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GENETIC AND ENVIRONMENTAL CONTROL OF TEMPORAL AND SIZE-DEPENDENT SEX ALLOCATION IN A WIND-POLLINATED PLANT

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Sex allocation in hermaphrodites can be affected by spatial and temporal variation in resources, especially in plants where size-dependent gender modification is commonplace. The evolution of sex allocation will depend on the relative importance of genetic and environmental factors governing patterns of investment in female and male function. In wind-pollinated plants, theoretical models predict a positive relation between size and male investment because of the fitness advantages associated with more effective pollen dispersal. Theory also predicts that the timing and allocation to each sex function should depend on available resources. We grew maternal half-sibling families of annual, wind-pollinated, *Ambrosia artemisiifolia* in sun and shade treatments to investigate these predictions. There was significant genetic variation for female and male flower production in both sun and shade treatments. Size-dependent sex allocation occurred in the direction predicted by theory, with male flower production increasing more rapidly in larger plants. The timing of sex function also varied, with significant genetic variation for dichogamy within environments and plasticity of this trait between environments. Protandry was expressed more commonly in the sun and protogyny in the shade. The occurrence of dynamic sex allocation with changing size and experimental treatment indicates the potential for adaptive responses under different ecological conditions.

KEY WORDS: **Dichogamy, genetic variation, phenotypic plasticity, quantitative genetics, sex allocation, wind pollination.**

In hermaphrodites, the optimal allocation of resources to female and male sex function can vary with environment, age, or size (Charnov 1982; Lloyd & Bawa 1984; Klinkhamer et al. 1997). Plasticity of allocation in relation to size or environment should be adaptive and thus be favored over fixed genetically determined allocation patterns (Lloyd & Bawa 1984; Zhang 2006). Natural selection should therefore favor individuals with the capacity to change patterns of resource allocation to match new conditions with optimal fitness maintained by phenotypic plasticity (Dudley & Schmitt 1996; Bazzaz 1997; Schmitt et al. 1999). Indeed, there is considerable evidence for plasticity in allocation patterns in response to environmental heterogeneity in both animals (reviewed in Schärer 2009) and plants (reviewed in de Jong & Klinkhamer 2005). Flexible sex allocation may be particularly feasible to implement in plants because of their modular growth and the production of multiple flowers, which in many species enables individuals to vary their sex expression. In this way, sex allocation has the potential to influence gender strategies and the timing of sex functions with consequences for mating and reproduction.

Models of sex allocation in hermaphroditic plants can explain varying patterns of investment in female and male function (Charlewsorth & Charlesworth 1981, 1987; Charnov 1982; Lloyd & Bawa 1984; Charlesworth & Morgan 1991; Morgan 1992). Sex allocation models typically use phenotypic gain curves, based on

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the shape of the relation between investment and fitness returns. Nonlinearity in either the female or male gain curve can cause the optimal allocation between sex functions to be unequal. Moreover, the female and male components of fitness can vary differentially among environments and between individuals of different size. Thus, variation in the patterns of sex allocation may arise when individuals are selected to alter their allocation in response to environmental conditions, or in response to increasing age or size (Lloyd & Bawa 1984; de Jong & Klinkhamer 1989; Zhang 2006). For wind-pollinated plants in particular, there are several key factors that can affect gain curves and the optimal allocation to female and male function. Local environmental conditions can affect relative plant height and successful pollen dispersal, and plant size has both direct and indirect consequences for patterns of sex allocation.

The role of environmental factors on sex allocation is important if there is plasticity in allocation to the two sex functions. The extent to which sexual traits are plastic or show genetic variation in sex allocation plasticity is seldom considered (but see Mazer & Schick 1991; Conner et al. 2003; Mazer et al. 2003; Caruso 2006; Bishop et al. 2010), despite the importance of determining the relative magnitude of genetic and environmental factors for predicting responses to selection on sex allocation. One environmental cue that is particularly relevant for wind-pollinated species is whether plants grow in closed canopy environments (shade) or in open environments (sun). These different habitats can have profound implications for the aerodynamic environment plants experience and the potential for pollen export and capture. For wind-pollinated plants with sex allocation plasticity, we might predict that closed canopy environments should favor allocation to female function and open environments male function.

Size-dependent sex allocation is beneficial when there are dissimilarities in fitness gain through female and male function with increasing size (de Jong & Klinkhamer 1989; Zhang 2006). In wind-pollinated species, plant size can affect fitness returns directly, representing one of the clearest examples of direct size-dependent effects on allocation (Niklas 1985; Burd & Allen 1988). Pollen dispersal is expected to be more effective with increased height because pollen travels further, thus increasing mating opportunities. A tall plant should function better as a pollen donor, whereas a diminutive plant may function better as a pollen recipient. Thus, contrary to the usual expectation of greater female allocation in larger plants (Lloyd & Bawa 1984), larger wind-pollinated plants should have greater male investment than smaller plants and indeed there is some evidence for this (e.g., Solomon 1989; Aizen & Kenigsten 1990; Ackerly & Jasienski 1990; Fox 1993; Dajoz & Sandmeier 1997; Pannell 1997; McKone et al. 1998; but see Koelewijn & Hunscheid 2000).

Plant size can also affect the resource status of plants so that the budget that can be invested in reproduction will differ for plants of varying sizes. If fitness gains are nonlinear for either female or male function (or both) then different allocation patterns are expected for small and large plants (Klinkhamer et al. 1997; de Jong & Klinkhamer 2005). Decelerating gain curves select for gradual shifts in allocation with size, with proportional allocation increasing to the sex function with the less decelerating curve. Additionally, theoretical models suggest that when female and male function are drawn from a rate-limited photosynthetic income, the timing and allocation to each function can vary in ways that differ from predictions based on marginal returns (Burd & Head 1992). Specifically, male function may present an opportunity cost in terms of photosynthetic income to female function, favoring a later staminate phase. Also, recent theory suggests that temporal changes in sex allocation, resulting in differences in the timing of female and male sex function (e.g., dichogamy), may serve as an adaptive response to environmental stochasticity in resource availability (Zhang 2006). However, empirical support for this idea is lacking and flexible environmentally induced changes in the expression of dichogamy have not been reported in the literature.

Sex allocation models typically use an evolutionary stable strategy approach to determine the optimal allocation of reproductive resources to female and male function (reviewed in Charnov 1979), implicitly assuming that female and male sexual function both share limiting resources. One predicted outcome of such resource sharing is the occurrence of trade-offs between sex functions and negative genetic correlations among reproductive traits. However, few studies have found negative genetic correlations between female and male allocation, despite significant genetic variation for most floral traits (Ashman & Majetic 2006). Negative genetic correlations between female and male traits may rarely be detected because variation in resource acquisition among plants can obscure them (van Noordwijk & de Jong 1986; Worley et al. 2003). Larger plants will have more resources to invest in reproduction, and so an overall positive correlation is often found between investment in female and male function.

Here, we investigate the effects of genetic and environmental factors on allocation to female and male flower production in common ragweed (*Ambrosia artemisiifolia*). Sex allocation in this species is variable and there is evidence that ecological conditions influence the relative production of female and male flowers (McKone & Tonkyn 1986; Ackerly & Jasienski 1990; Traveset 1992; Lundholm & Aarssen 1994; Paquin & Aarssen 2004). However, to what extent genetic variation also contributes toward gender expression in populations of *A. artemisiifolia* is poorly understood (although see Jones 1936). *Ambrosia artemisiifolia* is particularly suitable for investigating the genetic and environmental regulation of gender because it is monoecious, self-incompatible, and possesses an annual life history (Friedman & Barrett 2008). Monoecy allows for easy quantification of female and male allocation, and the presence of self-incompatibility simplifies interpretation of results because geitonogamous self-fertilization and its effects on fitness need not be considered. Finally, the annual habit enables an assessment of lifetime reproductive investment, without concern for future survival or reproduction.

Using an experimental approach, we examine the genetic and environmental components of temporal and size-dependent sex allocation in *A. artemisiifolia* in two environmental treatments (sun and shade). We investigate the predictions of sex allocation models that include the direct and indirect size-dependent effects on the timing of female and male investment by addressing three specific questions: (1) Is there evidence for quantitative genetic variation in female and male flower production and are these components of sex allocation genetically correlated? Quantitative genetic variation in sex allocation, including any interactions with environment, would enable populations to adjust their sex functions to prevailing ecological conditions. (2) Do contrasting sun and shade environments affect plant size and allocation to female and male flowers? By imposing treatments that alter plant size, our objective was to separate environmental and genetic causes of plant-size variation to assess whether size-dependent sex allocation occurs in the direction predicted by theory (Lloyd & Bawa 1984; de Jong & Klinkhamer 2005). (3) Is there evidence for genetic and environmental regulation in the temporal control of female and male function resulting in plastic dichogamy? If the marginal fitness returns for female and male allocation differ between sun and shade treatments, then the timing, duration, and sequence of female and male function should differ between environments (Zhang 2006).

Methods

STUDY SPECIES AND SAMPLING

Ambrosia artemisiifolia (Asteraceae) is native to eastern North America but has become invasive in several regions including Europe, Asia, and Australia. It grows abundantly on a variety of soil types in disturbed habitats, including arable land as a crop weed, early successional fields, roadsides, waste places and gardens. Thus populations experience a wide range of environmental contexts varying in plant density and light regimes (Bazzaz 1974; Raynal & Bazzaz 1975). Male flowers are born on terminal racemes on both the primary stem and on lateral branches, whereas female flowers occur in small clusters in the leaf and branch axils only (Payne 1963). For convenience throughout this article, we refer to the tight cluster of tiny male flowers or "composite head" as simply a flower. Individual female flowers were easier to count directly. Female flowers have a single ovule and plants are highly outcrossing with an estimated female outcrossing rate of $t = 0.95$ (Friedman & Barrett 2008). Because there is no vegetative propagation in *A. artemisiifolia* all reproduction occurs by seed.

We collected maternal seed families from an extensive population at the University of Toronto Koffler Scientific Reserve in Ontario (44°03′N, 79°29′W) in October 2006. The plants were located in several colonies occurring in abandoned fields, disturbed ground, and roadside edges. At least 30 seeds were collected from each of 80 randomly selected open-pollinated plants. Following a cold-stratification period (4°C and 24-h darkness), we germinated seeds in petri dishes at the end of May 2007 in a glasshouse at the University of Toronto under ambient sunlight.

EXPERIMENTAL DESIGN AND MEASUREMENTS

When seedlings had produced two fully opened cotyledons they were transplanted into 2.5 cm peat pots. Pots were filled with a soil mix consisting of 75% Pro-Mix, 20% sand and 5% topsoil. Plants were then placed into randomly predetermined locations in one of six blocks within either a sun or shade treatment. Two weeks later, we transplanted plants into 10 cm pots. Plants were watered as needed and treated with water-soluble fertilizer once (20:20:20 N:P:K, concentration 1.5g/L). We sprayed plants with a pesticide (Avid, Syngenta) to control for spider mites twice during the experiment.

The shade treatment was implemented by erecting three 1.2 m rectangular frames above glasshouse benches using 3.5 × 3.5 cm cedar posts, resulting in three blocks. We covered these frames with neutral-density shade cloth that reduced light levels by ~80%. The sun treatment also involved three blocks that received ambient full sunlight in the rooftop glasshouse. All blocks were in the same glasshouse. Individuals within each family were divided evenly between the two treatments (mean ± SE: 8.64 ± 0.21), and all families were represented in each of the three blocks per treatment. A total of 1523 plants were in the experiment, with 760 in the shade and 763 in the sun. The experiment ran for 118 days and was terminated when a majority of plants were fruiting and had started to senesce. The duration of plant growth was equivalent to the annual life cycle in field populations.

We recorded vegetative height, total height, and canopy width at the widest part of the plant biweekly, beginning seven days after transplanting. For each plant, we recorded the date the first female and first male flower commenced anthesis and the number of days between the start of each sex function. We counted the number of branches on each plant just prior to senescence. Four times during the experiment we counted the number of female flowers and estimated the number of male flowers on every plant by measuring the length of each male inflorescence. For a subset of 40 plants, randomly selected from each treatment and different families, we measured the length of inflorescences and counted the number of flowers and assessed the fit of a regression relating these two variables separately for each treatment. The fit of the regression was $R^2 = 0.89$ for sun plants and $R^2 = 0.87$ for shade plants. Using these regressions, we estimated the number of male flowers from the inflorescence length for each plant. Prior to senescence, we harvested all plants and weighed the aboveground dry biomass.

GENETIC AND ENVIRONMENTAL INFLUENCES ON SEX ALLOCATION

To examine if the environmental treatments affected the proportion of male flowers, we used generalized linear mixed models (GLMM: implemented in SAS PROC GLIMMIX; 9.1; SAS Inst. 2002). GLMM is a pseudo-likelihood statistical procedure specifically designed to analyze mixed model data with categorical or binomial response variables (Wolfinger & O'Connell 1993). The data were fit to a binomial distribution with a logit-link function, with treatment and block nested within treatment as fixed factors and family and family × treatment as random factors.

We determined the least-squares means and examined the effect of treatment on each measure of plant size (number of branches, dry biomass, vegetative height, and canopy width) separately using a mixed model analysis (SAS PROC MIXED). The models included treatment and block nested within treatment as fixed effects and family and family × treatment as random effects. We also investigated the relation between measurements of plant size (number of branches, dry biomass, vegetative height, and canopy width) and the number of female and male flowers by calculating phenotypic correlations among the traits for plants in each treatment. We used vegetative height, as opposed to total height, for all analyses because the terminal male inflorescence can often make up a large portion of total height.

We used a mixed model analysis (SAS PROC MIXED; 9.1; SAS Inst. 2002) to determine the effects of size on the number of female and male flowers. The full model included the effect of vegetative height as a covariate. We used restricted maximum likelihood (REML) to estimate the variance components of random effects involving family, and their significance was calculated from hierarchical log-likelihood ratio tests (Saxton 2004). We fitted a single model that included both levels of the trait (female and male flowers) as response variables, and allowed among-family and within-family variances and covariances to differ between treatments. We estimated genetic variance as four times the family variance component, and calculated the broad-sense heritability (H^2) as the estimated genetic variance divided by the total phenotypic variance (Lynch & Walsh 1998). We tested whether genetic variance of flower production (V_G) was significantly greater than zero by likelihood ratio tests and whether COV_A and r_A of female and male flower production differed significantly from zero by constraining them in the model and using a likelihood ratio test with one degree of freedom. To calculate the size-adjusted variance components, we used the residuals from a regression analysis of female and male flowers on vegetative height.

The estimates of genetic variance are based on seed from field-collected maternal families and therefore maternal environmental effects could inflate the estimates. To assess this possibility, we measured the size (length in millimeter) of a random subsample of eight seeds from each family prior to germination. The mean seed size across families ranged from 2.9 to 4 mm. For each treatment, we calculated correlation coefficients using family means between seed size and height one-week postgermination, final height, flowering date, number of branches, and final number of female and male flowers. In both treatments there was a significant positive correlation between seed size and height one week postgermination (shade: $r = 0.38$, $P < 0.05$; sun $r = 0.23$, $P < 0.05$). However, there were no significant correlations with any of the variables measured later in plant growth. This suggests that maternal environment is unlikely to strongly influence measures of sex allocation or its interactions with size and treatment.

DICHOGAMY AND TEMPORAL CHANGES IN SEX ALLOCATION

We used a random regression mixed model analysis in SAS PROC MIXED to investigate the production of female and male flowers in each treatment over time. We fit individual intercepts and slopes for each family and used a repeated measures design. Because the data did not show a linear increase in flower production over time, we included both the linear and quadratic effects of time.

To investigate the effect of treatment and family on the type and expression of dichogamy, we calculated the difference in day of opening of the first female and first male flower for each plant. A score of zero indicates no dichogamy (i.e., female and male flowers opened on the same day for a particular plant) whereas negative numbers indicate that plants were protogynous (female first), and positive numbers indicate that plants were protandrous (male first). The number itself represents the number of days separating sex functions. We then used a mixed model analysis in SAS PROC MIXED to determine the effect of treatment, block, family, and family × treatment on dichogamy scores. We used REML to estimate the variance components of random effects (those involving family), and their significance was calculated from log-likelihood ratio tests. We fitted a model that allowed among-family and within-family variances to differ between treatments. We estimated genetic variance as four times the family variance component and calculated the broad-sense heritability as the genetic variance divided by the total phenotypic variance.

To determine whether the degree of dichogamy (number of days between alternate sex functions) differed between protogynous and protandrous plants in each treatment, we classified plants as protogynous or protandrous and then examined the effect of this classification on the degree of dichogamy. We used a mixed model analysis in SAS with the absolute number of days separating sex functions as the independent variable and determined the effects of treatment, dichogamy class and their interaction. We used estimate statements to interpret the causes of significant interactions.

Results

VARIATION IN SEX ALLOCATION AND INDICES OF PLANT SIZE

Phenotypic correlations among indices of plant size indicated that they were all positively correlated with one another, and positively correlated with allocation to female and male flowers in both treatments (Table 1). All measures of plant size differed significantly between the two treatments (Table 2), with greater allocation to female flowers, male flowers, dry biomass, width, and branching in the sun treatment. Only final vegetative height was greater in the shade treatment.

THE EFFECT OF TREATMENT AND FAMILY ON SEX ALLOCATION

Overall, plants in the sun produced more female and male flowers than plants in the shade (Table 2). The proportion of male flowers differed significantly between the treatments ($F_{1,1257} = 636.31$, $P < 0.0001$), with plants in the sun making a larger fraction of male flowers than plants in the shade (proportion male flowers shade: mean = 0.48, SE = 0.02; sun: mean = 0.61, SE = 0.02, adjusted for other factors in the model).

We found a significant effect of treatment, family, and a family by treatment interaction in the allocation to female and male flowers. There was more variation among family means in female allocation in the shade treatment. In contrast, in the sun treatment this pattern was reversed with a greater amount of variation in male allocation (Fig. 1). There was also a significant interaction between family and treatment, but only for allocation to female flowers (Fig. 2). Families that had relatively higher allocation to female flowers in the shade treatment had relatively lower allocation to female flowers in the sun treatment and vice versa. Allocation to female and male flowers increased with vegetative height in both the sun and shade treatments (Table 3). In both the shade and sun treatments male flower production increased significantly faster than female flower production with vegetative height (shade: female vs. male $t_{665} = 3.57$, $P < 0.001$; sun: female vs. male $t_{669} = 8.52$, $P < 0.0001$; Fig. 3).

HERITABILITY AND GENETIC CORRELATIONS

There was significant genetic variation for female and male allocation in both treatments (Table 4; shade female: $\chi^2 = 37.8$, $P < 0.0001$; shade male: $\chi^2 = 23.4$, $P < 0.0001$; sun female $\chi^2 = 5.8$, $P < 0.05$; sun male $\chi^2 = 46.1$, $P < 0.0001$). Broad-sense estimates of heritability for allocation to female and male flowers were similar in the shade treatment (Table 4; $H^2 = 0.59$ and 0.45, respectively); however, in the sun treatment heritability was higher for male allocation ($H^2 = 0.63$; Table 4) and lower for female allocation ($H^2 = 0.21$; Table 4). We also found a positive genetic correlation between allocation to female and male flowers in both the sun and shade treatments ($r_G = 0.36$ and 0.45, respectively; Table 4). The genetic correlation in the shade treatment was significantly different from zero ($\chi^2 = 5.2$, $P < 0.05$), but the correlation in the sun was not ($\chi^2 = 2.1$, $P = 0.15$).

Heritability estimates for flower production were not greatly affected by accounting for vegetative height. Although size adjustments reduced the variation attributable to maternal family, they also reduced the residual variation (Table 4). However, controlling for plant size drastically changed the genetic correlations between female and male flower production in both the sun and shade treatments and removed the positive genetic correlations. In the shade treatment, the size-adjusted genetic correlation was −0.04 and in the sun treatment it was −0.30 (Table 4). Neither of these measures are significantly different from zero based on likelihood ratio tests.

FLOWER PRODUCTION AND CHANGES TO SEX ALLOCATION OVER TIME

The number of female and male flowers produced over time differed significantly between the two treatments (Table 5; Fig. 4). Plants in the shade treatment began flowering earlier and initially produced more female flowers than plants in the sun treatment. However, plants in the sun treatment accelerated female flower production toward the second half of the experiment (shade vs. sun for linear effect: $t_{808} = 32.18$, $P < 0.0001$; quadratic effect: $t_{1250} = 39.16$, $P < 0.0001$). Plants in the shade treatment also produced more male flowers early during growth, but by mid way through the growth period male flower production ceased. Plants in the sun treatment initially produced fewer male flowers, but flower production accelerated during the latter phases of growth (shade vs. sun for linear effect: $t_{954} = 27.85$, $P < 0.0001$; quadratic effect: $t_{1343} = 43.83$, $P < 0.0001$).

THE EFFECTS OF TREATMENT AND FAMILY ON DICHOGAMY

There was a significant effect of treatment on the type of dichogamy exhibited by plants ($F_{1,154} = 108.12$, $P < 0.0001$). Shade plants tended to be more protogynous with a mean = 1.25 (SE = 0.19, range 0–16) days between female and male flower anthesis. In contrast, plants in the sun were more protandrous with a mean = 1.82 (SE = 0.19, range 0–14) days between male and female anthesis. In both treatments, individuals that were protandrous, protogynous, or nondichogamous occurred (shade: proportion protandrous 0.23, protogynous 0.48, nondichogamous 0.29; sun: proportion protandrous 0.53, protogynous 0.16,

Table 1. Phenotypic correlations among measurements of plant size and sex allocation traits in *Ambrosia artemisiifolia* grown in sun and shade treatments in the glasshouse. Pearson correlations are shown above, and statistical significance (*P* values) are below.

	Shade						Sun					
	Dry biomass (*n*=666)	Vegetative height (*n*=668)	Branches (*n*=668)	Plant width (*n*=668)	Female flowers (*n*=670)	Male flowers (*n*=670)	Dry biomass (*n*=668)	Vegetative height (*n*=672)	Branches (*n*=674)	Plant width (*n*=673)	Female flowers (*n*=678)	Male flowers (*n*=678)
Dry biomass		0.44 0.0001	0.73 0.0001	0.61 0.0001	0.76 0.0001	0.74 0.0001		0.26 0.0001	0.47 0.0001	0.56 0.0001	0.46 0.0001	0.51 0.0001
Vegetative height			0.34 0.0001	0.23 0.0001	0.35 0.0001	0.31 0.0001			0.18 0.0001	0.05 0.166	0.16 0.0001	0.03 0.461
Branches				0.51 0.0001	0.66 0.0001	0.65 0.0001				0.31 0.0001	0.34 0.0001	0.48 0.0001
Plant width					0.46 0.0001	0.55 0.0001					0.18 0.0001	0.43 0.0001
Female flowers						0.57 0.0001						0.25 0.0001

Table 2. Least-squares means (SE) and test-statistics for treatment effects for seven traits in *Ambrosia artemisiifolia* grown in shade or sun treatments in the glasshouse. The values for dichogamy represent the mean number of days between the day of first male anthesis and the day of first female anthesis (i.e., negative values indicate protogyny, positive values indicate protandry). Values for female flowers, male flowers, number of branches, dry biomass, and vegetative height were recorded just prior to senescence. Values for plant width were recorded 2 weeks prior to senescence.

Trait	Shade	Sun	*F*-ratio	*P*-value
Dichogamy (days)	−1.25 (0.19)	1.82 (0.19)	$F_{1,154}$=99.57	<0.0001
Female flowers (*n*)	167.61 (4.02)	247.53 (3.74)	$F_{1,154}$=211.24	<0.0001
Male flowers (*n*)	279.66 (6.45)	462.19 (11.27)	$F_{1,154}$=197.40	<0.0001
Branches (*n*)	9.49 (0.28)	15.44 (0.20)	$F_{1,154}$=295.14	<0.0001
Dry biomass (g)	1.11 (0.03)	2.77 (0.04)	$F_{1,154}$=1165.26	<0.0001
Vegetative height (cm)	29.51 (0.54)	19.27 (0.41)	$F_{1,154}$=231.42	<0.0001
Width (cm)	18.32 (0.21)	19.15 (0.32)	$F_{1,154}$=4.66	<0.05

nondichogamous 0.31). Shade plants flowered earlier than sun plants, regardless of their type of dichogamy ($F_{1,154} = 92.95$, $P < 0.0001$), with the mean day of first flowering 63.55 (SE = 0.40) days postgermination, whereas the mean day of first flowering for sun plants was 69.13 (SE = 0.42) postgermination. The degree of dichogamy (number of days separating the sex functions) was affected by treatment ($F_{1,154} = 65.18$, $P < 0.0001$), and there was a significant interaction between the type of dichogamy and treatment ($F_{1,878} = 12.96$, $P < 0.001$). In each treatment, the prevalent type of dichogamy (protogyny in the shade, protandry in the sun) had a greater number of days between sex functions than the less common type.

There was a significant effect of family on dichogamy ($\chi^2 = 15.9$, $P < 0.0001$), but no significant family by treatment interaction ($\chi^2 = 3.4$, $P > 0.05$). We detected significant genetic variation for dichogamy in both treatments (Table 6; shade $\chi^2 = 52$, $P < 0.0001$; sun $\chi^2 = 29.4$, $P < 0.0001$) with families that were

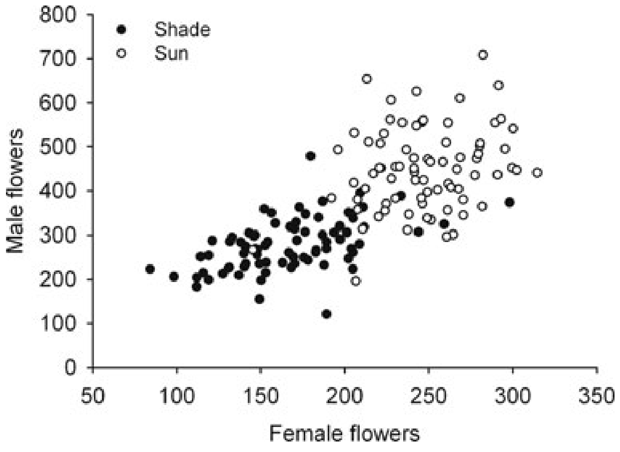

Figure 1. Relation between family means for standardized female allocation and standardized male allocation in shade (•) and sun (◦) treatments in *Ambrosia artemisiifolia* in the glasshouse.

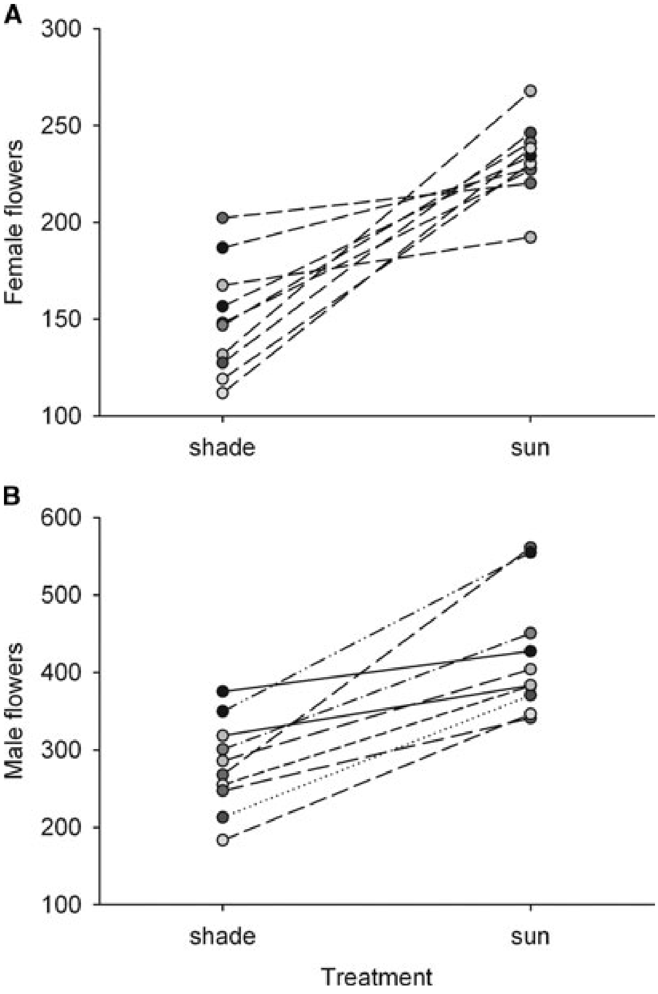

Figure 2. Relation between family means for 10 random families for (A) female and (B) male allocation across the two treatments (shade and sun) in *Ambrosia artemisiifolia* in the glasshouse. The effect of family × treatment is only significant for female allocation.

Table 3. **Significance tests for general linear models of flower production of *Ambrosia artemisiifolia* plants in sun and shade treatments in the glasshouse, with vegetative height as a covariate. See Figure 3 for depiction of fixed-effect three-way interaction.**

Source of variation	Test statistic
Block (treatment)	$F_{4,1277}=26.31^{***}$
Treatment	$F_{1,1184}=116.87^{***}$
Vegetative height	$F_{1,1353}=57.48^{***}$
Family	$\chi^2=2.3$
Flower sex	$F_{1,917}=69.42^{***}$
Family × treat	$\chi^2=0.7$
Family × flower sex	$\chi^2=6.7^{**}$
Treatment × flower sex	$F_{1,1145}=29.40^{***}$
Treatment × vegetative height	$F_{1,1261}=4.02^{*}$
Flower sex × vegetative height	$F_{1,1340}=1.49$
Family × treat × vegetative height	$\chi^2=7.1^{**}$
Treatment × flower sex × vegetative height	$F_{1,1270}=4.35^{*}$

$^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.0001$

most strongly protandrous in the sun the least protogynous in the shade and vice versa (Fig. 5). The heritability estimates for dichogamy in the shade and sun treatments were 0.45 and 0.34, respectively (Table 6).

Discussion

The main findings of our study are the demonstration of genetic and environmental control of sex allocation in *A. artemisiifolia,* including adjustments in the allocation to female and male function over time and in individuals of different sizes. As predicted, based on habitat effects on pollen dispersal, plants grown in the shade allocated relatively more resources to female flowers, whereas plants in the sun produced proportionately more male flowers. Significantly, we found a reversal in the magnitude of genetic variation for female and male allocation between sun and shade treatments. In the shade, there was significantly more genetic variation in female than male flower production, although this relation was reversed in the sun (Fig. 1). As predicted by theories on direct size-dependent sex allocation, we found a significant effect of plant size, with the strongest effect on male allocation in the sun treatment (Fig. 3). Finally, we detected significant genetic variation for dichogamy and a plastic response of this trait to environment. Plants in the shade tended to produce female flowers first in contrast to plants grown in the sun, which produced male flowers first (Fig. 5). To our knowledge, our study is the first to empirically demonstrate the quantitative relations between genetic and environmental components of temporal sex allocation and to show that dichogamy can be a plastic trait under genetic control. We now consider the ecological and evolutionary consequences of environmental and size-dependent influences

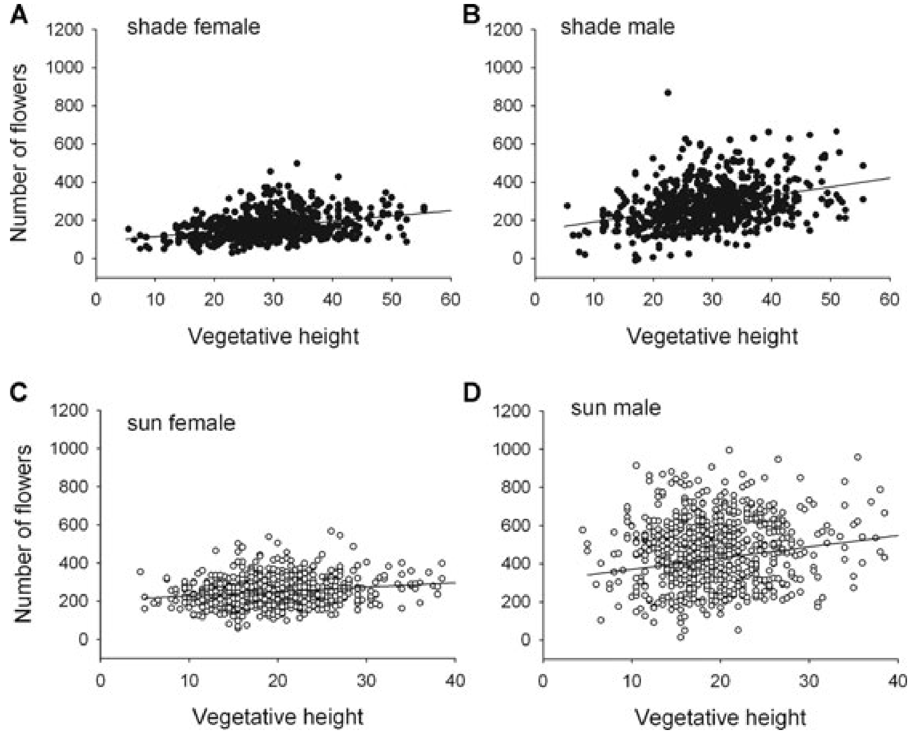

Figure 3. **The relations of female and male flower production to the size of plants in sun and shade treatments in *Ambrosia artemisiifolia* in the glasshouse. The lines illustrate the regression predictions based on vegetative height. Shade – Female $y = 87.19 + 2.71x$. Shade – Male: $y = 144.72 + 4.61x$. Sun – Female: $y = 205.22 + 2.29x$. Sun – Male: $y = 311.04 + 5.90x$. See Table 3 for statistical details.**

Table 4. **Genetic variance (V_G), environmental variance (V_E), broad-sense estimates of heritability (H^2), genetic covariance (COV_G), and maternal family genetic correlation (r_G) for measured and size-adjusted (parameter followed by $_{(adj)}$) allocation to female and male flowers in sun and shade treatments for *Ambrosia artemisiifolia* in the glasshouse. Standard errors are in parentheses and are calculated from the asymptotic covariance matrix. Asterisks represent values that are significantly different from zero, based on likelihood-ratio tests.**

	Shade		Sun	
	Female	Male	Female	Male
V_G	0.42 (0.12)***	0.19 (0.06)***	0.16 (0.08)*	0.64 (0.17)***
$V_{G(adj)}$	0.24 (0.07)***	0.11 (0.04)***	0.05 (0.04)^{NS}	0.45 (0.12)***
V_E	0.29	0.23	0.62	0.37
$V_{E(adj)}$	0.17	0.24	0.65	0.34
H^2	0.59 (0.07)***	0.45 (0.04)***	0.21 (0.04)*	0.63 (0.10)***
$H^2_{\ (adj)}$	0.58 (0.06)***	0.42 (0.03)***	0.07 (0.04)^{NS}	0.57 (0.11)***
COV_G	0.13 (0.06)*		0.11 (0.08)^{NS}	
$COV_{G(adj)}$	−0.01 (0.03)^{NS}		−0.04 (0.06)^{NS}	
r_G	0.45 (0.16)*		0.36 (0.24)^{NS}	
$r_{G(adj)}$	−0.04 (0.19)^{NS}		−0.30 (0.26)^{NS}	

$^{*}P<0.05$, ** $P<0.01$,*** $P<0.0001$

on sex allocation, and discuss how the presence of quantitative genetic variation for gender allocation may allow adaptive adjustments in the timing of allocation to sex function in hermaphroditic plants.

ENVIRONMENT-DEPENDENT SEX ALLOCATION

We have demonstrated that sex allocation in *A. artemisiifolia* is dependent on environmental conditions and can be adjusted plastically. The finding of relatively higher male flower production in the sun treatment supports predictions based on the optimal sex allocation for wind-pollinated plants growing in open environments. Based on aerodynamic arguments, in open habitats plants emphasizing male function should experience greater returns resulting from proficient pollen dispersal. This allocation pattern contrasts with studies of animal-pollinated species that have most often reported that shaded plants produce more male flowers whereas sun plants produce more female flowers (e.g., Gregg 1975; Bertin 1982; Solomon 1985; reviewed in Korpelainen 1998), presumably due to the greater resource costs of female function. A second explanation may also account for the increase in male flower production in the sun despite the greater resources available. Male allocation may not be as inexpensive relative to female investment as is often assumed, particularly in terms of nitrogen and phosphorous requirements (Bazzaz & Carlson 1979; Harris & Pannell 2008). Such costs could be especially important for a wind-pollinated species that invests heavily in pollen production such as *A. artemisiifolia* in which the pollen:ovule ratio can be as high as 60000:1 (Payne 1963), but where overall flower costs are low because plants have very small flowers and lack nectar and showy petals. Thus, the particular requirements of wind pollination should be considered when considering the expected patterns of environmentally induced changes in sex allocation.

Table 5. **Summary of REML general linear mixed-models of the influence of time on the number of female and male flowers produced by families of *Ambrosia artemisiifolia* in sun and shade treatments in the glasshouse.**

Response variable	Source of variation	Test statistic
Time – female flowers	Block (treatment)	$F_{4,668}=3.80^{*}$
	Treatment	$F_{2,158}=145.96^{***}$
	Treatment×time	$F_{2,315}=779.24^{***}$
	Treatment×time^2	$F_{2,673}=2931.53^{***}$
Time – male flowers	Block (treatment)	$F_{4,650}=24.48^{***}$
	Treatment	$F_{2,229}=80.40^{***}$
	Treatment×time	$F_{2,427}=389.88^{***}$
	Treatment×time^2	$F_{2,673}=1074.00^{***}$

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.0001$

GENETIC COMPONENTS OF SEX ALLOCATION

We detected significant genetic variation among plants in the production of female and male flowers in both the sun and shade treatments. After we adjusted for size differences among maternal families, significant genetic variation persisted for all traits except for female allocation in plants grown in the sun. However, size-adjustments reduced the variation attributable to maternal family. Significantly, we found a reversal in the magnitude of variation for female and male allocation between sun and shade treatments. In

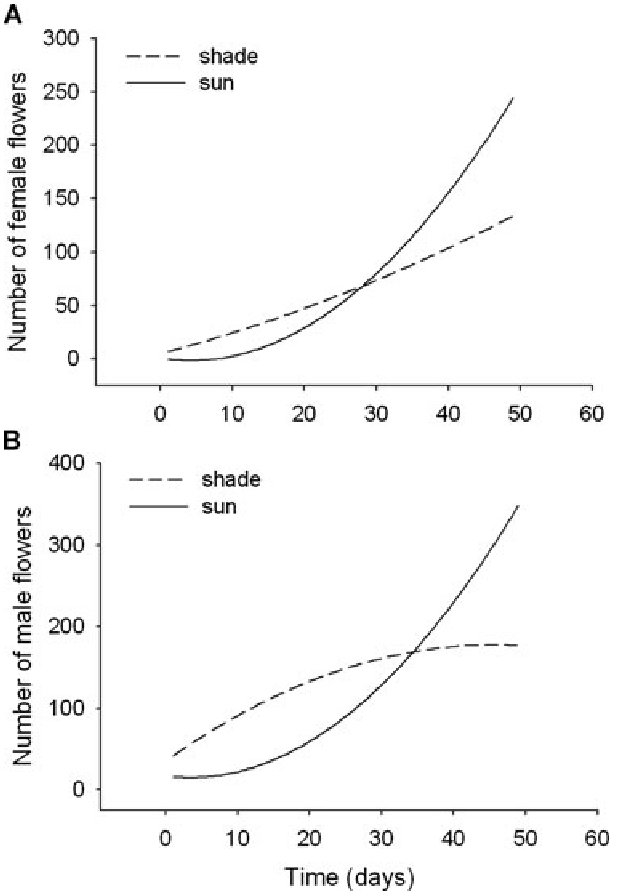

Figure 4. Flower production over time for a) female flowers (F) and b) male flowers (M) in sun and shade treatments in *Ambrosia artemisiifolia* in the glasshouse. The fitted lines depict quadratic regressions based on a repeated-measures random regression analysis for four sampling times (A) shade: $F = 5.07 + 1.73t + 0.02t^2$; sun: $F = 0.76 - 1.04t + 0.12t^2$; (B) shade: $M = 32.34 + 6.21t - 0.07t^2$; sun: $M = 16.28 - 1.08t + 0.16t^2$.

the shade, there was greater variation in female flower production than in male flower production, while this relation was reversed in the sun (Fig. 1). This finding indicates that the capacity of genotypes to adjust their allocation of resources to female versus male flower production depends on environmental conditions, and suggests that the lability of sex allocation is driven by opposite responses of the sex functions in the two environments. This result is consistent with adaptive arguments (i.e., plants in the sun are more variable in male allocation, plants in the shade are more variable in female allocation), and indicates that there is ample genetic variation to allow divergent selection on sex allocation in the two environments.

Genotypes of *A. artemisiifolia* responded differently in their allocation to female and male flower production in sun versus shade environments. We found a significant maternal family-by-environment interaction for female flower production, but not for male flower production. All families made fewer male flowers in the shade and the degree of adjustment was similar across families. However, genetic variation in phenotypic plasticity was evident in the allocation to female flowers in the two treatments (Fig. 2A). A significant G × E for sex allocation in the two environments resulted from differences in the relative investment to female flowers among families. Few studies have examined the genetic and environmental components to sex allocation (but see Mazer & Schick 1991; Dorken & Barrett 2004). Given the important role that environmental factors play in governing gender variation in monoecious species (Freeman et al. 1981; Bierzychudek 1982; Sarkissian et al. 2001; Dorken & Barrett 2003), establishing a genetic component to variation in sex allocation is important. Our results suggest that the phenotypic expression of genetic variation and consequently the response to selection will depend on habitat conditions.

If the phenotypes favored by natural selection are the same across environments, genotype × environment interactions can slow the response to selection, because the selected phenotypes are produced by different genotypes in each environment (Via & Lande 1985). However, in the case of *A. artemisiifolia* growing in sun and shade habitats, we expect the fittest phenotypes to differ between environments. In this way, genetic variation in phenotypic plasticity may actually accelerate the response to selection. If gender modification is to respond appropriately to changing environmental conditions, a population must not only have heritable genetic variation for gender expression but also heritable variation in the plasticity of allocation (Goldman & Willson 1986; Mazer

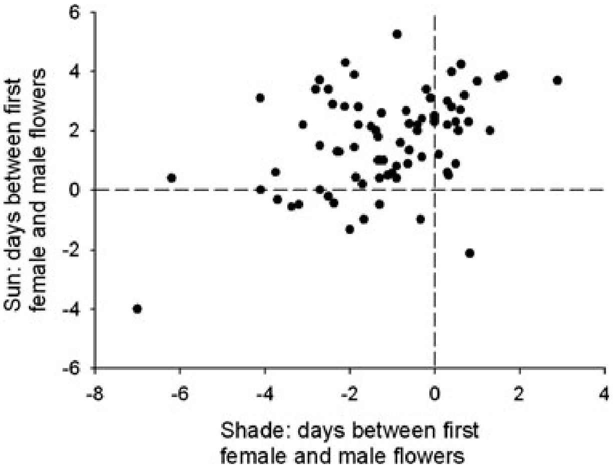

Figure 5. Family means of number of days between first anthesis of each sex function in sun and shade treatments in the glasshouse. The dotted lines indicate no dichogamy, values to the left or below the lines indicate protogyny, and values to the right or above the lines indicate protandry. Families differed significantly in their degree of dichogamy within each treatment. There was a consistent relation among families in the degree of dichogamy across the two treatments (i.e., no significant Family × Treatment interaction).

Table 6. Genetic variance (V_G), environmental variance (V_E), broad-sense estimates of heritability (H^2) for dichogamy (number of days separating anthesis in first female and male flowers) in each treatment for *Ambrosia artemisiifolia* in the glasshouse. Standard errors are calculated from the asymptotic covariance matrix. Asterisks represent values that are significantly different from zero, based on likelihood-ratio tests.

	Shade	Sun
V_G	7.69 (1.91)***	5.59 (1.66)***
V_E	3.80	6.61
H^2	0.45 (0.08)***	0.34 (0.06)***

$^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.0001$

1992; Vogler et al. 1999). Our results demonstrating significant heritability of plasticity in allocation in *A. artemsiisifolia* indicate that there is the potential for adaptive evolutionary changes to sex allocation in different environments.

EFFECTS OF SIZE ON SEX ALLOCATION

Size-dependent sex allocation in *A. artemisiifolia* is contingent on environmental conditions. In both experimental treatments, relatively larger plants of *A. artemisiifolia* produced more female and male flowers (i.e., slope: $b > 1.0$). However, investment into male flowers increased with size more rapidly than did female investment (Fig. 3). These results differ from those for animal-pollinated species, which generally show greater investment into female allocation with increased plant size (e.g., Wright & Barrett 1999; Ashman et al. 2001; Mazer & Dawson 2001; Sarkissian et al. 2001; Cao & Kudo 2008). Previous studies of wind-pollinated plants have relied on a correlative approach involving the detection of phenotypic correlations between plant size and sex allocation, thus confounding genetic and environmental sources of variation (but see Koelewijn & Hunscheid 2000). By manipulating the growth environment and replicating families across environments, we have demonstrated genetic variation in the ability of plants to adjust their sex allocation in response to size separate from any environmentally induced changes to allocation.

The observed patterns of gender variation revealed by this study are those expected for size-dependent sex allocation models that predict greater male investment in wind-pollinated plants with increasing size (Sakai & Sakai 2003). Pannell (1997) observed increased female allocation in wind-pollinated monoecious *Mercurialis annua* under high densities. He attributed this result to the smaller stature of plants making them less suited for effective pollen dispersal than those growing at low density. Interestingly, we found that although sun plants were significantly shorter than shade plants (but larger in every other index of size—Table 2), their pattern of size-dependent sex allocation was consistent with the direct benefits that size would give to pollen dispersal. Size-dependent sex allocation models have seldom considered the distinction between height and other indices of size (but see Bickel & Freeman 1993), although the measures need not have similar consequences for resource provisioning, pollination, mating, or seed dispersal. Wind-pollinated species may incur size-dependent benefits through both direct effects of increased pollen dispersal and indirect effects whereby larger plants have more resources available for reproduction (Lloyd & Bawa 1984; de Jong & Klinkhamer 1994; Zhang 2006). The gradual changes in sex allocation that we have demonstrated (Fig. 4) suggest the existence of not only direct effects of size-dependent sex allocation but also nonlinear gain curves for either female or male (or both) sex functions due to the indirect effects of plant size.

TEMPORAL CHANGES IN ALLOCATION AND FLEXIBLE DICHOGAMY

Despite the widespread occurrence of dichogamy in the flowering plants (Lloyd & Webb 1986) there is relatively little theoretical work on this floral strategy (but see Lloyd & Yates 1982; Burd & Head 1992; Sargent et al. 2006), especially consideration of when plants should be protogynous versus protandrous. Comparative evidence suggests that abiotically pollinated species are more likely to be protogynous, whereas biotically pollinated species are more likely to be protandrous (Sargent & Otto 2004). Also, protogyny is commonly associated with self-compatibility and protandry with self-incompatibility (Bertin 1993; Routley et al. 2004). The expression of dichogamy is seldom considered in the context of sex allocation theory and is usually treated as a fixed constraint (but see Burd & Head 1992; Zhang 2006). This may be, in part, because most plant species are either protandrous or protogynous with the exception of the small number of species that are polymorphic for the two conditions (heterodichogamy—see Gleeson 1982; Renner 2001; Pannell & Verdú 2006; Bai et al. 2007). In the literature, *A. artemisiifolia* is reported as protandrous but the results of this study have revealed a more complex situation involving plasticity in dichogamy. Plants were more often protogynous in the shade and protandrous in the sun, although some plants expressed the alternative condition in both environments. Significantly, we also found genetic variation for the expression of dichogamy in both environmental treatments (Table 6; Fig. 5).

The flexible pattern of dichogamy that we observed in *A. artemisiifolia* appears to be unreported in the plant literature and does not fit into the types of dichogamy typically described (reviewed in Lloyd & Webb 1986). Because the overlap between sex functions is large it is unlikely that this condition promotes disassortative mating between protandrous and protogynous morphs, as demonstrated for heterodichogamy (Bai et al. 2007). Flexible dichogamy is also unlikely to be an antiselfing mechanism, preventing transfer of pollen between flowers on the same plant, as *A. artemisiifolia* is strongly self-incompatible (Friedman &

Barrett 2008). The extensive overlap in sex expression evident within most plants and the relatively short duration in which they function exclusively as either a female or a male suggests that flexible dichogamy is unlikely to reduce the amount of geitonogamous pollen captured by stigmas to any significant degree.

Dichogamy in *A. artemisiifolia* may not function as a floral strategy per se but rather is best interpreted as part of a continuum in the temporal control of sex allocation. All size-dependent sex allocation models assume that plants can accurately assess their local conditions before they influence reproductive success (Lloyd & Bawa 1984). However, if a plant cannot accurately assess the total amount of resources available for reproduction, then resources at any time should be allocated to the sex that will yield the higher fitness return. In one of the first studies to link temporal displacement of sex functions to sex allocation theory, Zhang (2006) proposed that the duration of overlap in sex functions, and the timing of each sex function, will depend on the shapes and magnitudes of the fitness gain curves. Changes between female and male investment should occur when fitness returns are not equal per unit of investment in each sex function. If the gain curve for one sex function is linear but the other sex has a concave function, then the transition between them should be abrupt. If both sexes have saturating gain curves then the transition should be gradual as we observed, and depend on the relative rates of saturation. Although we expect that in *A. artemisiifolia* the male gain curve will be linear or nearly so, the lack of a sharp transition between sex functions may be explained because male flowers are photosynthetic and contribute to available resources (Bazzaz & Carlson 1979; Burd & Head 1992) and/or if the duration of staminate flowering is selected to optimize pollination opportunities. In the shade environment, allocation to female function increased more slowly over time than in the sun environment (Fig. 4A) and allocation to male function saturated (Fig 4B). Both the slower accumulation of female flowers and the saturation of male flowers in the shade may reflect reduced photosynthetic resources in this environment.

CONCLUSIONS

A key feature of fitness gain curves is that they are context dependent, varying between environments and sometimes between individuals of different resource status (Charnov 1982). Our experiment has shown that plants adjust their sex allocation in response to size and also to the contrasting environmental conditions that they commonly experience under field conditions. Given the aerodynamic requirements for successful wind pollination, plastic sex allocation allows *A. artemisiifolia* to adjust allocation for effective pollen dispersal. Our demonstration of flexible dichogamy highlights the need to integrate models of sex allocation with research on dichogamy. In some cases, dichogamy may be better viewed as a simple outcome of more general temporal changes in sex allocation rather than soley as a floral adaptation associated with pollination and mating.

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LITERATURE CITED

Ackerly, D. D., and M. Jasienski. 1990. Size-dependent variation of gender in high-density stands of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae). Oecologia 82:474–477.

Aizen, M. A., and A. Kenigsten. 1990. Floral sex ratios in scrub oak (*Quercus ilicifolia*) vary with microtopography and stem height. Can. J. Bot. 68:1364–1368.

Ashman, T.-L., and C. J. Majetic. 2006. Genetic constraints on floral evolution: a review and evaluation of patterns. Heredity 96:343–352.

Ashman, T.-L., J. Pacyna, C. Diefenderfer, and T. Leftwich. 2001. Size-dependent sex allocation in a gynodioecious wild strawberry: the effects of sex morph and inflorescence architecture. Int. J. Plant Sci. 162:327–334.

Bai, W. N., Y. F. Zeng, and D.-Y. Zhang. 2007. Mating patterns and pollen dispersal in a heterodichogamous tree, *Juglans mandshurica* (Juglandaceae). New Phytol. 176:699–707.

Bazzaz, F. A. 1974. Ecophysiology of *Ambrosia artemisiifolia*—a successional dominant. Ecology 55:112–119.

———. 1997. Allocation of resources in plants: state of the science and critical questions. Pp. 1–38 *in* F. A. Bazzaz and J. Grace, eds. Plant resource allocation. Academic Press, San Diego, CA.

Bazzaz, F. A., and R. W. Carlson. 1979. Photosynthetic contribution of flowers and seeds to reproductive effort of an annual colonizer. New Phytol. 82:223–232.

Bertin, R. I. 1982. The ecology of sex expression in red buckeye. Ecology 63:445–456.

———. 1993. Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. Am. J. Bot. 80:557–560.

Bickel, A. M., and D. C. Freeman. 1993. Effects of pollen vector and plant geometry on floral sex ratio in monoecious plants. Am. Midl. Nat. 130:239–247.

Bierzychudek, P. 1982. The demography of jack-in-the-pulpit, a forest perennial that changes sex. Ecol. Monogr. 52:335–351.

Bishop, E. J., R. B. Spigler, and T.-L. Ashman. 2010. Sex-allocation plasticity in hermaphrodites of sexually dimorphic *Fragaria virginiana* (Rosaceae). Botany 88:231–240.

Burd, M., and T. F. H. Allen. 1988. Sexual allocation strategy in wind-pollinated plants. Evolution 42:403–407.

Burd, M., and G. Head. 1992. Phenological aspects of male and female function in hermaphroditic plants. Am. Nat. 140:305–324.

Cao, G.-X., and G. Kudo. 2008. Size-dependent sex allocation in a monocarpic perennial herb, *Cardiocrinum cordatum* (Liliaceae). Plant Ecol. 194:99–107.

Caruso, C. M. 2006. Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. Am. J. Bot. 93:531–538.

Charlesworth, D., and B. Charlesworth. 1981. Allocation of resources to male and female functions in hermaphrodites. Biol. J. Linn. Soc. 15:57–74.

———. 1987. The effect of investment in attractive structures on allocation to male and female functions in plants. Evolution 41:948–968.

Charlesworth, D., and M. T. Morgan. 1991. Allocation of resources to sex functions in flowering plants. Philos. Trans. R. Soc. Lond. B 332:91–102.

Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. Proc. Natl. Acad. Sci. USA 76:2480–2484.

———. 1982. The theory of sex allocation. Princeton Univ. Press, Princeton, NJ.

Conner, J. K., R. Franks, and C. Stewart. 2003. Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. Evolution 57:487–495.

Dajoz, I., and M. Sandmeier. 1997. Plant size effects on allocation to male and female functions in pearl millet, a hermaphroditic wind-pollinated species. Can. J. Bot. 75:228–235.

de Jong, T. J., and P. G. L. Klinkhamer. 1989. Size-dependency of sex-allocation in hermaphroditic, monocarpic plants. Funct. Ecol. 3:201–206.

———. 2005. Evolutionary ecology of plant reproductive strategies. Cambridge Univ. Press, Cambridge.

Dorken, M. E., and S. C. H. Barrett. 2003. Gender plasticity in *Sagittaria sagittifolia* (Alismataceae), a monoecious species. Plant. Syst. Evol. 237:99–106.

———. 2004. Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. J. Ecol. 92:32–44.

Dudley, S. A., and J. Schmitt. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. Am. Nat. 147:445–465.

Fox, J. F. 1993. Size and sex allocation in monoecious woody plants. Oecologia 94:110–113.

Freeman, D. C., E. D. McArthur, K. T. Harper, and A. C. Blauer. 1981. Influence of environment on the floral sex ratio of monoecious plants. Evolution 35:194–197.

Friedman, J., and S. C. H. Barrett. 2008. High outcrossing in the annual colonizing species *Ambrosia artemisiifolia* (Asteraceae). Ann. Bot. 101:1303–1309.

Gleeson, S. K. 1982. Heterodichogamy in walnuts: inheritance and stable ratios. Evolution 36:892–902.

Goldman, D. A., and M. F. Willson. 1986. Sex allocation in functionally hermaphrodite plants: a review and critique. Bot. Rev. 2:157–194.

Gregg, K. B. 1975. The effect of light intensity on sex expression in species of *Cycnoches* and *Catasetum* (Orchidaceae). Selbyana 1:101–113.

Harris, M. S., and J. R. Pannell. 2008. Roots, shoots and reproduction: sexual dimorphism in size and costs of reproductive allocation in an annual herb. Proc. R. Soc. Lond. B 275:2595–2602.

Jones, K. L. 1936. Studies on *Ambrosia*, I. The inheritance of floral types in the ragweed, *Ambrosia elatior* L. Am. Midl. Nat. 17:673–699.

Klinkhamer, P. G. L., T. J. de Jong, and H. Metz. 1997. Sex and size in cosexual plants. Trends Ecol. Evol. 12:260–265.

Koelewijn, H. P., and M. P. H. Hunscheid. 2000. Intraspecific variation in sex allocation in hermaphroditic *P. coronopus* (L.) J. Evol. Biol. 13:302–315.

Korpelainen, H. 1998. Labile sex expression in plants. Biol. Rev. 73:157–180.

Lloyd, D. G., and K. S. Bawa. 1984. Modification of the gender of seed plants in varying conditions. Evol. Biol. 17:225–388.

Lloyd, D. G., and C. J. Webb. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. N. Z. J. Bot. 24:135–162.

Lloyd, D. G., and J. M. A. Yates. 1982. Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). Evolution 36:903–913.

Lundholm, J. T., and L. W. Aarssen. 1994. Neighbor effects on gender variation in *Ambrosia artemisiifolia*. Can. J. Bot. 72:794–800.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Mazer, S. J. 1992. Environmental and genetic sources of variation in floral traits and phenotypic gender in wild radish: consequences for natural selection. Pp. 281–325 *in* R. Wyatt, ed. Ecology and evolution of plant reproduction. Chapman and Hall, New York.

Mazer, S. J., and C. T. Schick. 1991. Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. II. Effects of planting density on phenotype and heritability estimates. Evolution 45:1888–1907.

Mazer, S. J., and K. A. Dawson. 2001. Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): ontogenetic and among-plant variation. Am. J. Bot. 88:819–831.

Mazer, S. J., D. E. Lowry, and T. Hansen. 2003. Effects of nutrient availability on primary sexual traits and their response to selection in *Spergularia marina* (Caryophyllaceae). J. Evol. Biol. 16:767–778.

McKone, M. J., and D. W. Tonkyn. 1986. Intrapopulation gender variation in common ragweed (Asteraceae, *Ambrosia artemisiifolia* L), a monoecious, annual herb. Oecologia 70:63–67.

McKone, M. J., C. P. Lund, and J. M. O'Brien. 1998. Reproductive biology of two dominant prairie grasses (*Andropogon gerardii* and *Sorghastrum nutans*, Poaceae): male-biased sex allocation in wind-pollinated plants? Am. J. Bot. 85:776–783.

Morgan, M. T. 1992. The evolution of traits influencing male and female fertility in outcrossing plants. Am. Nat. 139:1022–1051.

Niklas, K. J. 1985. The aerodynamics of wind pollination. Bot. Rev. 51:328–386.

Pannell, J. R. 1997. Variation in sex ratios and sex allocation in androdioecious *Mercurialis annua*. J. Ecol. 85:57–69.

Pannell, J. R., and M. Verdú. 2006. The evolution of gender specialization from dimorphic hermaphroditism: paths from heterodichogamy to gynodioecy and androdioecy. Evolution 60:660–673.

Paquin, V., and L. W. Aarssen. 2004. Allometric gender allocation in *Ambrosia artemisiifolia* (Asteraceae) has adaptive plasticity. Am. J. Bot. 91:430–438.

Payne, W. W. 1963. The morphology of the inflorescence of ragweeds (*Ambrosia franseria*: Compositae). Am. J. Bot. 50:872–880.

Raynal, D. J., and F. A. Bazzaz. 1975. Interference of winter annuals with *Ambrosia artemisiifolia* in early successional fields. Ecology 56:35–49.

Renner, S. S. 2001. How common is heterodichogamy? Trends Ecol. Evol. 16:595–597.

Routley, M. B., R. I. Bertin, and B. C. Husband. 2004. Correlated evolution of dichogamy and self-incompatibility: a phylogenetic perspective. Int. J. Plant Sci. 165:983–993.

Sakai, A., and S. Sakai. 2003. Size-dependent ESS sex allocation in wind-pollinated cosexual plants: fecundity vs. stature effects. J. Theor. Biol. 222:283–295.

Sargent, R. D., and S. P. Otto. 2004. A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. Evol. Ecol. Res. 6:1183–1199.

Sargent, R. D., M. A. Mandegar, and S. P. Otto. 2006. A model of the evolution of dichogamy incorporating sex-ratio selection, anther-stigma interference, and inbreeding depression. Evolution 60:934–944.

Sarkissian, T. S., S. C. H. Barrett, and L. D. Harder. 2001. Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? Ecology 82:360–373.

Saxton, A. M. 2004. Genetic analysis of complex traits using SAS. SAS Publishing, Cary, NC.

Schärer, L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. Evolution 63:1377–1405.

Schmitt, J., S. A. Dudley, and M. Pigliucci. 1999. Manipulative approaches to testing adaptive plasticity: phytochrome-mediated shade-avoidance responses in plants. Am. Nat. 154:S43-S54.

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

———. 1989. Size-dependant sex ratios in the monoecious, wind-pollinated annual, *Xanthium strumarium*. Am. Midl. Nat. 121:209–218.

Traveset, A. 1992. Sex expression in a natural population of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae). Am. Midl. Nat. 127:309–315.

van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128:137–142.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522.

Vogler, D. W., S. Peretz, and A. G. Stephenson. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in *Campanula rapunculoides* (Campanulaceae). Am. J. Bot. 86:482–494.

Wolfinger, R., and M. O'Connell. 1993. Generalized linear mixed models: a pseudo-likelihood approach. J. Stat. Comput. Simulation 4:233–243.

Worley, A. C., D. Houle, and S. C. H. Barrett. 2003. Consequences of hierarchical allocation for the evolution of life-history traits. Am. Nat. 161:153–167.

Wright, S. I., and S. C. H. Barrett. 1999. Size-dependent gender modification in a hermaphroditic perennial herb. Proc. R. Soc. Lond B 266:225–232.

Zhang, D.-Y. 2006. Evolutionarily stable reproductive investment and sex allocation in plants. Pp. 41–60 *in* L. D. Harder, and S. C. H. Barrett, eds. Ecology and evolution of flowers. Oxford Univ. Press, Oxford.

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