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Genetic differences among populations in sexual dimorphism: evidence for selection on males in a dioecious plant

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Abstract

Genetic variation among populations in the degree of sexual dimorphism may be a consequence of selection on one or both sexes. We analysed genetic parameters from crosses involving three populations of the dioecious plant *Silene latifolia*, which exhibits sexual dimorphism in flower size, to determine whether population differentiation was a result of selection on one or both sexes. We took the novel approach of comparing the ratio of population differentiation of a quantitative trait (Q_{ST}) to that of neutral genetic markers (F_{ST}) for males vs. females. We attributed 72.6% of calyx width variation in males to differences among populations vs. only 6.9% in females. The Q_{ST}/F_{ST} ratio was 4.2 for males vs. 0.4 for females, suggesting that selection on males is responsible for differentiation among populations in calyx width and its degree of sexual dimorphism. This selection may be indirect via genetic correlations with other morphological and physiological traits.

Introduction

The extent to which populations differ from one another as a result of natural selection is a question of long-held interest to evolutionary biologists. Phenotypic divergence is necessary but not sufficient to invoke selection as the cause of differentiation, as founder effects, drift and gene flow are likely to influence trait means and variance (Lande, 1976). Approaches to this question therefore include combinations of reciprocal transplant experiments between divergent populations, estimates of contemporaneous selection on traits in divergent populations, experimental studies of selection gradients and environmental characteristics, phylogenetically based comparison studies and correlations between genes and phenotypes across clines (e.g. Clausen *et al.*, 1948; Wade & Kalisz, 1990; Zamudio, 1998; Badyaev *et al.*, 2000; Savolainen *et al.*, 2007). In addition, whether among-population variation in a trait has been caused by drift or natural selection can be evaluated by quantifying Q_{ST} and F_{ST}, where Q_{ST} estimates population differentiation of quantitative traits (Spitze, 1993) and F_{ST} estimates population differentiation of neutral genetic markers (Wright, 1951). Although this approach does not allow identification of the selective force, when Q_{ST} exceeds F_{ST}, this can be taken as evidence that the observed differences among populations for the study trait(s) are a result of selection for different phenotypes in different populations or on genetically correlated traits (Leinonen *et al.*, 2008). Such measures have been used to evaluate whether natural selection has led to local adaptation (Merila & Crnokrak, 2001), as well as the extent to which invasions are promoted by adaptive evolution (Keller & Taylor, 2008).

Here, we utilized this latter approach to ask questions about the evolution of sexual dimorphism in a trait that differs among populations in both its mean and the extent to which the sexes differ, a topic that has been addressed in a variety of taxa using some of the more traditional approaches mentioned earlier (e.g. Johnston & Selander, 1973; Zamudio, 1998; Badyaev *et al.*, 2000; Kraushaar & Blanckenhorn, 2002; Kwiatkowski & Sullivan, 2002). We were interested in comparing males and females, rather

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than obtaining an absolute comparison of Q_{ST} and F_{ST}. We chose a floral-size trait that is highly sexually dimorphic and is known to be genetically integrated with many other traits (Delph *et al.*, 2004a, 2005, 2010).

We performed a quantitative-genetic, among-population crossing experiment with the dioecious plant *Silene latifolia* to evaluate whether population differentiation was a consequence of drift or local differences in selection on males, females or both sexes. *Silene latifolia* is found throughout Europe, where it is native, and widespread in the central portion of North America, where it has been introduced and appears invasive (Taylor & Keller, 2007). Several characteristics, including its widespread occurrence, indicate that this species is ideal for investigating among-population variation and its causes (see also Taylor & Keller, 2007; Keller *et al.*, 2009). Sexual dimorphism exists for many traits in *S. latifolia* and has a genetic basis (Delph, 2007; Delph *et al.*, 2010). For example, males make numerous small flowers compared to females (Meagher, 1992; Carroll & Delph, 1996) and have higher rates of photosynthesis and respiration (Gehring & Monson, 1994; Laporte & Delph, 1996; Delph *et al.*, 2005). Moreover, several sexually dimorphic traits have been shown to vary in their means among populations, as shown with common-garden experiments (Delph *et al.*, 2002; Delph & Bell, 2008). Although population variation in the extent of sexual dimorphism is less variable (as predicted, Lande, 1980), it nevertheless occurs for some floral-size traits, including calyx width (Delph *et al.*, 2002; Delph & Bell, 2008).

We made crosses using individuals from three populations, one from North America and two from Europe. We subsequently grew and measured the offspring in a common-garden setting and estimated phenotypic and genetic variance parameters using the linear animal model of quantitative genetics (Lynch & Walsh, 1998). This allowed us to quantify the effects of sire, dam, sire × dam interactions, heritability and Q_{ST}. We took estimates of F_{ST} from published accounts and compared the Q_{ST}/F_{ST} ratio for males and females. Our results show that calyx width and the degree to which it is sexually dimorphic are genetically variable among the three study populations and that this divergence is likely to have been caused by local differences in selection on males.

Materials and methods

Population crosses and measurement of calyx width

Seeds from three populations of *S. latifolia* were grown to flowering in a greenhouse at Indiana University: VIR (from Giles County, Virginia, USA), CRC (Cabo de Roca, Portugal) and ZAG (Zagreb, Croatia). Once flowering began, crosses were made between individuals (sire × dam), including all three within-population crosses (VIR × VIR, CRC × CRC and ZAG × ZAG) and all six possible among-population crosses (VIR × CRC, CRC × VIR, VIR × ZAG, ZAG × VIR, CRC × ZAG and ZAG × CRC). Crosses were made by hand, by rubbing the anthers from several open flowers of a given sire across the styles of each dam, to saturate them with pollen. A small paper jewellers' tag was placed around the pedicel of each pollinated flower to mark the cross and the fruit was allowed to fully mature, at which time the seeds were collected. Two flowers were pollinated for each sire × dam combination.

Table 1 The number of offspring of each sex (F, female, M, male) measured for the nine types of crosses made.

Dam population	Sire population: VIR F	VIR M	CRC F	CRC M	ZAG F	ZAG M	Total offspring F	Total offspring M
VIR	87	67	149	87	94	97	330	251
CRC	120	69	114	77	81	110	315	256
ZAG	128	64	132	107	69	73	329	244
Total	335	200	395	271	244	280	974	751

CRC, Cabo de Roca; VIR, Virginia; ZAG, Zagreb.

Four dams and four (VIR, ZAG) or five (CRC) sires were used per population, such that each dam was crossed to 12 or 13 sires and each sire was crossed to 12 dams. This crossing scheme produced 145 full-sib families. Because we required individuals of both sexes from each family to estimate the sexual dimorphism in calyx width, we used a relatively large family size to insure that there would be a high chance of rearing at least two males and two females per family. Thus, we allotted our measuring efforts towards larger numbers of offspring per family and fewer half-sib families per population. Although the relatively small number of sires per population limits our ability to estimate the heritability for each population, the number of sires was sufficient for our main purpose of determining whether there were genetic differences among populations in sexual dimorphism. We grew a mean of 11.9 offspring per family to flowering (ranging from 8 to 12 offspring per family, see Table 1 for specific numbers of offspring for each cross type) in a 1 : 1 mixture of commercial potting mix (Metromix, Scotts Horticultural Products, Marysville, OH, USA) and sterilized, composted soil.

Seeds were planted into celled trays for germination. Once they obtained their first true leaves, they were transplanted into four-inch plastic pots and randomly assigned to three adjacent greenhouse benches with supplemental lighting (16 h light : 8 h dark). Plants were watered automatically twice per day from below (ebb and flood system) and were fertilized weakly every month (half-strength 20 : 20 : 20 Peter's solution, Scotts Horticultural Products). Plants were treated with pesticides for thrips and aphids on an as-needed basis.

Plants were monitored daily when bolting began, and the main inflorescence stalk was tied to a thin metal stake inserted into the pot for support. The sex of each plant

was recorded at flowering. Calyx width was measured with digital callipers (to 0.1 mm) at its widest point for the third, fourth and fifth flower to open on each plant. By always measuring the same set of flowers, we hoped to minimize any effect of plant architecture on these measures. Mean calyx width was calculated as the average of the three measurements from each plant.

Data analyses

Mean calyx width data from 1925 plants were analysed using the mixed model procedure (MIXED) of SAS® (SAS 1996). This program was used to determine which fixed effects should be included in the initial model. The data were analysed separately for males and females. The initial model included a fixed effect for the sire population, dam population and the interaction between the sire and dam population. An interaction between sire and dam population would be evidence of a contribution of epistasis or heterosis to the genetic differences between populations (Goodnight, 2000; Drury & Wade, 2011). Additional comparisons were made to determine the importance of maternal and paternal influence and to determine the effect of the three populations on mean calyx width and sexual dimorphism. The model also included a random effect for the sire, dam and residual error. The mean sexual dimorphism was calculated for each population as the difference between the average calyx width of males and females in that population. In analysing the phenotypic variation among populations, the model for sexual dimorphism included a fixed effect for the sire population, dam population and the interaction between the sire and dam population.

Genetic variance in mean calyx width and sexual dimorphism

For calyx width, we estimated the genetic variation among individuals using the linear animal model as implemented in the ASReml software package (Lynch & Walsh, 1998; Gilmour *et al.*, 2002):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

in which $\mathbf{y}$ is a vector of observations on individual mean calyx width for either males or females; $\mathbf{b}$ is a vector of fixed effects, with incidence matrix $\mathbf{X}$ linking observations to the fixed effects; $\mathbf{a}$ is a vector of additive genetic effects ('breeding values'), with incidence matrix $\mathbf{Z}$ linking observations on individuals to their breeding value; and $\mathbf{e}$ is a vector of random residuals. The fixed effects in $\mathbf{b}$ account for systematic differences among observations and included sire population, dam population and an interaction between the sire and dam population. Covariance structures of the random effects were $\mathrm{Var}[\mathbf{a}] = \mathbf{A}\sigma_A^2$ where $\mathbf{A}$ is a matrix of coefficients of relatedness between individuals and σ_A^2 is the additive genetic variance, and $\mathrm{Var}[\mathbf{e}] = \mathbf{I}\sigma_e^2$, where $\mathbf{I}$ is an identity matrix and σ_e^2 is the residual variance. Two generations of pedigree were included in the calculation of the relationship matrix ($\mathbf{A}$). A natural logarithm of the mean calyx width for both males and females was taken to investigate whether differences between males and females were caused by a scaling effect. Results showed that there was no scaling effect, so there was no need to transform the data. For calyx width, this analysis yields estimates of the additive genetic variance, phenotypic variance and heritability for each sex.

The above model cannot be applied directly to sexual dimorphism, because sexual dimorphism is not expressed in a single observation but is the difference between observations. We used two approaches to estimate genetic parameters for sexual dimorphism. First, we estimated genetic parameters for sexual dimorphism by calculating the mean dimorphism observed within each full-sib family, $y_{d,\mathrm{fam}} = \bar{y}_{M,\mathrm{fam}} - \bar{y}_{F,\mathrm{fam}}$, and subsequently analysing this trait using the linear animal model given above. In this analysis, $y_{d,\mathrm{fam}}$ is a property of a family, and the relationship matrix ($\mathbf{A}$) contains the pedigrees of each family. Second, genetic parameters for sexual dimorphism were derived from results of the bivariate analysis of calyx width of both sexes. Sexual dimorphism in this case is the difference between the calyx width of an individual male and a female, $d = y_M - y_F$. As a single individual cannot express both traits, the environmental correlation between males and females is zero by definition. Thus, phenotypic variance in dimorphism equals $\sigma_{P_d}^2 = \mathrm{Var}(y_M - y_F) = \sigma_{P_M}^2 - r_A\sigma_{A_M}\sigma_{A_F} + \sigma_{P_F}^2$, additive genetic variance in dimorphism equals $\sigma_{A_d}^2 = \mathrm{Var}(A_M - A_F) = \sigma_{A_M}^2 - r_A\sigma_{A_M}\sigma_{A_F} + \sigma_{A_F}^2$ and the heritability in dimorphism equals $h_d^2 = \sigma_{A_d}^2 \Big/ \sigma_{P_d}^2$. The difference between the first and the second approach is in the residual ('environmental') variance in dimorphism. In the second approach, dimorphism refers to the difference between a single male and female, whereas in the first approach, an average is taken per family. The first approach, therefore, averages the environmental effects on dimorphism, which reduces the residual variance and therefore increases heritability. Residual variance equals $(\sigma_{e_M}^2/n_M) + (\sigma_{e_F}^2/n_F)$in this case, where n_M is the number of male offspring and n_F the number of female offspring of a family. Hence, heritability depends on the number of records per family.

We calculated Q_{ST} for each sex, a measure of population differentiation for quantitative traits defined as the ratio, $\sigma_{G_{\mathrm{Among}}}^2 \Big/ \left(\sigma_{G_{\mathrm{Among}}}^2 + 2\sigma_{G_{\mathrm{Within}}}^2\right)$ (Goudet & Buchi, 2006; Goudet & Martin, 2007; Martin *et al.*, 2008), where $\sigma_{G_{\mathrm{Among}}}^2$ is the genotypic variance among populations and $\sigma_{G_{\mathrm{Within}}}^2$ is the average genetic variance within populations. To calculate the Q_{ST}, we only used the information on the purebred populations (VIR × VIR, CRC × CRC and ZAG × ZAG). In the model, we included a random effect for the population and the individual. When used in

conjunction with estimates of the genetic differentiation (F_{ST}) among populations, Q_{ST} can lend insight into whether the phenotypic differences observed among populations were caused by random genetic drift or by natural selection (Goudet & Buchi, 2006).

Results

Phenotypic variation in mean calyx width

For all populations, flowers from females had calyces that were significantly greater in width than flowers from males [12.31 ± 0.04 mm (mean ± SE) vs. 8.49 ± 0.03 mm, respectively; $P < 0.001$]. The population that both the sire and the dam came from had a significant effect on the mean calyx width of flowers of both female and male offspring, with the sire population having a larger effect than the dam population (Table 2). In addition, mean squares for the effect of population, either sire or dam, are higher for males than females. The sire × dam population interaction was not significant for either sex, indicating that there was no significant heterosis or epistasis (Table 2). As seen in Fig. 1 (which shows the average calyx width of offspring across all crosses for the three populations) and Table 3, crosses involving individuals from the CRC population led to significantly wider calyces in most comparisons to crosses involving individuals from VIR or ZAG, regardless of whether CRC was the dam or the sire or whether the flowers were from females or males. Similarly, crosses involving ZAG usually led to significantly smaller calyces, especially for males.

To determine whether reciprocal F_1s have similar calyx widths, reciprocal crosses were compared (e.g. VIR × CRC vs. CRC × VIR). Calyces of offspring from the CRC sire × VIR dam cross were significantly larger than those in the reciprocal cross (Table 3), for both females and males ($P < 0.001$ and 0.05, respectively). Differences in the mean calyx width of the offspring from reciprocal crosses involving the other two populations were not significant.

Table 2 Mean squares for calyx width of female (F) and male (M) offspring.

Source of variation	d.f.	Sex	Calyx width
Sire population (SP)	2	F	38.14**
		M	97.93**
Dam population (DP)	2	F	17.81**
		M	67.47**
SP × DP	4	F	1.91
		M	1.20
Random sire	1	F	0.013**
		M	0.006**
Random dam	1	F	0.006**
		M	0.003**
Residual	742	F	1.15
	965	M	0.60

**$P < 0.01$.

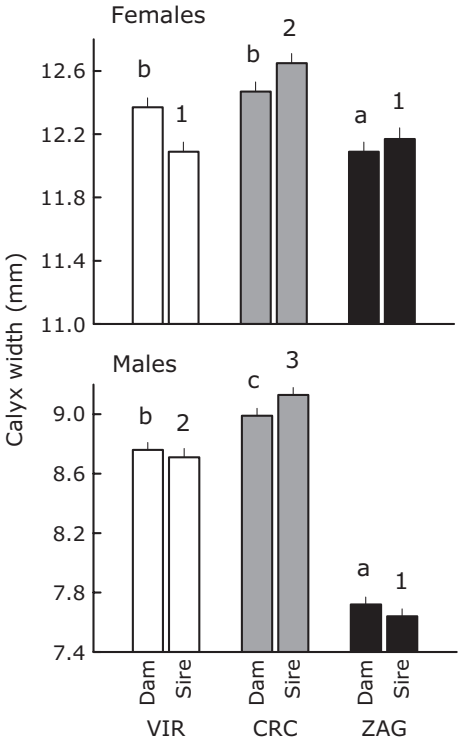

Fig. 1 Mean (±SE) calyx width (mm) of flowers of female and male offspring for each of the three study populations when acting as a dam or a sire. Within the graphs for each sex, significant differences among means are indicated with different letters (dams) or numbers (sires) above the means.

Table 3 Least square means (±SE) for calyx width for females and males for each cross combination.

	Sire population		
Dam population	VIR	CRC	ZAG
Females			
VIR	12.03^{ab} ± 0.12	12.78^{d} ± 0.09	12.30^{bc} ± 0.11
CRC	12.23^{bc} ± 0.10	12.77^{d} ± 0.10	12.40^{c} ± 0.12
ZAG	12.03^{ab} ± 0.10	12.40^{c} ± 0.09	11.82^{a} ± 0.13
Males			
VIR	8.99^{d} ± 0.10	9.49^{e} ± 0.08	7.81^{b} ± 0.08
CRC	9.16^{d} ± 0.09	9.54^{e} ± 0.09	8.27^{c} ± 0.07
ZAG	7.98^{b} ± 0.10	8.35^{c} ± 0.08	6.83^{a} ± 0.09

Means within each sex that are not significantly different from one another ($P < 0.05$) share the same superscript.
CRC, Cabo de Roca; VIR, Virginia; ZAG, Zagreb.

Genetic variance in mean calyx width

The overall heritability of calyx width, 0.41, was greater than zero (Table 4). The heritability of calyx width in

Table 4 Phenotypic and genetic variation for mean calyx width and the extent of sexual dimorphism.

	σ_p^2	σ_a^2	σ_e^2	h^2
Calyx width				
Total	0.98 ± 0.07	0.41 ± 0.13	0.58 ± 0.07	0.41 ± 0.11
Females	1.25 ± 0.11	0.58 ± 0.20	0.67 ± 0.11	0.47 ± 0.12
Males	0.67 ± 0.07	0.41 ± 0.13	0.26 ± 0.07	0.61 ± 0.14
Sexual dimorphism				
Family	0.50 ± 0.09	0.43 ± 0.17	0.07 ± 0.09	0.86 ± 0.21
Individual	2.20 ± 0.25	0.42 ± 0.16		0.19 ± 0.07

σ_p^2 = phenotypic variance, σ_a^2 = additive variance, σ_e^2 = environmental variance, h^2 = heritability.

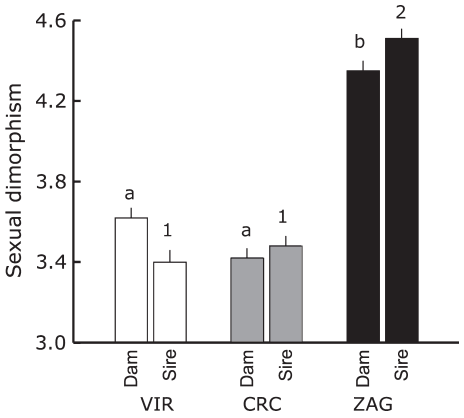

Fig. 2 Mean (±SE) sexual dimorphism in calyx width (mm) (calculated as the mean for females minus the mean for males) for offspring for each of the three study populations when acting as a dam or a sire. Significant differences among means are indicated with different letters (dams) or numbers (sires) above the means.

males was larger than that of females, but they were not significantly different from each other. As we found above and as is evident from Fig. 1, mean male calyx width varies among populations to a much greater extent than does mean female calyx width. We calculated the male and female values of Q_{ST}, the fraction of the genetic variance that is among populations, using the sex-specific data on the genetic variation within (0.37 for males vs. 0.74 for females) and among (1.96 for males vs. 0.11 for females) the three purebred populations, VIR, CRC and ZAG. For males, Q_{ST} equals 0.726, meaning that almost 73% of the total genetic variation in calyx width on males arises from differences among populations. Similarly, the Q_{ST} of calyx width for females equals 0.069; less than 7% of the total genetic variation in females is attributable to differences among populations. The Q_{ST} for males is an order of magnitude greater than that for females.

Sexual dimorphism

There was significant variation among populations in sexual dimorphism, with offspring from both ZAG dams and sires being significantly more sexually dimorphic than those from VIR or CRC (all $P < 0.001$; Fig. 2). Both the sire and the dam population had an effect on the extent of sexual dimorphism, and its heritability, based on the family mean dimorphism, 0.86 ± 0.21, was significantly greater than zero (Table 4). We also estimated the heritability of sexual dimorphism defined for a single pair of observations (h_d^2), using methods described above, as 0.19 ± 0.07 (Table 4). Using both methods, the genetic variance was almost the same and equalled 0.43.

Discussion

Our study revealed that three populations of *S. latifolia* studied here display highly heritable differences in both mean calyx width and the degree to which males and females differed from each other. Although males had smaller calyces in all three populations, the degree of sexual difference in calyx width varied significantly among populations. Moreover, based on the partitioning of variation, it appears that the populations differ for this trait because of selection on males rather than females.

Both dams and sires contributed significantly to variation in calyx width. Whereas reciprocal crosses between CRC and VIR led to offspring with means similar to the sire, the calyx width of offspring from reciprocal crosses between CRC and ZAG and between VIR and ZAG tended to be similar to that of the dam. However, because all parents in this experiment were grown from seed of plants reared in the greenhouse under similar conditions, it is unlikely the latter similarities were caused by a maternal environmental effect. Indeed, our methods were effective, because, when we included maternal effects in our analyses, the estimates were exceedingly small (< 0.005) and statistically insignificant (data not shown).

The direction of sexual dimorphism was found to be consistent across all three within-population crosses and all six between-population crosses: the calyx width of flowers on females is always greater than those on males. This contrasts with some insect studies, in which the pattern of within-population sexual dimorphism has been observed to be reversed in between-population hybrids (e.g. Wade *et al.*, 1994). Our estimate of the heritability of sexual dimorphism, 0.19, is similar to the range of estimates from animals, e.g. weight in mice (0.07 in Hanrahan & Eisen, 1973), body depth in the three-spine stickleback (0.26 in Lester *et al.*, 2008) and weight in tilapia (0.26 in Lester *et al.*, 2008). Estimated values of the heritability of sexual dimorphism tend to cluster well below 0.50, reflecting the relatively high genetic correlations for many phenotypic traits observed between the sexes. Indeed, the between-sex genetic correlation for calyx width in *S. latifolia*, calculated from both artificial selection and within-population crosses,

has been shown to be high [ranging from 0.82 (Steven *et al.*, 2007) to > 1.0 (Delph *et al.*, 2004a)]. Hence, with either disruptive selection for calyx width on the two sexes (constrained by positive genetic correlations) or direct selection among families for sexual dimorphism (constrained by low heritability), evolution towards sex-specific optima would be expected to be slow in natural populations.

The sire and dam population mean squares for males are greater than those for females, but, given the absence of a significant sire × dam population interaction, there is no evidence that epistasis or heterosis contributes to the differences in calyx width in either sex (see also Drury & Wade, 2011). This suggests that populations differ genetically from one another in calyx width on males to a much greater extent than they do on females and that the observed differences can be adequately explained by an additive model of gene action. We see this clearly in the mean differences in Fig. 1. If we compare only the three within-population cross means with one another, the average difference between the populations in mean male calyx width is 1.81 mm, whereas that for females is 0.63 mm. The variation among populations in calyx width on males is three times greater than that of females. We can express this among-population variation in units of phenotypic standard deviations (Lande, 1980) using data on the phenotypic variance within populations for males and females. As the phenotypic standard deviation for males, 0.844, is smaller than the corresponding value of 1.369 for females, populations differ from one another by 2.13 standard deviations of the male calyx width but by only 0.46 standard deviations for female calyx width. This is nearly a five-fold difference on the standardized scale. We can also compare our estimates of the phenotypic differentiation among populations, Q_{ST}, with Wright's (1951) genetic measure of population differentiation F_{ST}. When Q_{ST} exceeds F_{ST} and as long as F_{ST} is estimated using neutral or weakly selected loci, one can infer that local natural selection has been involved in the differentiation of the mean trait values among populations (e.g. Goudet & Buchi, 2006). Several estimates of F_{ST} obtained from allozyme studies for *S. latifolia*, as well as other species in the genus, have been published. Using seven polymorphic allozyme loci, McCauley (1994) estimated F_{ST} as 0.13 for *S. latifolia* (nee *Silene alba*) in a study of populations within a 25-km^2 region of Virginia. One of our populations, VIR, was from this same region. For the island endemic, *Silene hifacesis*, a species with more conspicuous population structure, Prentice *et al.* (2003) estimated a somewhat lower F_{ST} of 0.117 using 12 polymorphic loci. Van Rossum *et al.* (1997) estimated F_{ST} for marginal populations of *Silene nutans* from two geographical regions as 0.148 and 0.133, using five polymorphic loci.

The best estimate of F_{ST} for comparison with Q_{ST} in our study comes from data furnished by P.D. Fields, S.R. Keller, & D.R. Taylor (unpublished data). That data set consists of 40 populations (including European and North American populations as in our study), comprising 393 individuals genotyped at 16 microsatellite loci. Their estimate of global F_{ST} (cf. Weir & Cockerham, 1984) is 0.173, with a 95% CI of 0.127–0.244. Comparing our estimate of Q_{ST} for male calyx width to this F_{ST} value, we find that Q_{ST} is 4.2 times greater than F_{ST}. The same comparison for females, however, reveals a ratio of 0.40. This comparison is consistent with the inference that selection has been acting more strongly on calyx width of males than of females and is responsible for the phenotypic differentiation among these populations. Although a lack of genetic variation can affect Q_{ST} (and, for example, cause it to be lower than F_{ST} [Merila & Crnokrak, 2001]), this is unlikely to be the case here, as the heritability for calyx width was not found to differ significantly between males and females.

Taken together, our evidence suggests not only that local selection is responsible for the differentiation of these populations but also that the selection has produced a much larger response in males than in females. Given comparable heritabilities for the two sexes, we infer that selection acting on males has been stronger than that acting on females. This kind of sex difference in the strength of selection (although not always in the response to selection) is commonly observed in animal studies (cf. Shuster & Wade, 2003). For example, selection was found to be or inferred to be greater on male dung flies (Kraushaar & Blanckenhorn, 2002) and male horned lizards (Zamudio, 1998), although studies of house finches have found that selection is divergent and of similar strength for males and females among populations that vary in their degree of sexual dimorphism (Badyaev *et al.*, 2000).

We choose to investigate the cause of among-population variation in mean calyx width and the extent to which it was sexually dimorphic, in part because both have been found to vary for this trait (Delph *et al.*, 2002; Delph & Bell, 2008). Variation in the mean width of the calyx among populations could be a result of selection directly on calyx width; however, direct, pollinator-mediated selection is unlikely, as the showy petals, rather than the calyx, attract pollinators. Moreover, calyx width and petal size have been shown to be genetically uncoupled (Delph *et al.*, 2004b), so even direct selection on petal size leading to a correlated response in calyx width is an unlikely explanation. Alternatively, it could result from a correlated response to selection on other traits. The latter scenario is likely given that calyx width has been shown to covary with a host of other traits, including flower number, allocation traits, leaf ecophysiological traits and plasticity in fertility (Meagher, 1994; Delph *et al.*, 2004a, 2005; Herlihy & Delph, 2009).

Variation in the extent of sexual dimorphism in calyx width could result from the fact that genetic covariances involving calyx width differ for males and females (Steven *et al.*, 2007), which could produce differences

in the correlated responses to selection within each sex. In general, males tend to be more genetically integrated than females and the covariances are stronger (Steven *et al.*, 2007; Delph *et al.*, 2010; Delph & Steven, unpublished data), making it likely that habitat differences among populations could differentially impact trait variation in the two sexes. For example, both phenotypic and genetic correlations between flower number and leaf thickness (specific leaf area) are greater for males than for females (unpublished data), and both are correlated with calyx width (Delph *et al.*, 2005) and share overlapping male-specific quantitative trait loci (Delph *et al.*, 2010). Hence, males that make a relatively large number of flowers also have relatively thin leaves, likely to result in greater water use, and thereby indirectly select against small calyces in dry habitats. The result of such indirect selection could lead to a change in the extent of sexual dimorphism in some populations compared to others.

In conclusion, we have shown that variation in the degree of sexual dimorphism among populations in calyx width occurred because of selection on males rather than selection on females or because of drift. Moreover, this population divergence occurred in spite of a strong between-sex correlation for this trait and relatively low heritability for sexual dimorphism, suggesting that the selection was either relatively consistent over many generations, strong or both. Our approach of comparing the ratio of Q_{ST} to F_{ST} between males and females has proven powerful in understanding the cause of variation in the degree of sexual dimorphism and is one that could be applied to any system with sexually dimorphic, quantitative traits.

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