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# AN EXPERIMENTAL TEST OF THE EFFECTS OF RESOURCES AND SEX RATIO ON MATERNAL FITNESS AND PHENOTYPIC SELECTION IN GYNODIOECIOUS *FRAGARIA VIRGINIANA*

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Resources, sex ratio, and seed production by hermaphrodites covary among natural populations of many gynodioecious plant species, such that they are functionally "more dioecious" as resources become more limiting. Strong correlations among these three factors confound our understanding of their relative roles in maintaining polymorphic sexual systems. We manipulated resource availability and sex ratio and measured their effects on relative fertility and phenotypic selection through the maternal fitness of females and hermaphrodites of *Fragaria virginiana*. Two results were particularly surprising. First, hermaphrodites showed little variability in fecundity across resource treatments and showed strong positive and context-dependent selection for fruit set. This suggests that variation in hermaphrodite seed production along resource gradients in nature may result from adaptation rather than plasticity. Second, although females increased their fecundity with higher resources, their fertility was unaffected by sex ratio, which is predicted to mediate pollen limitation of females in natural populations where they are common. Selection on petal size of females was also weak, indicating a minimal effect of pollinator attraction on variation in the fertility of female plants. Hence, we found no mechanistic explanation for the complete absence of high-resource high female populations in nature. Despite strong selection for increased fruit set of hermaphrodites, both the strength of selection and its contribution to the maintenance of gynodioecy are severely reduced under conditions where females have high relative fecundity (i.e., low resources and high-female sex ratios). High relative fertility plus high female frequency means that the evolution of phenotypic traits in hermaphrodites (i.e., response to selection via seed function) should be manifested through females because most hermaphrodites will have female mothers. Fruit set was never under strong selection in females; hence, selection to increase fruit set in hermaphrodites will be less effective in maintaining their fruiting ability in natural populations with low resources and high female frequency. In sum, both sex ratio and resource availability influence trait evolution indirectly—through their effects on relative fertility of the sexes and patterns of selection. Sex ratio did not impose strong pollen limitation on females but did directly moderate the outcome of natural selection by biasing the maternal sex of the next generation. This direct effect of sex ratio on the manifestation of natural selection is expected to have far greater impact on the evolution of traits, such as seed-producing ability in hermaphrodites and the maintenance of sexual polymorphisms in nature, compared to indirect effects of sex ratio on relative fertility of the sexes.

**KEY WORDS:** Dioecy, *Fragaria*, gynodioecy, natural selection, plant gender, relative fertility, Rosaceae, sex-differential plasticity, sex ratio, sexual dimorphism.

Natural selection is sensitive to ecological context because individual reproductive success is often strongly affected by abiotic and biotic conditions (Wilson 1995; Totland and Eide 1999; Herrera 2000; Caruso 2000, 2001; Caruso et al. 2003; Maad and

Alexandersson 2004; Alonso 2005). Understanding how ecological variables influence natural selection can help explain patterns of trait expression, and may be particularly informative in cases in which trait variation is strongly associated with

environmental factors. Two ecological factors are likely to be particularly important—the availability of resources and the availability of compatible mates.

Resource availability and sex ratio covary negatively in a considerable number of gynodioecious plant species, suggesting an important role of these two ecological factors in the evolution of gender and sexual dimorphism in plants (reviewed in Ashman 2006). First, females increase in frequency as environmental conditions become more stressful, for example, as soil water or nutrients decrease in abundance (e.g., Delph 1990; Costich 1995; Puterbaugh et al. 1997; Ashman 1999; Vaughton and Ramsey 2002, 2004; Asikainen and Mutikainen 2003; Barr 2004; Case and Barrett 2004). This creates a gradient between low-resource high-female (LRHF) populations and high-resource low-female (HRLF) populations. Among these same populations, the fruit and seed production of hermaphrodites also varies, with individuals producing fewer fruits and seeds both as resources become more limited (e.g., Ashman 1999 and unpubl. data; Delph and Carroll 2001; Vaughton and Ramsey 2004; Barr 2004; Case and Barrett 2004; Delph and Wolf 2005) and as females become more abundant (e.g., Delph 1990; Wolfe and Shmida 1997; Ashman 1999; Delph and Carroll 2001; Vaughton and Ramsey 2002, 2004; Case and Barrett 2004; Asikainen and Mutikainen 2003, 2005). This affects sexual system expression, such that populations are functionally gynodioecious in high resources, and increasingly dioecious with lower resources. These patterns underscore the context-dependent sex-specific nature of selection, and its importance for the evolution of plant sexual systems—maintaining gynodioecy under high resources and selecting for dioecy under low resources.

Several hypotheses have been posed to explain the stress-dioecy relationship, aimed at understanding: (1) why female frequencies increase under low resources and decrease under high resources, and (2) why hermaphrodites exhibit reduced fruiting ability with low resources and high female sex ratio (Delph and Wolf 2005; Ashman 2006). Particularly with respect to hermaphrodite fruit production, it is not clear whether the proximate mechanism generating variation in gender and sexual dimorphism involves resources or sex ratio or both. If hermaphrodites reduce their seed output because they cannot maintain both sex functions when resource-limited, then variation in resource levels generates this pattern in nature (Delph 2003) either by plasticity or by natural selection for reduced reproductive cost. If natural selection favors hermaphrodites that are “more male” wherever many females are present, then a reduction in fruit set by hermaphrodites is also adaptive at high female sex ratios.

This paper describes an experiment to measure context-dependent and sex-specific selection in the gynodioecious Virginian wild strawberry (*F. virginiana* Rosaceae). We addressed the effect of resource availability and sex ratio on phenotypic selec-

tion by manipulating both factors and measuring morph-specific selection in each ecological context. We focused on four traits likely to be targeted by selection—ovules per flower, petal size, flower number, and fruit per flower (fruit set)—and likely to differ among contexts and between the sexes.

Context-dependent changes in female fertility could result from altered seed-making opportunities (ovules per flower, total flower number, or fruit set) or variation in pollination success (petal size). Although we expected all plants to increase reproductive allocation with greater resources, increases in these four traits are likely to be sex specific. Hermaphrodites divide resources and fitness between pollen and seed, often unevenly in the presence of females (Lloyd 1976; Delph 2003; Delph and Wolf 2005). Barring resource competition between the sex morphs (not relevant to our study), sex ratio cannot directly influence premating allocation, but female bias can reduce seed set by lowering the proportion of pollen-producing plants in a deme (Lewis 1941; Ashman and Stanton 1991; McCauley and Brock 1998), and by decreasing pollinator visitation by insects, which strongly prefer hermaphroditic flowers in many species (Bell 1985; Ashman and Stanton 1991; Eckhart 1991; Delph and Lively 1992) including *F. virginiana* (Ashman 2000; Ashman and Diefenderfer 2001).

Variation in fecundity of females relative to hermaphrodites (i.e., relative seed fertility) is the basis of phenotypic selection through maternal fitness. In general, hermaphrodites are expected to exhibit greater lability of maternal function than females because most of their fitness is gained through pollen (Lloyd 1976; Delph 1990; Delph and Lloyd 1991; Delph 2003; Delph and Wolf 2005). Hermaphrodites that can self-pollinate and suffer little inbreeding depression at the seed fertilization stage, like *F. virginiana* (T-L. Ashman et al., unpubl. data), are also unlikely to be affected by pollen limitation. Therefore, we expected hermaphrodite seed fertility to be affected by resources but not by sex ratio, and we expect little selection on petal size in hermaphrodites relative to selection on ovule production and fruit set. In contrast, the seed fertility of females is likely to be affected by both resources and sex ratio, either by altering allocation to ovules and seed maturation (resource effect only) or by changes in pollen availability, hence the likelihood of reduced seed set because of pollen limitation (combined effects of resources and sex ratio).

Because total selection on a given trait reflects a balance of selection between maternal and paternal sex functions, which may differ in both strength and direction, sex-specific and context-dependent selection should play an important role in phenotypic evolution (Morgan and Ashman 2003). In our experiment, the importance of selection via seed fertility in hermaphrodites is likely to be minimized where females are both abundant and relatively fecund, because the majority of hermaphrodites in the next generation will have female mothers, hence their phenotypic evolution ( $\Delta\bar{z}_H$ ) will be primarily in response to selection in females.

This concept was made concrete by Morgan and Ashman (2003) as the parameter “fertility-weighted sex ratio” which is a multiplicative function of the mean seed production of each sex and the sex ratio under a given sex determination system. It essentially represents the proportion of hermaphrodite offspring in each generation produced by female versus hermaphrodite mothers (parameters  $a$  and  $b$  respectively; see Methods and Morgan and Ashman 2003). For example, when females produce all the seeds in a population, then the next generation of hermaphrodites has only female mothers, so  $a = 1$ , and  $b = 0$ . In this case, only female-specific selection ( $\beta_F$ ) would contribute to  $\Delta\bar{z}_H$ , and selection via hermaphrodite seed fitness would not contribute to hermaphrodite’s phenotype in the next generation. Unlike sex ratio, variation in resource availability is not an explicit parameter in this model. Yet many studies have shown a direct effect of soil resources on the seed fertility of females relative to hermaphrodites (e.g., Ashman et al. 2001; Barr 2004; Asikainen and Mutikainen 2005). Therefore, ecological context may not only alter the strength and direction of selection, but may also determine how much of that selection is translated into phenotypic change through its effects on the fertility-weighted sex ratio.

In our experiment, we specifically addressed the following questions:

- (1) Is relative seed fertility of the sexes affected by resources and sex ratio? Specifically, is there evidence for sex-specific responses of seed fertility across treatments?
- (2) What are the agents (resources vs. sex ratio), putative targets (flower and ovule number, petal size, fruit set), and opportunity for selection in each context? Do these differ between the sex morphs?
- (3) How does the fertility-weighted sex ratio in each context modify predictions about the ecological effects of sex ratio and resources on phenotypic evolution?

## Methods

### STUDY SYSTEM

*Fragaria virginiana* (Rosaceae), the Virginian wild strawberry, is a creeping perennial herb native to eastern North America (Staudt 1989), which commonly grows in meadows, old fields, and along road or forest edges. Gynodioecy is under nuclear control with male sterility (femaleness) dominant to male fertility (Ahmadi and Bringhurst 1989), meaning that homozygous recessive hermaphrodites can produce only hermaphrodite seed offspring, whereas females produce offspring of both sexes in equal proportions. Wild strawberries over-winter as rosettes, reproduce sexually via seed and asexually via stolons. Flowering and fruiting occur from late April to early June and stolons are produced throughout the summer in western Pennsylvania. Floral visitors include small solitary bees, flies, butterflies, and ants (Ashman

and Diefenderfer 2001; Ashman and King 2005). Although pollinators prefer perfect flowers, which are substantially larger than pistillate flowers (Ashman 2000), females have very high fruit set (~90%) whereas that of hermaphrodites is low (<20%) even under optimal greenhouse conditions (Staudt 1989; Ashman 2003; this study).

### SOURCE POPULATION FOR GENOTYPES

The genotypes used for this experiment originated from a single natural population of wild strawberry in western Pennsylvania (population P in Ashman 1999). This source population contained a relatively high proportion of fruiting hermaphrodites, providing broad variation in fruiting ability for our study populations. Each year, plants were cloned from runners under greenhouse conditions in the summer and fall, and over-wintered at the Pymatuning Laboratory of Ecology (PLE, Crawford Co., PA; 41°34'N, 80°27'W) until the start of the experiment (April 30 in both years).

We assigned genotypes to treatments based on sex-specific reproductive trait values measured on one to two clones per genotype under greenhouse conditions. We assigned a broad distribution of reproductive phenotypes within treatments to maximize opportunities for and detection of selection, and to create an even distribution of phenotypes among treatments to standardize starting conditions. We selected females based on petal size and ovule number per flower, and hermaphrodites based on fruit set and pollen number per flower.

### IMPOSITION OF RESOURCE TREATMENTS

We maintained a threefold difference in resource availability to individual plants in low resource (LR) versus high resource (HR) treatments for three critical resources: (1) space, that is, pot size (75 mL for LR vs. 200 mL pots for HR), (2) fertilizer (Fall: one dose for LR vs. weekly 50 ppm dose of liquid 20–20–20 NPK for HR; Spring: two Osmocote pellets per pot for LR vs. six pellets for HR), and (3) water (Fall: watered to saturation every three days for LR vs. daily for HR; Spring: ~50 mL per pot every three days for LR vs. ~100 mL per pot every other day for HR). We chose these levels of resource addition to create in a twofold difference in total plant biomass between treatments at the time of flowering (Ashman et al. 2001 and see online Supplementary Appendix A).

### IMPOSITION OF SEX RATIO TREATMENTS

In each of two years, four experimental gardens were created that consisted of 80 plants. One of each HR and LR treatment was assigned as a high female (HF, 60% female) and one to a low female (LF, 15% female) sex ratio treatment, resulting in HRF, HRLF, LRHF, and LRLF treatment combinations. These sex ratios span the range observed among natural populations of wild strawberry in western Pennsylvania (Ashman 1999). The HF gardens were assigned 48 female and 32 hermaphrodite genotypes whereas the

LF gardens were assigned 12 female and 68 hermaphrodite genotypes (total of 320 experimental plants per year). The arrangement of plants within each garden was stratified by sex and random with respect to genotype.

### GROWTH AND MAINTENANCE OF EXPERIMENTAL GARDENS IN THE FIELD

Experimental gardens consisted of 1.2 m<sup>2</sup> sunken plots in four sites that were at least 500 m apart from each other in a farm field at PLE. Spacing between sites was chosen to minimize gene flow between them (T-L. Ashman, unpubl. data). Before placing plants into the field gardens, we nested the experimental plants (in their original pots) into larger pots filled with inert dry clay chip (Turface™; Profile Products LLC, Buffalo Grove, IL) with no nutrient content. The nested pots minimized site effects by preventing roots from extending into the native soil, spaced the plants evenly at a natural interplant density (11–13 cm apart, T-L. Ashman, unpubl. data), and standardized height so that crowns of plants in both small and large pots would be at ground level. Each site hosted one treatment combination (e.g., resource level × sex ratio) in each year. Because our objective included measuring selection via male fertility (T-L. Ashman, unpubl. data), space and time constraints did not allow for replication within as well as between years.

### MEASUREMENT OF REPRODUCTIVE TRAITS

We measured one vegetative and four reproductive traits that were expected to contribute heavily to maternal fitness through increased resource availability for reproduction (vegetative plant size), increased opportunities for making seeds (ovules per flower, flowers per plant, fruit set), and increased pollen receipt (petal size).

We estimated total plant size at the onset of flowering as total leaf number × width of the central leaflet of the largest fully expanded leaf (Ashman 1999). A single flower at the secondary position of the first inflorescence per plant was marked and petal area (length × width) was measured to the nearest 0.1 mm with digital calipers. These flowers are good indicators of the average flower size across all positions and inflorescences (T-L. Ashman, unpubl. data). At the end of fruiting, we counted the ovules in all marked flowers, and counted total flower number, total fruit, and total seed produced by each plant.

### DATA ANALYSES

We replicated treatments between years rather than within years to increase number of genotypes per garden in each year, and maximize our power to detect selection on individuals within each context. Because of the sample sizes we required to measure selection, it would have been logically impossible to carry out

the experiment with treatments replicated within years. This presented two main statistical issues. First, we could not simultaneously test for treatment effects while accounting for differences between study years. Preliminary analyses indicated that the study years did differ significantly for most traits (see online Supplementary Appendix A), and most importantly for fertility (Table 1). So, although “year” was not a factor in our statistical models, we have presented the data and selection analyses separately for each study year. Second, it was important to avoid pseudoreplication in assessing the effect of the sex ratio treatment on allocation and fertility, which, unlike resource availability, cannot be applied to individuals. Therefore, we used split-plot ANCOVAs (Potvin 2001) on all traits and for both resource and sex ratio treatments to conservatively assess the effects of our treatments.

### *Response to ecological context*

We used split-plot ANCOVAs to (1) confirm the effects of resource treatment on premating allocation traits, and (2) assess the effects of resources and sex ratio on female fecundity. Resource and sex ratio treatments were tested at the level of each garden, differences between sexes were tested among individuals, and initial plant size was included as a covariate. We used Bonferroni correction on all *P*-values to account for multiple tests. We found resources to have mostly positive effects on premating traits (significant plant size, resource, and resource × sex interactions), whereas sex ratio had no effect, as expected. In addition, there were significant sex effects on petal size and ovules per flower (see online Supplementary Appendices A and B).

We calculated the ratio of female to hermaphrodite fertility (F:H) from the average number of seeds produced by each sex morph for each resource × sex ratio × year replicate (i.e., garden) separately. The opportunity for selection (*I*; Wade 1979) within each sex morph was estimated as the variance in relative seed fertility for each resource × sex ratio × year, where relative seed fertility was calculated as absolute seed production divided by the mean seed production for each sex. This represents an upper bound on the strength of selection, potentially limiting the extent to which traits can evolve in each sex and context. Fertility-weighted sex ratio (parameter “*a*”) was calculated as per Morgan and Ashman (2003):

$$\frac{(1/2)\overline{W_F}x_F}{(1/2)\overline{W_F}x_F + \overline{W_{H_f}}x_H},$$

where *x<sub>F</sub>* and *x<sub>H</sub>* are the frequencies of females and hermaphrodites, and  $\overline{W_F}$  and  $\overline{W_{H_f}}$  are mean seed production of females and hermaphrodites, respectively.

### *Phenotypic selection*

Selection gradients were estimated from multiple regression of the four plant traits on relative seed fitness within each sex morph.

**Table 1.** Patterns of (A and B) relative seed fertility between sex morphs, (C and D) opportunity for selection (variance in relative seed production), and (E) fertility-weighted sex ratio for females (parameter "a" [fertility-weighted sex ratio of hermaphrodites "b" is equivalent to  $1 - a$ ; Morgan and Ashman 2003]) in four ecological contexts in two years for female (F) and hermaphrodite (H) plants of *Fragaria virginiana*.

	Year	HRHF	HRLF	LRHF	RLRF
A. Proportion of seeds produced by F (95% CI from 1000 bootstraps)	2001	0.95 (0.92–0.98)	0.63 (0.51–0.76)	0.92 (0.88–0.96)	0.69 (0.58–0.78)
	2002	0.97 (0.96–0.99)	0.64 (0.53–0.75)	0.93 (0.90–0.96)	0.70 (0.60–0.81)
B. Relative F:H seed fertility (95% CI from 1000 bootstraps)	2001	12.6 (12.3–12.9)	9.8 (9.6–10)	7.5 (7.3–7.7)	12.1 (11.9–12.3)
	2002	22.1 (21.5–22.7)	9.9 (9.7–10.1)	9.0 (8.9–9.1)	13.5 (13.2–13.8)
C. Opportunity for selection (I; F)	2001	0.36	0.32	0.39	0.26
	2002	0.33	0.49	0.46	0.42
D. Opportunity for selection (I; H)	2001	1.68	2.09	1.46	1.80
	2002	1.71	1.68	1.38	1.84
E. Fertility-weighted sex ratio of females ("a") (95% CI from 1000 bootstraps)	2001	0.904 (0.902–0.908)	0.470 (0.467–0.473)	0.850 (0.847–0.853)	0.521 (0.514–0.524)
	2002	0.945 (0.942–0.948)	0.470 (0.467–0.473)	0.871 (0.869–0.874)	0.547 (0.545–0.550)

We conducted the selection analyses separately by sex because selection is expected to be sex specific, because maternal fitness represents total fitness of female plants and only a portion of total fitness for hermaphrodites, and each contributes uniquely to response to selection (Morgan and Ashman 2003).

To minimize correlations between traits and fitness that are caused by a common dependence on resource status, we removed the effects of total plant size from all traits and from fitness for each sex and year using PROC REG in SAS and used the residuals of these regressions in the selection analyses. We standardized values for each trait (but not relative fitness) to a mean of 0 and standard deviation of 1 using PROC STDIZE in SAS (SAS Institute 1999). We conducted the regressions separately for each sex  $\times$  resource  $\times$  sex ratio  $\times$  year and corrected significance for multiple tests ( $\alpha = 0.05/8 = 0.00625$ ).

The distribution of hermaphrodites' maternal fitness was not normal because a large proportion of hermaphrodites produced no seed. Thus, we used resampling to assess the significance of individual selection gradients. We bootstrapped total seed production and reran the multiple regressions on each resampled dataset. We generated 1000 resampled datasets for each sex  $\times$  resource  $\times$  sex ratio  $\times$  year using the JACKBOOT macro in SAS and recalculated each selection gradient for each, as well as means and 95% confidence intervals for relative seed fertility, and the fertility-weighted sex ratio.

To statistically compare patterns of selection among treatments and years, we used ANCOVA to assess heterogeneity of slopes between each trait and fitness across treatment groups. Significant interaction effects between traits and treatments in the ANCOVA indicate significant differences in selection for a particular trait between treatments. Type III sums of squares were calculated separately for each sex (two ANCOVAs). The full model included the nonstandardized residuals for all four plant traits, class variables (resources, sex ratio, and year), interactions among class variables (e.g., resources  $\times$  sex ratio), and between class variables and traits (e.g., petal size  $\times$  resources  $\times$  sex ratio). We used backwards elimination and applied Bonferroni correction as above. We used absolute rather than relative fitness as the response variable because, unlike for the regressions, seed counts were necessarily pooled across treatments for the ANCOVAs, and relative fitnesses calculated within treatments are not comparable between treatments.

## Results

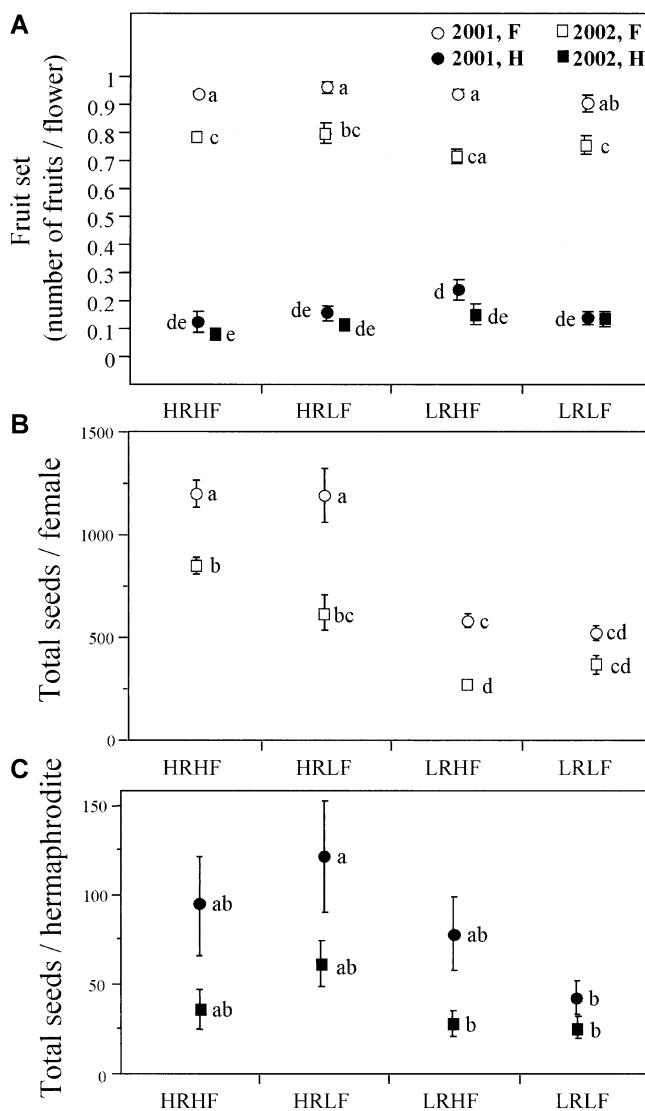
### RESPONSE TO ECOLOGICAL CONTEXT

#### Treatment effects on seed fertility

Across treatments, the sexes differed dramatically in their production of fruits ( $F_{1,621} = 1847, P < 0.0001$ ; Fig. 1A) and seeds ( $F_{1,617} = 934, P < 0.0001$ ; Fig. 1B, C). Females matured 90–96% of their flowers into fruits in 2001 and 71–80% in 2002 (Fig. 1A),

with individual female plants making 7–22 times the number of seeds as hermaphrodites, and collectively contributing 63–70% (in LF) and 92–97% (in HF) of the offspring to the next generation (Fig. 1B, C; Table 1A, B). Hermaphrodite's fruit set varied more widely in both years, averaging 12–23% fruit set in 2001 and 8–15% in 2002 (Fig. 1A).

HR increased seed production by females in both years (Fig. 1B), but had little detectable effect on seed production by hermaphrodites (Fig. 1C). This sex-specific response to resources was observed both with (resource  $\times$  sex: ANCOVA  $F_{1,617} = 152$ ,  $P < 0.0001$ ) and without adjusting for total plant size (Fig. 1C).



**Figure 1.** Variation among resource and sex ratio treatments in means ( $\pm 1$  SE) of (A) fruit set and (B) seed fertility of females (open symbols) and (C) hermaphrodites (filled symbols) in two years (2001 circles, 2002 squares). Lower case letters indicate means that are statistically indistinguishable from each other across and within years.

and contributed to treatment-specific patterns of relative seed fertility (Table 1B). In both years, relative seed fertility followed the pattern: HRHF > LRLF  $\gg$  HRLF > LRHF. This means that LR should hasten the spread of females in populations in which they are rare (LF), converting LRLF to LRHF populations. But because HR increased the fertility advantage of females when they were already abundant (HF), HRHF populations should be maintained in the absence of countervailing forces.

## PHENOTYPIC SELECTION

### Treatment effects on opportunity for selection

The opportunity for selection through seed fitness ( $I$ ) differed substantially between the sexes, with  $I$  for hermaphrodites greater than for females ( $F$ -test for equal variances:  $P < 0.0001$ ), but was statistically similar among all treatments and between years (all  $P > 0.10$ ; Table 1C, D).

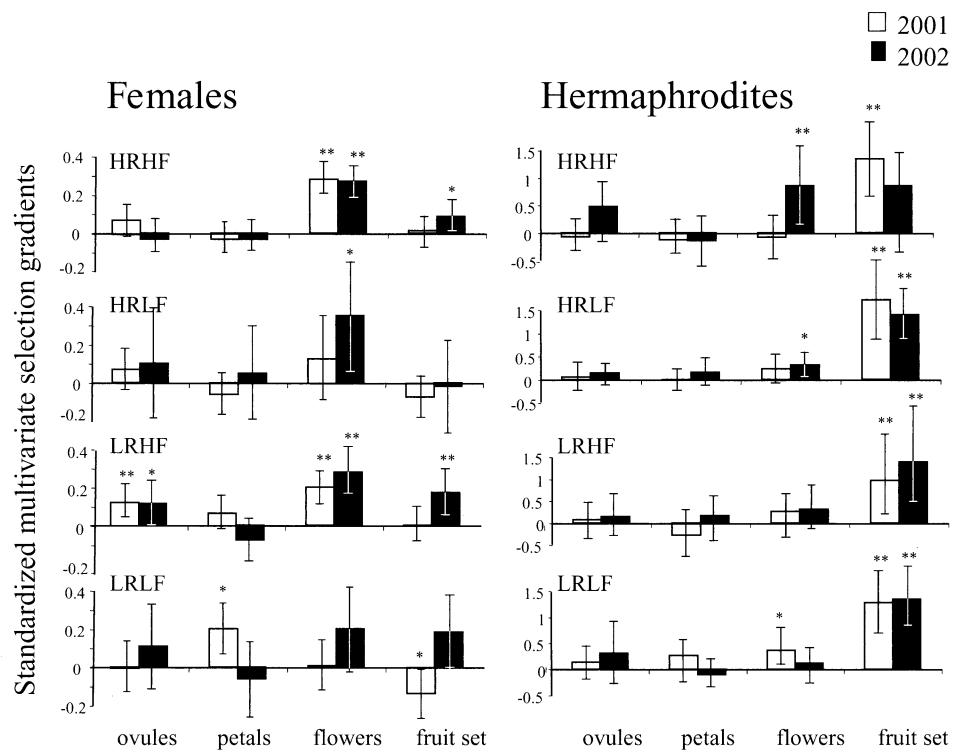
### Treatment effects on selection

After Bonferroni correction, all significant selection gradients were positive. The strongest selection we detected favored greater total fruit production in both sexes, but did so by sex-specific means. Given the invariably high fruit set among females, selection favored increased total flower number; this increases total fruit production because every flower produced by females is very likely to become a fruit (see Fig. 1A). In contrast, given relatively low and highly variable fruit set among hermaphrodites, selection favored increased fruit set (Fig. 2). The fact that we detected significant selection on females only in the HF treatments may reflect a lack of statistical power in LF treatments ( $n = 12$  females per garden).

Overall, we detected selection on all four reproductive traits in females, but only on flower number and fruit set in hermaphrodites (single trait effects in online Supplementary Appendix C). Several trait  $\times$  treatment interactions (Fig. 2) indicate that selection gradients differ significantly across treatments, particularly on fruit set in hermaphrodites (Fig. 2).

In females, selection on ovules per flower was detected only in 2001 (ovules  $\times$  year: ANCOVA  $P < 0.0059$ ). There was some evidence of selection on petal size (ANCOVA  $P < 0.0467$ ), and the petal size  $\times$  resources  $\times$  year interaction (ANCOVA  $P < 0.0286$ ) reflects selection to increase petal size in LR in 2001 (Fig. 2). We detected contrasting effects of resources on selection on flower number versus fruit set—stronger selection for fruit set in the LR than HR treatments, but stronger selection for flower number in HR versus LR (Fig. 2).

In hermaphrodites, selection for increased fruit set varied significantly among treatments and years (online Supplementary Appendix C). Although strong in all contexts, selection in hermaphrodites was significantly stronger under HR (fruit set  $\times$  resources: ANCOVA  $P < 0.0001$ ), particularly in LF treatments



**Figure 2.** Patterns of multivariate phenotypic selection via maternal fitness in four ecological contexts for two sexes (females on left, hermaphrodites on right) in two years (2001 in open bars, 2002 in black bars). Standardized multivariate selection gradients ( $\pm 95\%$  confidence intervals) are shown for ovules per flower, petal size, total number of flowers per plant, and fruit set (fruits per flower). Significance and confidence intervals were determined by bootstrapping. Asterisks indicate gradients significantly different from zero ( $P < 0.05$ ). Double asterisks remain significant after Bonferroni correction for multiple tests ( $\alpha = 0.05/8 = 0.00625$ ).

(fruit set  $\times$  resources  $\times$  sex ratio: ANCOVA  $P < 0.0051$ ) and in 2001 (fruit set  $\times$  year  $\times$  resources: ANCOVA  $P < 0.0024$ ), and stronger in 2001 vs. 2002 (fruit set  $\times$  year: ANCOVA  $P < 0.0001$ ).

**Treatment effects on contribution to selection response**  
Ecological context affected not only the strength and direction of selection, but also the realized contribution of selection to trait evolution. In both years, the fertility-weighted sex ratios differed significantly between resource  $\times$  sex ratio treatments: HRHF > LRHF  $\gg$  LRLF > HRLF (Table 1E). Both LF treatments were close to 0.5, meaning the relative contributions of female- versus hermaphrodite-specific selection to hermaphrodite trait evolution were more or less equivalent, but only HRLF were significantly biased in favor of selection on hermaphrodites. In HF treatments, fertility-weighted sex ratios were closer to 1, meaning that the relative contribution of sex-specific selection to hermaphrodite trait evolution was grossly biased toward selection in females, significantly more so in HRHF. Therefore, sex ratio variation combined with resource effects on relative seed fertilities makes selection on traits in hermaphrodites in HRLF populations more likely to result in phenotypic change than selection on traits in hermaphrodites in other resource or sex ratio contexts.

## Discussion

Natural selection in gynodioecious wild strawberry populations is context dependent and sex specific. Both resource availability and sex ratio affect the maternal fitness of females and hermaphrodites, and do so in a sex-specific manner, largely because of sex differences in the opportunity for selection. Surprisingly, seed production by hermaphrodites did not increase with high resources, suggesting that selection and adaptation rather than plasticity to resources may be responsible for ecological gradients of seed-producing ability in hermaphrodites. Our data suggest that both the maintenance of HRLF populations and the conversion of LRLF to LRHF populations in nature are largely driven by sex ratio effects. Contrary to expectation, we found no evidence for female plants being disadvantaged in HRHF because of pollen limitation, and therefore have no data to explain the absence of these populations in nature.

## SEX-SPECIFIC EFFECTS OF CONTEXT ON FERTILITY AND PHENOTYPIC SELECTION

Females clearly outperform hermaphrodites as mothers, producing 7–22 times the number of seeds per plant as hermaphrodites, and the vast majority of the total seeds in each garden. Variance in relative fitness was consistently lower for females than for

hermaphrodites, thus there was greater opportunity for selection ( $I$ ) in all contexts and higher selection gradients in hermaphrodites compared to females. However, hermaphrodites' seed fertility was not affected by resource treatment to the same degree as females, a result contrary to what is often predicted (Delph and Lloyd 1991; Delph 2003), and found in other *F. virginiana* studies (Ashman 2006).

Our results are consistent with the hypothesis that the relationship between stress and gender dimorphism under the conditions of this study results from selection for hermaphroditic genotypes with greater or lesser fruiting ability, and not by plasticity alone. Studies reporting plasticity of fruit production in males base this inference largely on correlations between fruit production and vegetative size, trade-offs between seed and pollen allocation, or increases in fruit production when cuttings are moved from the field to greenhouse (reviewed in Delph and Wolf 2005). Two recent studies supplemented field plants with resources (Barr 2004; Asikainen and Mutikainen 2005) and observed increased seed production by hermaphrodites. Although these patterns are common and fit well with predictions from sex allocation models, these types of data often confound several factors related to allocation, such as architectural constraints in the case of cuttings, potential pollen limitation, or other soil characteristics in field settings. Resource limitation can still play a significant role in the maintenance of fruiting ability in gynodioecious species without plasticity, if it selects for male-biased genotypes.

It is also possible that plasticity to resources is itself variable across environments and among genotypes, and still contributes to environmental gradients in hermaphrodite fruit production in nature. Ashman (2006) found evidence for variation in fruit set plasticity among natural populations of wild strawberry, and that the *degree* of plasticity for fruiting ability was negatively correlated with both female frequency and with pollen production per flower. She concluded, given the potential costs of plasticity to siring success, that selection should favor fixed genotypes under LR, especially with HF, but more plastic genotypes under HR, especially with LF. The source population for the plants in this study (population P in Ashman 1999) is a very large HRLF population. It was chosen because we could maximize the genotypic variation in fruiting ability among hermaphrodites for the detection of selection, and indeed we found strong selection for fruit set in hermaphrodites. However, the role of plasticity versus adaptation in mediating the stress–dioecy correlation needs further experimental assessment. In particular, experiments with replicated genotypes across resource gradients that can specifically assess the relative roles of plastic versus fixed phenotypes.

Phenotypic selection through the maternal fitness of females and hermaphrodites was differentially manifested. Patterns of selection were largely consistent across ecological contexts for females, that is, all four reproductive traits were apparent targets.

Increased investment into ovules per flower, flower number, and fruit set resulted in greater relative seed fitness among females by increasing the opportunity for making seeds. Selection to increase fruit set was stronger in LR than HR treatments. However, if female seed set had been strongly pollen limited, we would have expected the opposite pattern—negative selection on allocation to ovules but likely positive selection on petal size to increase attractiveness to pollinators. Selection on petal size was detectable and positive, but weak, in females. Selection gradients for petal size only approached significance in only one treatment in one year (LRLF 2001). We had expected females to be most pollen limited in HF sex ratio treatments, yet we did not see patterns of selection indicative of pollen limitation. In contrast, selection via maternal fitness of hermaphrodites was significantly affected by treatments and years, and primarily targeted one trait—fruit set. Unlike all other reproductive traits and seed production, mean fruit set by hermaphrodites did not vary among treatments or between years, but selection did. The causes of sex-specific selection reflect dramatic sex differences in total allocation to seeds, opportunity for selection, and differential responses of seed fertility to changes in resources and sex ratio contexts.

The targeting of fruit set in hermaphrodites suggests limited avenues for increasing their maternal fitness compared with females. Fruit set was under strong positive selection in all hermaphrodites, and selection for increased fruit set was stronger in HR than LR and stronger LF than HF treatments. Fruit set in hermaphrodites is heritable, and shows no significant between-sex genetic correlation, thus can evolve independently in each sex (Ashman 2003). Therefore, two factors determine whether selection will result in increased hermaphrodite fruit set in subsequent generations—the nature of selection on fruiting ability through male fitness, which can be antagonistic to female fitness (T-L. Ashman, unpubl. ms), and on the proportion of hermaphrodites in the each generation with female mothers (e.g., fertility-weighted sex ratio; Morgan and Ashman 2003).

We did not include any potential context-dependent effects on rates of self-fertilization or inbreeding depression in our estimates of maternal fitness (Charlesworth and Charlesworth 1978). Low pollen availability and/or pollinator visitation (Alonso 2005) or LR and HF conditions may increase the likelihood of self-pollination in hermaphrodites, and LR may intensify inbreeding depression (Dudash 1990; Byers and Waller 1999; Heschel et al. 2005). Although previous studies of *F. virginiana* showed low visitation in HF contexts (Ashman and Diefenderfer 2001), hermaphrodites showed little evidence of inbreeding depression at the seed formation stage (T-L. Ashman, unpubl. data). Therefore, it seems that fitness estimates based on seed quantity might accurately reflect maternal fitness in this study. However, if there is a cost of self-fertilization in terms of pollen fitness (i.e., pollen discounting; Barrett 2003), this may exacerbate any effects of pollen

limitation of seed set on females, which is already expected to be high in LR and HF contexts. Given our results, hermaphrodites in natural LRHF populations produce few seeds even when out-crossed; we might speculate that the effects of resources and sex ratio on sexual system evolution via seed fitness may outweigh their effects on mating system parameters. However, this conclusion awaits quantitative verification.

### SEX-SPECIFIC EFFECTS OF CONTEXT ON TRAIT EVOLUTION

Two factors must be accounted for to understand the contribution of sex-specific and context-dependent patterns of selection to phenotypic evolution. First, because femaleness is caused by a dominant nuclear allele, homozygous recessive hermaphrodites produce only hermaphrodite seed offspring, but females can be mothers to offspring of both sexes. Consequently, selection via the maternal fitness of hermaphrodites cannot influence traits in females, but selection in both sexes can effect change in hermaphrodites (under constraints imposed by  $G$ ; Ashman 2003; Morgan and Ashman 2003). Second, the contribution of sex-specific selection on a given trait to phenotypic change depends on the relative fertility of the sexes combined with the population sex ratio. Ultimately, this means that resource availability can only have an *indirect* effect on the realization of selection through its effects on relative fertility, whereas sex ratio can have both a *direct* and *indirect* effect.

In our study, we found interactive effects of resources and sex ratio on both relative fertility and on the fertility-weighted sex ratio. Relative (F:H) fertilities in HRHF and LRLF were substantially greater than in HRLF and LRHF. We expected hermaphrodites in LF to gain a larger proportion of their total fitness via seed, on average, relative to hermaphrodites in HF treatments for two reasons (Lloyd 1974, 1975). Females produce many more seeds than hermaphrodites, reducing the marginal fitness value of each seed produced by hermaphrodites. Second, hermaphrodites serve as fathers for all seeds produced by females, which tips the balance of their reproductive success in favor of pollen. HR should exacerbate this effect because each of the females made twice as many seeds. Our expectation of relative fertilities was met under HR, where HF > LF, but the reverse was true under LR. In short, hermaphrodites in LRLF made fewer seeds than we had predicted, and females in HRHF were more fecund than we had predicted.

Although relative fertility is a component of the fertility-weighted sex ratio, these two parameters did not covary among treatments. This fertility-weighted sex ratio parameter essentially weights selection coefficients for each sex, either amplifying or diminishing the importance of each to trait evolution. This is a critical component to the evolution of gender and sexual dimorphism in gynodioecious species. Relative fertility of the sexes

is important for maintaining a particular sex ratio in the next generation, particularly in species with nuclear sex determination (Lloyd 1976). This is because only females can produce female seed offspring, thus greater relative fertility of females will drive the sex ratio closer to unity, whereas greater relative fertility of hermaphrodites will increase the number of hermaphrodites in subsequent generations. The consequences of variation in fertility-weighted sex ratio are quite different from variation in relative fertility of the sexes. The former incorporates relative fertilities and current sex ratios, and determines the extent to which natural selection on phenotypes is mediated through female versus hermaphrodite mothers. This has dramatic implications for the evolution of traits in hermaphrodites, which can be produced by mothers of either sex. Therefore, the response to selection of any trait for which selection differs between the sexes will depend on both the relative fertility and the current sex ratio environment.

Selection via the maternal fitness of hermaphrodites is tempered in populations in which females are abundant (i.e., HF). It is important to note that this is not because they gain most of their fitness through pollen, but because they contribute so many fewer seed offspring to the next generation compared with females. This means that selection to increase the fruiting ability of hermaphrodites is almost completely ineffective in HF populations, regardless of how strong that selection is or how heritable fruit set is. And because increased fruiting ability is not often under strong selection in females, fruiting ability is unlikely to be maintained in hermaphrodites wherever the fertility-weighted sex ratio is biased toward females. This phenomenon likely contributes to the rapid loss of female fertility of hermaphrodites with increasing female frequency (i.e., as a positive feedback mechanism). In LF populations, selection via maternal fitness in both sexes contributes more or less equally to phenotypic change (fertility-weighted sex ratio approx. 0.50), which could explain why we see hermaphrodites with greater fruiting ability where females are rare. Therefore, contrary to common expectation, the greater contribution of selection in hermaphrodites when females are rare does not depend on the relative maternal:paternal fitness of the average hermaphrodite, but instead depends on the relative maternal fitness of the two sexes.

In most gynodioecious species, natural populations vary along a continuum between HRLF and LRHF (but see LRLF populations in Barr 2004). HRHF populations are not typically observed. Barr (2004) argued that the maximum female frequency in gynodioecious populations may decrease with soil resources if constraints on maternal fitness shift from resource limitation to pollen limitation, which would disproportionately affect females relative to hermaphrodites in HR (Asikainen and Mutikainen 2005). Comparisons of the degree of pollen limitation between females in HRHF and HRLF suggest that potential pollen limitation at HR had little affect on fertility or on patterns of selection,

and that pollen limitation may have a stronger affect at LR than HR (Case and Ashman, unpubl. ms.). Hence, our data suggest that sex-ratio-dependent pollen limitation should not prevent the existence HRF populations in nature. Instead, it appears from our data that populations starting at HRF are likely to stay that way unless pollen limitation is more severe than we found in our study.

## CONCLUSIONS

Resources, sex ratio, and seed production by hermaphrodites covary among natural populations of many gynodioecious plant species, resulting in high-resource populations with few females and high-fruited hermaphrodites versus low-resource populations with many females and little seed production by hermaphrodites. Overall, our results for maternal fitness in *F. virginiana* suggest that the primary role of resource availability in maintaining this correlation is to strengthen positive natural selection on fruit set in hermaphrodites. The primary effect of sex ratio is to bias the maternal sex of the next generation, therefore altering the translation of sex-specific natural selection into phenotypic change. We found no evidence that high female frequency reduces female seed production, and no mechanistic explanation for the absence of high-female high-resource populations in nature. Instead, we found evidence for an important role of population sex ratio in maintaining correlations between high female frequencies and low hermaphrodite seed production through its effect on the fertility-weighted sex ratio. Perhaps most importantly, our results indicate that changes in the fruiting ability of hermaphrodites among populations can result from adaptation and context-dependent natural selection. Whether this occurs at the expense of or in addition to plastic responses to resource availability remains to be seen. Complementary studies addressing context-dependent selection via paternal fitness are needed to fully understand the role of ecological context in the maintenance of gender and sexual polymorphism.

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## Supplementary Material

The following supplementary material is available for this article:

**Appendix A.** Variation among treatments in total plant size and five reproductive traits for females and hermaphrodites *Fragaria virginiana*. Values in the first four rows present least squares means (LSM) for each sex, resource treatment (high, HR, vs. low, LR), and sex ratio treatment (high female, HF, vs. low female, LF). See text for analytical details; full model results are presented in digital Appendix B. For each row (traits), means sharing superscripts within rows are not significantly different from each other following Turkey's pairwise comparisons of res\*sex ratio\*sex LSMs. Values in the last four rows are raw means showing differences years for each trait (unadjusted for total plant size).

**Appendix B.** Split-plot ANCOVA of reproductive traits of females and hermaphrodites of *F. virginiana* under differing resource (R) and sex ratio (SR) contexts in two study years. We used a split-plot design because treatments were applied at the garden level. The effects of high versus low resources ( $df = 1$ ) and high female versus low female sex ratio ( $df = 1$ ) on each trait were tested relative to garden ( $df = 4$  gardens per treatment per year). The effect of plant sex (female vs. hermaphrodite) was a split-plot-level treatment and was tested over the error term ( $df = 618 - 624$ ). Asterisks (\*) indicate significant  $F$ -values at  $P = 0.05$ ; those in **boldface** remain significant after Bonferroni correction for six separate analyses ( $\alpha = 0.05/6 = 0.0083$ ).

**Appendix C.** Analysis of covariance of the relationship between traits and fitness of females and hermaphrodites of *F. virginiana* under varying resource and sex ratio contexts in each of two study years (see text for details of the selection analysis). Significant differences among the relationship between the traits and absolute fitness were determined by ANCOVAs conducted separately for each sex across traits and years. We used type III sums of squares. The ANCOVA models were optimized using sequential backwards elimination of higher- to lower-order ( $P$ -to-remove = 0.10); factors not included in each model are either not listed or indicated by dashes. Interactions exceeding the threshold to remove were not removed if higher-order interactions containing those factors were retained.  $P$ -values in **boldface** remain significant after Bonferroni adjustment for the two models ( $\alpha = 0.05/2 = 0.025$ ), which tested treatment effects on selection in each sex separately.

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