowering onset (standardized) on relative male fitness when plants are self-incompatible (a) under varying strengths of fruit set decline within plants, and (b) under varying trends in within-plant variation in flower femaleness (βa), as estimated in a numerical model.

Figure S2.5 Effect of timing of flowering onset (standardized) on relative male fitness under varying strengths of decline in within-plant fruit set probability when (a) the number of successful seeds saturates with total seed production ($\alpha < 1$) and (b) representation in the pollen pool saturates with increasing pollen production per day ($\gamma < 1$), as estimated in a numerical model.

Figure S2.6 Effect of timing of flowering onset (standardized) on relative male fitness when plants are (a) protandrous, or (b) protogynous, as estimated in a numerical model.

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