Review Paper

Explaining phenotypic selection on plant attractive characters: male function, gender balance or ecological context?

Tia-Lynn Ashman^{1*} and Martin T. Morgan²

¹Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

²School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA (mmorgan@wsu.edu)

It is widely agreed that the flowers of hermaphrodite plants evolve in response to selection acting simultaneously through male and female sexual functions, but we know very little about the pattern of gender-specific selection. We review three current hypotheses for gender-specific selection by viewing them within a single phenotypic selection framework. We compile data from phenotypic selection and manipulative studies and evaluate the fit between empirical data and the hypotheses. In this preliminary analysis, we find that neither the male-function hypothesis nor the gender-balance hypothesis is well supported. However, the context-dependence hypothesis is supported by the documented diversity of gender-specific selection and by evidence that selection through female fertility is significantly correlated with pollen limitation of seed production. Future studies contributing to our understanding of selection through male and female function in plants need to quantify and manipulate the ecological context for reproduction, as well as describe male and female fitness responses to fine-scale trait manipulation.

Keywords: natural selection; male function; floral evolution; phenotypic selection; pollination; pollen limitation

1. INTRODUCTION

While most would agree that attractive floral displays in hermaphrodite plants evolve in response to both female functions (seed production) and male functions (pollen export), there is less agreement about the relative strength of gender-specific selection. Two decades ago, parallels with sexual selection in animals (Bateman 1948; Willson 1979) led many researchers to conclude that floral features could evolve mainly in response to selection through male fertility (e.g. Bell 1985; reviewed in Burd & Callahan 2000). Echoes of this original claim continue to be heard today, even though supporting evidence is scarce (references below), competing hypotheses have been proposed (Morgan & Schoen 1997b) and the importance of reproductive context (e.g. pollen limitation of female fertility) in shaping selection is becoming increasingly apparent (Baker 1967; Wilson *et al.* 1994; Ashman & Diefenderfer 2001). We show how three current hypotheses can be described within a single phenotypic selection framework. In doing so, we elucidate how the ecological context is responsible for generating variation in the relative strength of selection through male and female fertility. We then assemble data from published phenotypic selection and manipulative studies to summarize our current understanding of the relative roles of female and male fertility in shaping floral traits. Finally, we provide guidance for future studies aimed at gaining a better understanding of the ecological circumstances that will generate male-

biased, female-biased, unbiased or balancing selection on floral attractive traits.

2. PHENOTYPIC SELECTION ANALYSIS PROVIDES A FRAMEWORK FOR UNDERSTANDING SELECTION THROUGH MALE AND FEMALE FERTILITY

Phenotypic models of selection on quantitative characters (Lande 1976, 1979) extend to selection in hermaphrodites (Morgan 1992) and provide an ideal framework for empirical research programmes into the selection of reproductive characters. The trait value (phenotype) of an individual, z , influences fitness through female $W_F(z)$ and male $W_M(z)$ contributions to reproductive success:

$$W(z) = \frac{1}{2} \left(\frac{W_F(z)}{\bar{W}_F} + \frac{W_M(z)}{\bar{W}_M} \right). \quad (2.1)$$

Here, $\bar{W}_F$, $\bar{W}_M$ are population average male and female fertilities, and the formulation in terms of relative fertilities ($W_F(z)/\bar{W}_F$, $W_M(z)/\bar{W}_M$) ensures equal average male and female fertilities. The formulation allows for many different phenotypes and emphasizes the symmetry of opportunities for reproductive success through female and male functions (Morgan & Schoen 1997a). Lande's formulation connects with statistical analysis because the fitness function of equation (2.1) describes gender-specific selection gradients (gender-specific differences in average trait value before and after selection, normalized by standing phenotypic variation; univariate gradients are symbolized as β_F , β_M , multivariate gradients as β_F , β_M). Specifically, the between-generation change in average trait value $\Delta \bar{z}$

* Author for correspondence (tia@pitt.edu).

is equal to the additive genetic variation of the trait G multiplied by the average of the selection gradients through male and female fertilities (Morgan 1992),

$$\Delta\bar{z} = G\frac{1}{2}(\beta_F + \beta_M). \quad (2.2)$$

Selection gradients are readily estimated as the slope of the regression of relative fitness (e.g. $W_F/\bar{W}_F, W_M/\bar{W}_M$) on trait value (Lande & Arnold 1983). Statistical uncertainties surrounding selection gradients are simply those of the regression analysis, facilitating straightforward hypothesis testing (e.g. asking whether selection gradients equal zero $\beta_F = 0, \beta_M = 0$, or one another, $\beta_F = \beta_M$). The intimate relationship between evolutionary and statistical methodology provides a firm theoretical foundation for understanding selection on plant reproductive characters. These methods extend to multiple characters and to different components of the life history (e.g. path analysis; Scheiner *et al.* 2000). Lande's model assumes continuous, quantitative variation influenced by many genes of small effect; the genetics of size-related floral characters are likely to approximate these assumptions, but several well-characterized polymorphisms influencing attraction (e.g. flower colour in *Ipomoea*; Schoen & Clegg 1985) are controlled by a few genes, each of large effect. For such characters, selection gradients (i.e. regression of relative fertility on trait value) accurately describe the change in average phenotype value within a generation, but the between-generation evolutionary equation (2.2) may be inappropriate.

3. THREE HYPOTHESES FOR SELECTION ON ATTRACTIVE TRAITS

The 'male-function' hypothesis (figure 1*b*) suggests that attractive features evolve primarily through selection on male fertility (recently reviewed in Burd & Callahan 2000). This hypothesis assumes that female fertility is resource limited rather than pollen or pollinator limited. In the selection framework presented here, selection is stronger through male than female function ($|\beta_M| \gg |\beta_F|$) as would occur if the average trait value expressed by a hermaphrodite population is closer to the female fitness optimum than the male fitness optimum (as in figure 1*a* with 1*b*), or if the female fitness surface is very flat compared to the male fitness surface.

A contrasting view recognizes that, at evolutionary equilibrium, selection through male and female function is equal in magnitude but opposite in sign ($\beta_F = -\beta_M$; Morgan 1992). This follows from equation (2.2) when there is no change in trait value ($\Delta\bar{z} = 0$), provided additive genetic variation exists ($G > 0$). This 'gender-balance' hypothesis (figure 1*c*) implies that attractive traits in hermaphrodites represent an evolutionary compromise where selection through male and female functions balances.

A third view originates with the observation that sexual reproduction depends on reproductive strategies of other individuals in the population, as well as on the type and behaviour of pollen vectors. With this in mind, the 'context-dependence' hypothesis (figure 1*d*) suggests that the relative strength of selection through male and female function depends on the pollinator context (Wilson *et al.* 1994; Ashman & Diefenderfer 2001). Not only can pollinator context generate selection that is stronger via male

than female fitness (II in figure 1*d*), or equal and opposite via the two genders (III in figure 1*d*), but some pollinator contexts can lead to selection that is stronger through female than male function (IV in figure 1*d*), or comparable via both genders (I or V in figure 1*d*). For instance, a trait that enhances pollinator visits may be weakly selected via female function when pollinators are abundant, but strongly selected when pollen limitation increases the variation in female fertility. Note, however, that changing *average* female fertility does not alter the strength of selection. Similar dynamics are expected for pollinator limitation of male fertility.

4. EMPIRICAL PATTERNS OF SELECTION AND PRELIMINARY TESTS OF HYPOTHESES

To assess our current understanding of selection on attractive traits and to view this in light of the three hypotheses, we collected data from published studies of phenotypic selection and experimental manipulations. First, we compared the covariation between male and female selection using seven studies that reported quantitative estimates of selection on floral traits through male and female fertilities (i.e. univariate β_F, β_M or multivariate β_F, β_M selection gradients). Male fertility was estimated directly from genetic data in one study (Morgan & Conner 2001), from pollinia/pollen removal in five studies, and dye export in one study (Campbell 1991). For each species, population or year combination, a single coefficient representing selection on a trait associated with flower size (e.g. corolla or labellum width, spur length, flower or petal area) was included. Figure 2 shows the pattern of covariation between selection via male and female function derived from these studies.

If the male-function hypothesis is correct then we expect selection through male function to be greater than that through female function. When we performed a paired *t*-test on the absolute value of selection via male and female fertility, the strength of selection ($\pm$ s.e.) through male fertility was slightly but not significantly greater than through female fertility (0.182 ± 0.034 versus 0.146 ± 0.032 ; $t = 0.97$; $p = 0.34$; $n = 29$). By contrast, if the gender balance hypothesis is correct we expect male and female selection gradients to be equal and opposite in sign. As a preliminary test of this idea we performed a sign test, and found that approximately one-third (11/29) of the pairs of selection gradients were opposite in sign, but this did not represent a significant deviation from random expectation ($\chi^2 = 0.86$; d.f. = 1; $p > 0.20$). In fact, the data weakly support a preponderance of positive selection via both male and female function (13/29; $\chi^2 = 6.7$; d.f. = 3; $p = 0.08$). Our survey of selection gradients provides little support for the male-function hypothesis and cannot reject the gender-balance hypothesis. The survey shows that selection on attractive traits reflects many gender-specific scenarios, including many of those depicted in figure 1*d*.

To further assess the pattern of fertility response to attractive traits we tallied studies that manipulated traits and recorded components of male and female fitness. We found 10 studies on 10 species where a floral (size, colour, nectar level) or inflorescence (flower number) trait was manipulated. Some studies contained more than one

Figure 1. Description of the three hypotheses within a phenotypic selection framework. (a) Male fitness (solid lines) and female fitness (dashed lines) surfaces for the (b) 'male-function' and (c) 'gender-balance' hypotheses. The location of the phenotypic trait distribution relative to the fitness surfaces generates gender-specific selection, i.e. $|\beta_M| \gg |\beta_F|$ in (b) and $\beta_F = -\beta_M$ in (c). The 'context-dependence' hypothesis is shown in (d) where various combinations of fitness surfaces (upper I–V) generate different combinations of gender-specific selection gradients (lower I–V) for the same phenotypic distribution. Note that an identical set of fitness surfaces with the gender identity of the surfaces reversed is possible, and would generate an additional set of gender-specific selection gradients.

manipulation, resulting in 12 different tests. We classified these with respect to the effect of the manipulation on male and female function in figure 3. If the male-function hypothesis is correct, then male function should be more responsive to modest alteration (i.e. reduction or augmentation) of floral traits than female function. By contrast, if reproductive traits reflect the balance of selection through male and female function as predicted by the gender-balance hypothesis then the same manipulation should induce opposite responses through the sex functions. Overall, a similar number of tests found a significant effect of manipulation on female (9) or male (6) function. Nearly equal numbers of tests represent significant effects on both (5) and female but not male (4) fitness components, but in only one test was there an effect on male but not female fitness components, and in two tests there was no effect on either. The pattern of effects does not differ from random expectation (Fisher's exact test: $p = 0.41$). While half of the tests showed a response in one sex but not the other, no manipulation increased one sex function while decreasing the other. The small number of manipulative studies conducted to date provides no compelling

evidence to distinguish between the male-function or gender-balance hypotheses, but do suggest a continuum of possible effects of floral trait manipulation, including those that affect both genders, and female but not male fitness.

Our survey shows a variety of combinations of gender-specific selection, but does not provide the information needed to evaluate the context-dependence hypothesis. However, several studies have reported both female selection gradients for flower size traits and degree of pollen limitation, allowing us to determine whether selection through female function varies with ecological context in a predictable way. Using data from 12 studies, we calculated an index of pollen limitation as $1 - \text{control/supplemental}$ for those that employed supplemental pollination, and as $1 - \text{reduction/control}$ for those that involved reductions in pollen deposition or pollinator visitation resulting from the presence of other flowering plants (Caruso 2000) or experimental enclosures (Mazer & Meade 2000). If the context-dependence hypothesis is correct then selection on attractive traits through female function should increase with strength of pollen limitation.

Figure 2. Pattern of phenotypic selection on floral attraction traits through male and female fertility. Univariate (triangles) and multivariate (circles) selection gradients are standardized to a mean of zero and a unit standard deviation. Data are from seven studies on eight species that reported both male- and female-specific selection on a flower-size trait (Campbell 1989, 1991; Wilson 1995; Conner 1996; Morgan & Schoen 1997b; O'Connell & Johnston 1998; Maad 2000; Ashman & Diefenderfer 2001; Morgan & Conner 2001).

Figure 3. Distribution of the effects of attractive trait (floral or inflorescence) manipulation on male (M) and female (F) fertility. Data are from 10 studies (12 species-trait combinations) where the response of both male and female fertility to manipulation was documented (Cresswell & Galen 1991; Mitchell & Waser 1992; Podolsky 1992; Broyles & Wyatt 1995; Johnson *et al.* 1995; Wilson 1995; Conner 1997; Johnston & Steiner 1997; Melendez-Ackerman & Campbell 1998; Jones & Reithel 2001).

Figure 4. Relationship between strength of selection through female fertility and degree of pollen limitation calculated as described in the text ($r = 0.53$; $p = 0.004$). Selection strength is represented as the absolute value of standardized univariate (triangles) and multivariate (circles) selection gradients (Campbell 1989; Johnston 1991a,b; Andersson 1996; O'Connell & Johnston 1998; Caruso 2000; Maad 2000; Mazer & Meade 2000; Ashman & Diefenderfer 2001; Totland 2001; Alexandersson & Johnson 2002; T.-L. Ashman and A. L. Case, unpublished data).

We found a strong positive relationship between the absolute strength of selection through female fertility and degree of pollen limitation (figure 4; $r = 0.53$; $p = 0.004$). This scenario is likely to be common, as pollen limitation is widespread and strong: two large surveys (Burd 1994; Ashman *et al.* 2004) have found that the majority of empirical studies detect significant pollen limitation in at least some years or locations. While this evidence for the context-dependence hypothesis for selection through female function is compelling, we still do not know how the pollination environment alters selection through male function. To fully understand how ecological context shapes selection both genders must be assessed.

5. WHAT EXPLAINS THE PATTERN OF SELECTION AND HOW CAN WE TEST THIS?

The pattern of selection summarized above does not provide support for either the male-function or the gender-balance hypothesis. This could indicate that additional biological complexity was not accounted for, or that methodological problems associated with measuring fertility and comparing selection gradients obscure true patterns. In the following sections, we discuss these alternatives and provide recommendations to clarify the action of selection on attractive characters.

(a) *Biological complexity can lead to false conclusions about pollinator-mediated selection*

The fact that selection can act indirectly through a number of phenotypically correlated and possibly unmeasured characters represents a serious challenge for all observational studies of selection (Lande & Arnold 1983). For instance, suppose plants with greater access to resources produce larger flowers and at the same time increase fruit production. The underlying resource variation leads to the false conclusion that the reproductive character itself benefits female fitness. Apparent selection is likely to be a particularly prominent problem in studies of plant reproductive characters (Burd & Callahan 2000). If male and female fertility are affected similarly by plant size, then this may explain the preponderance of studies finding positive selection through both male and female fertility (figure 2). Statistical (e.g. identifying appropriate proxies for 'overall size') and experimental (e.g. standardizing resource status) approaches can address these issues to some extent (Mitchell-Olds & Shaw 1987; Burd & Callahan 2000).

Ecological and demographic trade-offs can also obscure selection on attraction. For instance, flowering and fruiting trade-offs are important for female function (Seger & Eckhart 1996; Sakai & Harada 2001). Suppose there is strong selection on floral display for the fertility component associated with pollen donation and receipt. If there are life-history trade-offs between flowering and fruiting (e.g. because investment in flowering decreases resources for fruit maturation), a female selection gradient incorporating fruit production might indicate that there are no benefits of floral display for female function. While correct in one sense (net effects of floral display on female function are limited) this conclusion obscures the comparable contributions of floral display to male and female fertility during pollination. Likewise, unmeasured countervailing selection pressures can affect the detection of selection. For instance, larger inflorescences increase pollinator attraction but may also decrease pollen export to other plants and increase self-pollination via geitonogamy (Klinkhamer & de Jong 1993). Similarly, large displays can increase pollinator visitation but may also increase damage by seed and flower predators (e.g. Brody & Mitchell 1997). Thus, measuring selection on sequential components of fitness (e.g. Campbell 1991) may be required to accurately compare gender-specific selection on attractive traits.

(b) *Fertility estimation complicates comparison of selection gradients*

Measuring selection requires some method of assessing fertility. Seed and fruit production are readily measured proxies for female fertility. However, if there are seed quantity–quality trade-offs then the number of germinating seeds (or surviving offspring) may be better predictors of female fertility than seed production (Campbell 2000). Male fertility is even harder to estimate because obvious measures such as pollen removal can be poor indicators of reproductive success. For instance, reproductive traits enhancing pollinator attraction may increase pollen removal and appear to increase male fertility, but fertility benefits may be low if geitonogamy is increased (Rademaker *et al.* 1997). Recent developments use genetic

markers coupled with paternity assignment techniques to assess male fertility. An advance has been to directly estimate selection gradients from genetic data (Smouse *et al.* 1999; Morgan & Conner 2001; Burczyk *et al.* 2002) rather than estimating male fertility contributions (e.g. via fractional paternity assignment) prior to estimating selection. Differences in methodology inherent in estimating male and female selection make it difficult to directly compare selection gradients or other statistics that might summarize gender-specific selection. A partial solution is to use highly variable microsatellite markers to determine paternity conclusively; this solution is likely to be restricted to small populations (less than 200) because of the extensive investment in genotyping required for conclusive paternity (Morgan & Conner 2001).

6. IS GENDER-SPECIFIC SELECTION CONTEXT DEPENDENT?

The patterns observed in our surveys could reflect context dependence of gender-specific selection rather than the experimental or statistical artefacts described above. Evidence that selection through female fertility is correlated with pollen limitation supports this idea (figure 4). If we are to understand the mechanisms that underlie the diversity of selection scenarios, we need to make rigorous connections between selection and context, i.e. combining selection gradient estimation with demonstrations of mechanism and process. Many of the studies reviewed above implicitly assume that pollinators are the agents of selection and the measured floral traits are the targets of selection, but these assumptions are rarely tested and in at least one case are known to be false (Caruso 2001). Selection analyses are not complete without an attempt to quantify the agency of selection and to verify its causal role in generating the gender-specific pattern of selection. Moreover, manipulations of putative targets of selection should be designed to address the more detailed question of whether there are gender-specific responses to variation in the trait. These suggestions are not new, but greater emphasis and refinement are needed to understand what causes gender-specific patterns of selection on attractive traits. Below, we briefly illustrate the value of these approaches.

(a) *Quantify the agency of selection*

Manipulation of context in conjunction with data from natural populations can reveal the importance of different agents of selection. For instance, Galen (1996) verified that selection on corolla flare was mediated by bumblebees by showing that selection disappeared when bumblebees were excluded or plants were hand pollinated. Similarly, by measuring selection on floral traits in *Ipomopsis aggregata* under several contexts Caruso (2000, 2001) revealed that selection through female fertility was mediated by competition for pollination when in the presence of a particular co-flowering species. Greater insight can be gained by direct manipulation of the context and measurement of both the strength of selection and a quantitative value of the context, for example pollen limitation or pollinator abundance (T.-L. Ashman and A. L. Case, unpublished data). When manipulation is not possible, it is important to quantify aspects of the selective context

that are likely to influence the value of attractive traits to male and female fertility. The test of the context-dependence hypothesis above is a case in point. The hypothesis was well supported with analysis of data from studies spanning a wide range of pollen limitation. It appears that regardless of its genesis, the consequence of pollen limitation is to increase selection through female fertility. We envision that similar patterns may be found for other aspects of the pollinator or ecological context (e.g. herbivores, resource availability).

(b) *Describe male and female functional response to putative targets of selection*

Because phenotypic selection analysis is an observational and correlational approach, it is particularly important to validate traits suspected to be targets of selection (Mitchell-Olds & Shaw 1987; Wade & Kalisz 1990). Gross manipulation of traits can provide compelling support for putative targets. For example, Conner *et al.* (2003) used anther manipulations to determine that anther dimorphism had no effect on female fertility but increased male fertility by restricting single-visit pollen removal. A series of small incremental manipulations (e.g. Cresswell 2000) can not only validate the target of selection but also better elucidate differential functional response by male and female fertility. Here, an analysis of covariance approach could be used to directly compare the relative impact of a manipulation on male and female fertility. By describing the shape of the relationship between fertility and trait value one can determine if gender-specific selection observed in phenotypic selection studies reflects a differential proximity of the population to the optimal trait value for a given gender (figure 1d), or different shapes of the male and female fitness surfaces. In either case, such an approach will provide a more powerful confirmation of phenotypic selection.

7. CONCLUSIONS

Our review and analysis of observational and manipulative studies indicates that the pattern of gender-specific selection is best explained by the context-dependence hypothesis. A fuller test requires more studies of selection through both male and female fertility that provide a standard metric for comparative analyses (selection gradients). To be maximally useful these studies need to quantify the agency of selection and confirm gender-specific selection with manipulative functional response experiments. This information, plus that on context frequencies and population trait means in the wild, will eventually allow us to determine whether selection on attractive traits in wild populations more often reflects a male-function, a gender-balance or a context-dependent scenario, and thus provide a fuller understanding of the forces shaping the evolution of attractive traits in plants.

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