THE QUANTITATIVE GENETICS OF MATE CHOICE EVOLUTION: THEORY AND EMPIRICISM

A Dissertation

by

NICHOLAS LAYNE RATTERMAN

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Approved by:

Co-Chairs of Committee,	Adam G. Jones
	Gil G. Rosenthal
Committee Members,	Ginger E. Carney
	Lee Fitzgerald
Head of Department,	Uel Jackson McMahan

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ABSTRACT

The evolution of mate choice remains one of the most controversial topics within evolutionary biology. In particular, the coevolutionary dynamics between ornaments and mating preferences has been extensively studied, but few generalizations have emerged. From a theoretical standpoint, the nature of the genetic covariance built up by the process of mate choice has received considerable attention, though the models still make biologically unrealistic assumptions. Empirically, the difficulty of estimating parameters in the models has hindered our ability to understand what processes are occurring in nature. Thus, it is the goal of this dissertation to contribute to the field both theoretically and empirically.

I begin with a review of the evolution of mate choice and demonstrate how the lack of cross-talk between theoretical and empirical pursuits into studying mate choice has constrained our ability to extract basic principles. The review is followed by a new model of intersexual selection that relaxes some of the critical assumptions inherent in sexual selection theory. There are two empirical studies whose goal is to measure mating preference functions and genetic correlations in a way that can be related back to theory. Finally, I conclude by setting the stage for future endeavors into exploring the evolution of mate choice.

The results presented herein demonstrate four things: (i) a lack of communication between theoretical and empirical studies of mate choice; (ii) genetic drift plays a much larger role in preference evolution than previously demonstrated; (iii) genetic

correlations other than those explicitly modeled are likely to be important in preference evolution; and (iv) variation in mating preferences can eliminate intersexual selection altogether. From these four findings it can be concluded that a tighter link between theory and empiricism is needed, with a particular emphasis on the importance of measuring individual-level preference functions. Models will benefit from integrating the specific phenotypes measured by empiricists. Experimentation will be more useful to theory if particular attention is paid to the exact phenotypes that are measured. Overall, this dissertation is a stepping stone for a more cohesive and accurate understanding of mate choice evolution.

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CHAPTER I

INTRODUCTION: DISCORDANCE BETWEEN THEORETICAL AND EMPIRICAL STUDIES OF MATE CHOICE

Introduction

The process of mate choice has drawn substantial scientific attention mainly because the phenotypic characters involved in sexual selection are often conspicuous, bizarre, and extravagant. However, from a behavioral and psychological standpoint, the behavior involved in mate choice is composed of multiple components that interact in a complex manner. The intricacy of these interacting behavioral traits can be understood by decomposing mate choice into understandable and measurable components to evaluate the behavioral architecture of mating decisions. A clear picture of the structure of these behavioral attributes will simultaneously shed light on their ability to evolve and the nature of selection on secondary sex ornaments. Unfortunately, most empirical studies seem to fall short of this target, and theory lacks models that integrate the constituent parts of mating behavior that are likely to be of evolutionary importance. We seek to identify the potential causes of these shortcomings of mate choice empiricism and theory, and we also provide a framework for a more cohesive understanding of the phenotypic basis of the behaviors involved in mating decisions.

A thorough review of the literature highlights at least three important problems that need to be addressed. First, the terminology used to describe mate choice is inconsistent. The same term can be used to describe two or more distinct phenotypes, or

different terms are used to describe the same phenotype. This problem becomes exacerbated in the literature because references are cited but are often describing completely different phenotypes but calling them the same thing. Similarly, general conclusions about the behavioral architecture of mate choice are obscured when two studies measure the same attribute but call them different things. Second, some parameters in models are inestimable, resulting in predictions that cannot be tested. Models that make untestable predictions are of little to no use when it comes to understanding natural phenomena. Finally, empiricists often make measurements that are not directly tied to theory. In turn, conclusions are drawn based on theoretical predictions, though these predictions refer to entirely different phenotypic characters than the ones that were empirically measured. Thus, our understanding of what occurs in the natural world is sometimes grounded in misappropriated theory.

Our review begins by identifying and dissecting the problems that impede a focused and collective effort to understand intersexual selection on both theoretical and empirical grounds. Our efforts are mainly centered on terminological issues. We then propose solutions to these problems. We propose a set of strictly operational definitions that identify the measurable components of mate choice. Next, we provide a guide to how mate choice is modeled, followed by how mate choice is empirically measured. We urge theorists to expand the current models to include multiple measurable components of mate choice that accurately portray individual behavioral attributes that lead to mating decisions. Finally, we direct empiricists to the specific variables that are explicitly treated in current theory to facilitate closer ties between total parameter space and

biologically realized parameter space. Arnold (1983) captures the essence of the roles of the two sub disciplines: "The goal of a model is to determine what *can* happen: only empirical work can determine what actually *does* happen in the real world."

Terminological issues

A first step to ameliorating the issues discussed to this point is to standardize the terminology used to describe the components of choice. In doing so, theoreticians and field workers alike will be able to correctly identify and communicate the exact behavioral attributes under investigation. Assays aimed at measuring mating preferences are fairly similar and should provide data that are clearly comparable across different systems. However, a thorough review of the literature demonstrates the scrambled terminology used to refer to components of mate choice. In many cases, the same term is used to describe different measurements. For example, choosiness has been operationally defined by Gray & Cade (1999), but subsequent studies used operational definitions that range from slightly different (Brooks and Endler 2001) to entirely different measures (Berglund et al. 2005). In other instances, the same measurement has multiple different definitions. Hedrick & Weber (1998) operationally defined selectivity as a measure of dispersion about the mean response of individual females (coefficient of variation), which is nearly identical to the definition of choosiness used above (Table 1.1). This problem is rampant in the literature, and may have significant effects on our ability to draw generalities about the important of different components of choice in intersexual selection.

On the utility of operational definitions

We propose a set of explicit and operational definitions to describe the components of mate choice. Specifically, we suggest that the nature of mate choice assays confines measurements to occur in specific categories. From these types of measurements we are able to categorize the data in a way that allows different research groups to communicate clearly about the patterns emerging from measured behaviors. Our selection of terms takes into account historical contingency of the use of the term to avoid increasing the already jumbled set of definitions. Our goal is to consolidate and canonize the current vernacular to provide standardized definitions with direct ties to which measurements are being made. Thus, our selection also provides distinction between the terms we suggest (Table 1.1) and their use in other fields. For example, in the mate-choice literature, 'discrimination' often refers to a measure of dispersion about the mean response to stimuli (Brooks and Endler 2001; Bailey 2008). However, in psychophysics, discrimination refers to the capacity to perceive a noticeable difference between stimuli (Levine and Shefner 1991; Shettleworth 2009). Thus, our proposed definitions are aimed to avoid confusion by being distinct from terms used in other fields. Finally, we stress the importance of operational definitions in our canonized terms. Conceptual definitions are very useful in helping to identify the particular realm in which a phenomenon resides. Unfortunately, conceptual definitions lack explicit details regarding how a particular term is measured and parameterized.

Perhaps an example best contrasts conceptual and operational definitions. Jennions & Petrie (1997) use the following decomposition of terms:

> Following Heisler et al. (1987), we define 'mating preferences' as the sensory and behavioural properties that influence the propensity of individuals to mate with certain phenotypes…We further subdivide 'mating preferences' because there are two properties that can be distinguished conceptually and, more importantly, sometimes empirically. We define 'preference functions' as the order in which an individual ranks prospective mates ceteris paribus; and 'choosiness' as the effort or energy that an individual is prepared to invest in assessing mates, both in terms of the number of mate sampled and the amount of time spent examining each mate.

What can we make of this if we go out to measure preferences? What measure captures the propensity of individuals to mate with certain phenotypes over others? According to their definition, it is either the preference function or choosiness. As far as preference functions are concerned, what is the scale upon which individuals are ranked? With respect to choosiness, what measurements quantify effort or energy used in mate assessment? They propose number of mates sampled and time spent examining each mate as estimates of choosiness, though both proxies are quantified by completely different processes, which may vary independent of one another (i.e., no phenotypic correlation between mate sampling and time spent sampling each individual).

Before we can begin to condense the mate choice vernacular into a set of operational definitions it is imperative to understand the difference between the terms used in models of mate choice versus data collected empirically. As we discussed above, theory generally allows a single attribute of female choice to vary and either excludes all

others or holds them constant. Thus, the behavioral attribute called "preference" is presented as either a maximum of a unimodal function (Fig. 1.1C), with a fixed width (i.e., tolerance), or as the coefficient an exponential function (Fig. 1.1A). Conversely, empirical studies of individual-level mating preferences have partitioned female response data into a mean (responsiveness), measures of dispersion about the mean (choosiness), the maximum (peak preference), and regression coefficients (preference functions) (Table 1.1, Fig. 1.2). This disconnect between the parameters describing mate choice results in ill-communication between empirical estimates and theory. While theory has inspired empiricists to measure distinct attributes of one sex's behavioral responses of to the other, these measurements are only loosely tied to what the theoretical parameters represent. Moreover, the two different models of preference functions use the same term (preference), which makes relating data to theory even more difficult. We propose to use the empirical data to define components of mate choice and for these different phenotypic qualities of individuals to be integrated into a more comprehensive and complete theory describing the evolution of mate choice.

A proposed set of operational definitions

Our proposed operational definitions are based on the data collected when individuals are tested with multiple stimuli, each replicated multiple times. Thus, each of the terms can either be expressed at the ***individual-level*** or ***population-level*** and distinction between these two levels is of utmost importance. Each of the terms below is defined on the individual-level, but pooling data among individuals will result in population-level measures. Contrasting individual- and population-level components of

choice defines variation in individual-level measures as the cause of the magnitude and direction of population-level measures. The mean value of response across all stimulus values provides an estimate of an individual's ***responsiveness*** (Fig. 1.2A). Individuals that respond either to a greater proportion of stimuli, or with a greater magnitude of response are said to be more responsive. This attribute is often tied to reproductive motivation or overall sex drive (Reinhold et al. 2002). Measures of dispersion about the mean (e.g., variance, standard deviation, coefficient of variation) provide information about the degree to which individuals prefer one stimulus over another, or ***choosiness*** (Fig. 1.2B). Individuals with small measures of dispersion are less choosy because they respond to stimuli in a relatively similar fashion: there are small differences in response to different stimuli. On the other hand, large values of dispersion come from individuals that choose certain stimuli with much greater intensity than others (Fig. 1.2). The most extreme response value (maximum for proportion, minimum for latency) is the ***peak-preference*** (Fig. 1.2C), representing the trait value that an individual most prefers. Linear regression of response measures on stimulus values returns a ***linear preference function*** (Fig 1.2D), which is analogous to a linear selection gradient (β) on the male trait (Lande and Arnold 1983). Quadratic regression coefficients provide an estimate of nonlinear selection acting on the ornament and are called ***quadratic preference functions*** (Fig 1.2E). Quadratic preference functions are analogous to quadratic selection gradients (γ) on the male ornaments (Lande & Arnold 1983), though more advanced methods of analysis are often used to characterize nonlinear and correlational selection surfaces. Finally, ***thresholds*** (Fig. 1.2F) occur when particular ornament values

elicit no response. Thresholds can occur for either small or large ornament values, and are indicated as x-intercepts on graphs plotting responses against ornament values. Note that we are not delving into the realm of mate searching and we have chosen not to do this for several reasons. First, the experimental designs used to detect searching strategies differ from those used to explicitly test preference components, per se. Second, mate searching has largely been treated separately in theory and empirical studies that characterize choice in a way that allows it to be decomposed into its component parts (e.g., responsiveness, choosiness, etc.). Theoretically, mate searching models do not take into account preference functions, as they mainly assume that preference for mate quality is open-ended (Janetos 1980; Real 1990). Empirically, there are studies that attempt to fit an observed search pattern to one of the expected patterns predicted by theory. However, none of these studies, to our knowledge, have measured phenotypic variation in individual-level components of choice. Without an estimate of phenotypic variation in the behavioral elements of mate choice there is no way to understand the evolutionary process of either male traits of female behaviors. Finally, the mate searching terminology is fairly well-defined and does not need a revision the way that mate choice terminology does.

Preference functions describe mate choice

Preference functions describe the probability that an individual will mate with a member of the opposite sex bearing an ornament of a given size, and provide the most biologically meaningful and comprehensive portrayal of mate choice. To this end, we focus on theory that models preferences in this way and find such treatments only in

quantitative genetic models of preference evolution. There are three additional reasons for focusing solely on quantitative genetic models. First, polygenic models are more biologically realistic than models involving only a few Mendelian loci (i.e., "oligogenic" models). Empirical measures of mate choice indicate that most preferences are continuously distributed, which suggests polygenic inheritance. Second, quantitative genetic models provide a framework for direct estimation of parameters describing the inheritance of preferences, facilitating an integration of empiricism and theory. In principle, key parameters of oligogenic models could be measured in empirical systems, but such an endeavor would require allele frequencies at the individual loci affecting preferences and thus far the genes underlying mate choice have not yet characterized in any natural system. Third, evolutionary dynamics differ between the two types of models, and we regard the quantitative genetic models as being more realistic. In oligogenic models, fixation of alleles is the end-result of trait-preference coevolution, whereas continued trait elaboration, often accompanied by the maintenance of genetic variation, is a likely outcome of a quantitative genetic model.

Turning to quantitative genetic models, Fisher (1930) first outlined a verbal model of indirect selection on mating preferences, which became a cornerstone of thought on the evolution of traits and preferences (Fisher 1930). Fisher's ideas were formalized by Lande (1981), whose treatment served as the foundation of all subsequent quantitative genetic theory on preference evolution (Mead and Arnold 2004). One important aspect of this model that was passed on to subsequent models of preference evolution was that Lande (1981) defined several types of preference functions describing

how females respond to phenotypic variation in ornamental traits. Lande (1981) posited two major types of preference functions, the parameters of which represent vastly different aspects of female behavior, though they are often treated as interchangeable. Figure 1.1 shows the preference functions for both population- and individual-level measures.

The psychophysical model specifies an open-ended preference function with all females in the population responding maximally to males with the largest ornaments ($\psi(z|y) \propto e^{yz}$), where *y* and *z* represent individual phenotypic values for females and males, respectively. Lande (1981) states "Individual females are assumed to differ in the degree of discrimination in mate choice, *y*." (p. 3722). A testable prediction emerging from this model is that all females rank the male traits in the same order of preference, and this prediction differentiates the psychophysical model from the unimodal preference model (see below; Fig. 1.1). Even though females do not vary in the rank order of males in the psychophysical model, they do vary in the likelihood of mating with males differing in ornament size, where some females show large differences per unit change in male ornament values (large *y*-values) and others show smaller increases in propensity to mate as male ornaments become larger (small *y*-values) (Fig 1.1B). Nevertheless, the fact that all females prefer the largest ornaments possible means that populations exhibiting open-ended preference functions will invariably experience directional intersexual selection on male ornaments (provided that the preferences translate into mating events).

In contrast, unimodal preferences are approximated by Gaussian functions and come in two forms that differ in the assumption of how the females choose among males. In the first case, absolute unimodal preferences, a variable, y, represent a female's peak preference: $\psi(z|y) \propto e^{-(z-y)^2/2v^2}$, and v is termed the "tolerance" for males that deviate from her peak preference (Fig. 1.1C). Another way to understand unimodal preferences is that each female has a particular ornament value (given by y) that maximizes her likelihood of mating. The probability that a female will mate with males deviating from her peak preference (y) is determined by her tolerance (v), which is usually assumed to be constant across all females. Thus, the parameter describing female preference values is a most-preferred male ornament value, which is an entirely different way of describing female preferences compared to the exponential regression coefficient used in the psychophysical model.

The second manifestation of the unimodal model assumes that preferences are relative, where the attractiveness of each male to a given female depends on his distance from the mean male phenotype of the population: $\psi(z|y) \propto e^{-[z-(\bar{z}^* + y)]^2/2v^2}$, where $\bar{z}^*$ is the mean male phenotype (Fig. 1.1E). Here, each female's trait value specifies the phenotypic distance of her peak preference from the mean male phenotype in the population (Fig 1.1F). As in the unimodal absolute preference function, the probability of mating with males that deviate from the peak preference decreases symmetrically according to the tolerance parameter. An added assumption of relative unimodal preferences is that all females have identical and accurate estimates of the mean male phenotype in the population.

The behavioral phenotype differs dramatically for each type of preference function model. Discrimination among males is an entirely different behavior than the ornament value that maximizes female response. Moreover, if females assess the mean male phenotype in the population, then preferences become relative and female preference values become much smaller (Fig. 1.1). We stress the importance of differentiating among these different preference functions when measuring individual-level preferences, which we discuss in the next section.

Empirical treatments of mate choice

In short, preferences can be measured on two scales: at the population-level and at the individual-level. We begin with a description of the basic experimental design for measuring mate choice and the type of data that are collected. Then we will demonstrate how population-level preferences can be estimated and contrast this to the estimation of individual-level preferences. Next we will introduce the various tools for analyzing preferences. Finally, we will demonstrate how empirical measures can be tied back to predictions generated by mate choice theory.

How are components of choice measured experimentally?

In 1998, Bill Wagner wrote a seminal paper on the measurement of mating preferences, where he explored the various advantages and limitations to different experimental techniques for measuring mate choice. Our goal here is not to rehash the content in Wagner (1998) and we encourage all students of intersexual selection to study his paper. Instead, we will provide a brief explanation of the types of assays that can be

used to phenotype mate choice behaviors, but for a detailed treatment of the topic, we direct readers to Wagner (1998).

There are a plethora of different types of mate choice assays, each of which incorporates its own assumptions and limitations. The basic approach is to expose a focal individual of one sex (usually females) to stimuli emitted by the opposite sex (usually males) and measure the focal individual's behavioral, physiological, or neural response. The number of stimuli can vary from a single-stimulus, or "no-choice" test, to multiple-stimuli "choice" tests. Stimuli can be entire individuals or artificial representations of putatively important phenotypic characters. The individuals may be physically separated in a way that only allows the transmission of stimuli in a particular sensory modality (e.g., two fish in different tanks that are allowed to see each other but not communicate via sound, chemical, tactile, or electric signals).

The data produced by a single assay can be either continuous or discrete. Discrete data often come in the form of presence or absence of response, represented by a binary variable. Conversely, continuous responses are often recorded as the latency to respond or the amount of time spent associating with a particular stimulus. In some situations, a continuous datum is converted into an index, which is often a proportion or ratio (e.g., time spent associating with individual *n* / total time spent associating). When replicated, the responses to a given stimulus may result in different variable types. Regardless of the details of each of these different methods, each will yield slightly different types of data each with implicit assumptions (e.g., association time = degree of preference vs. number of responses = degree of preference). Because these data are later

made analogous to preferences as they are modeled, their original form is going to be important.

There have been some studies that contrast the results based on the type of data used to record individual responses to stimuli. Bailey (2008) recorded a binary variable (response vs. no response) as well as a continuous variable (response latency) in a no-choice design. One important result that emerged from this study is that the form of sexual selection varies depending on the measure of fitness. Bailey (2008) reports linear selection when a binary measure was used and nonlinear selection when a continuous measure was used as the response variable. Drawing on our own work, we found that multiple results were qualitatively different based on the proxy for preference. In a fashion similar to Bailey (2008), we measured a continuous variable (copulation latency) and a binary variable (copulatory success). At the population-level, male phenotypic characters were more likely to have a significant effect on the binary variable than the continuous variable (Ratterman et al. 2012). Interestingly, when evaluated on the individual-level, the opposite is true: the continuous response variable yielded many more traits that had significant effects (Ratterman et al. 2012). Which of these two types of variables more accurately depicts preference? With respect to the natural setting of these lab-reared animals, preferences could be more important if they determine how fast a pair mates. However, if a trait will never lead to copulation, then it is unnecessary to know how long it will take before the onset of mating. For this reason it is important to measure both aspects of response to gain a fuller picture of how the behavioral responses are manifested.

Mate choice is often measured to infer the form of selection on ornamental characters or to estimate the nature of selection acting on mating preferences (Brooks et al. 2005; Chenoweth and Blows 2005). Population-level measures of mate choice incorporate individual-level variation in choice behaviors. For example, individual-level preferences for a given trait may be present and strong, but may result in no net population-level preference function: if individuals exhibit the same magnitude of preferences that differ in direction, the end result is a population-level preference function of zero: the preferences cancel each other out. However, if the distribution of choice phenotypes changes such that one type becomes more common, then population-level preference functions may become strong very rapidly. All too often a coarse approach of measuring mate choice at the population-level is employed and unwarranted evolutionary inferences are made with inadequate data, an all too common type-II error.

We stress the importance of measuring individual-level components of mate choice with as much precision (i.e., replication) at as many stimulus values as possible. This approach is laborious and time-consuming, but ultimately pays dividends. Without ample replication of individual-level measures, we are unable to quantify phenotypic variation occurring both within and among individuals. Estimates of heritability for behaviors are fairly low (~ 0.2: (Mousseau and Roff 1987), meaning that there is a considerable influence of the environment on phenotypic variation. Unfortunately, precise and accurate estimates of the behavioral components of mate choice require high replication within individuals and obtaining preference functions with considerable resolution requires testing multiple stimulus values. The majority of studies attempting

to characterize variation within individuals for mate choice behaviors provide two replicates which simply is not enough: population-level parameters are often replicated on an order of magnitude more than this. Individuals are known to be highly variable in their responses to identical stimuli, and increased levels of replication are needed for accurate estimates of each individual's mean response value.

Experimental procedures determine what type of data are collected, and when individuals are measured repeatedly with the same stimulus values, the data can take on a different form and be analyzed in various ways. For instance, Ritchie (1996) tested females four times with each stimulus value, recording a binary variable for each test. When the binary scores were summed across all four trials, each individual's preference for each stimulus was represented as an ordinal score. Thus, it is entirely possible that a binary measure can be converted into an ordinal score, which results in different analytical techniques. Furthermore, the type of data collected determines the validity of inferences that are made to theoretical representations of mate choice. Thus, care needs to be taken to appreciate the effects of experimental design on the nature of the data, and how this will impact the translation of empirically measured preferences to models of mate choice.

Analytical techniques for characterizing preference components

Provided that individuals are repeatedly tested with multiple different stimulus values, the data lend themselves to various types of analyses. Remembering that preference functions are intersexual selection surfaces on ornamental traits, it is possible to employ the extensive set of tools developed by formal selection theory in the analysis

(Lande and Arnold 1983; Brodie et al. 1995; Janzen and Stern 1998; Chenoweth and Blows 2006). However, the preference functions are only one of many behavioral characters contributing to mate choice (responsiveness, choosiness, etc.), necessitating the use of other, often more basic, analytical techniques.

For each individual with multiple scores, their mean response to all stimuli in all tests describes their responsiveness. Means are easily analyzed using analysis of variance with significant differences among individuals indicating that responsiveness varies among individuals. Estimates of dispersion about the mean (e.g., standard deviation) provide a proxy for individual choosiness. If individuals respond with high intensity to some stimuli and low intensity to others, then they can be described as being choosy with respect to the ornamental differences among those individuals. Choosiness measures vary considerably with respect to their computation. Gray & Cade (1999) calculated choosiness as the maximum response value minus the mean response (responsiveness) and divided this value by the standard deviation in response to all stimuli. Brooks & Endler (2001) computed choosiness (they call it discrimination) as the standard deviation of all responses. Bailey (2008) compared these two measures and found that they were significantly correlated with each other ($r = 0.702$), though we did not find the same result with our data ($r = 0.326$, $p = 0.358$). It should be noted that Bailey (2008) divided the difference in mean and maximum responses by the standard deviation in the maximum response, not the total response to all stimuli. Hedrick & Weber (1998) used the coefficient of variation as a measure of choosiness (they call it selectivity) and Fowler-Finn & Rodriguez (2012) squared the coefficient of variation as

an estimate of choosiness, drawing reference to the relevance of this measure to the computation of cubic splines (Schluter 1988). Thus, choosiness is quantified in multiple ways, which may have consequences for comparing this trait across different experiments and systems. As the goal of measuring the different components of mate choice is to gain estimates of independent aspects of behavior generating intersexual selection, we are at odds with using the coefficient of variation, as it integrates the mean into its computation. Thus, measures of responsiveness and choosiness become highly correlated and the two traits are unable to be treated independently.

Preference functions are estimated by equations relating variation in stimulus values to female responses, and hence, preference. These equations are returned from linear and quadratic regressions of response measures on stimulus values, and describe the shape of female preferences. The regression coefficients from these models are treated as the trait values for individuals and they report information on both the direction and magnitude of the preference function. Linear preference functions assume that female responses scale linearly with male trait values and describe open-ended preferences. On the other hand, quadratic preference functions allow for curvature to be included in the shape of the preference by examining how responses scale with the squared values of stimuli. Positive quadratic regression coefficients are interpreted as disruptive preferences, where extreme values of the ornament are more attractive than intermediate values. Conversely, negative quadratic regression coefficients denote stabilizing preferences, where intermediate values of the ornament are most attractive. These two regressions are part of the well-established methodology of estimating

selection on various traits (Lande & Arnold 1983) and are widely used (Kingsolver et al. 2001). Both forms of preference can be acting simultaneously. If, for example, there are significant positive linear and negative quadratic regression coefficients, this means that while the largest stimulus values are more attractive than the smallest, intermediate values are most attractive. In other words, smaller intermediate values are less attractive than larger intermediate values.

A major advantage of measuring individual-level preference functions is that the data required to do so will also provide estimates for individual-level responsiveness and choosiness. Taken together, these trait values can be organized into variance-covariance matrices as a way to evaluate phenotypic integration of the different components of choice. Unfortunately, some experimental designs do not lend themselves to this approach, especially when individuals are not measured for all of the components. Specifically, using isogenic lines to estimate breeding values for the different components of choice means that each individual is tested only once. While this approach has the advantage of independence among tests and no carryover effects of repeated testing, each individual of a given genotype only contributes one datum to the estimate. Thus, all of the traits are attributes of the line and not individuals, making variance-covariance matrices impossible to estimate.

McGuigan et al. (2008) employed function-valued trait analysis (Kirkpatrick and Heckman 1989) as another means of sidestepping the problem of repeated testing of individuals. This type of analysis uses a random-coefficient model to analyze preference functions and can be extended to multivariate phenotypes where multiple ornaments are

assessed simultaneously (McGuigan et al. 2008). In this case, individuals are tested once, and the preference functions are estimated by repeated measures of families. Random-coefficient modeling provides estimates of variance-covariance matrices, though none have included components of choice other than preference functions into the analyses, though this method seems promising for future studies.

Relating empirical results back to theory

Some studies make direct comparisons to theory – they identify peak preferences and treat them as an individual's preference value, or they compute choosiness and treat that as the trait value to test for genetic variation or the fitness consequences of different behavioral phenotypes (Gray and Cade 1999; Qvarnstrom et al. 2006; Shaw and Lesnick 2009). Problems arise, however, when incorrect parallels are drawn. For example, if the shape of a preference function is unimodal, then the preference value in relation to theory is the peak preference. If the shape of the preference function is open-ended, then the trait value for each female is, strictly speaking, the exponential regression coefficient when the ornament values are analyzed on a log scale (Lande 1981). No study, to our knowledge, has used an exponential regression to estimate preference functions, so direct tests of the psychophysical model are nonexistent. It would be inappropriate to use the peak preference as the measure of the individual preference if the shape of the preference function is open-ended. Conversely, individual preference values are sometimes described by regression coefficients when the preference functions assume unimodal shapes. This inappropriate assignment of trait values may not be a big problem; analytical theorists suggest that the actual form of the preference function does

not change the dynamics of the models (Lande 1981; Kirkpatrick 1985, 1986; Hall et al. 2000). However, we think that this should be tested experimentally: what happens when different components of choice are added to models of sexual selection? To be sure, only two of our five proposed choice components is explicitly modeled in quantitative genetics theory (peak-preference and open-ended preferences). The discordance between theory and data on mate choice can be resolved by more accurate models of elements of behavior contributing to choice, and to more empirical estimates of the traits that are explicitly modeled.

Concluding remarks

Intersexual selection is an interesting topic and there are many unanswered questions, which leaves the field ripe for future studies. Collectively, there is a huge potential to unveil general patterns pertaining of the evolution of mate choice, but this requires a concerted effort focused in the same direction. Addressing these questions as a scientific community necessitates agreement among individual research groups on the terms used to describe components of mating behavior, what needs to be measured, how they need to be measured, and how to analyze them. Progress in the field has been hindered by miscommunication and lack of a common set of terms used to describe components of mating preferences. All too often valuable data sets go unanalyzed with respect to a standing problem in the study of mate choice, though they have the necessary data and power to make a substantial contribution to a the field. Similarly, many researchers do not collect data in a way that is informative to theory. Theory forms the basis for asking empirical questions, as all hypotheses are generated in some sort of

theoretical framework. Thus, to better understand why phenotypes have evolved to be the way they are, theory needs to be tested and refined. Theoreticians will benefit from modeling measurable parameters that are unambiguous and potentially suggest a measuring strategy in their papers to guide empiricists. Given the increasing number of labs working on mate choice, the power to address long-standing questions in evolutionary biology is Herculean. However, the realization of this power within our discipline becomes weak if we do not make a concerted effort to accomplish a unified goal.

CHAPTER II

THE FISHERIAN MECHANISM OF SEXUAL SELECTION: A PARAMETER SPACE ODYSSEY

Introduction

Though he touched on it in *On the Origin*, Darwin was delayed twelve years in unveiling his book on sexual selection, which served as an explanation for bizarre traits like a peacock's tail. Sexual selection, Darwin posited, can come in two forms depending on which sexes interact (Darwin 1871). *Intra*sexual selection arises from fitness variation caused by interactions between members of the same sex, whereas *inter*sexual selection arises from fitness variation caused by interactions between the sexes (Andersson 1994). Intrasexual selection remained the leading explanation for the evolution of secondary sex traits, though many contemporaries rejected intersexual selection on the grounds that females, as the inferior sex, did not have the mental faculties to actually choose their mates (Prum 2012).

An automatic consequence of the process of mate choice is that offspring inherit their parents' genetic predispositions that led them to pair. The progeny of a given pairing will inherit alleles from their father that made him attractive to their mother. Similarly, alleles contributing to their mother's mating preference will be inherited by both sons and daughters. If there is heritable variation in both phenotypes, then in a single generation a genetic correlation forms simply due to the process of mate choice: alleles contributing to the ornaments and preferences are coinherited, which results in

linkage disequilibrium (Lande 1981). When two traits are genetically correlated, selection on one trait can cause a correlated response in the other trait (Lande 1980, 1981; Lande and Arnold 1983). With regard to mate choice phenotypes, this means that preferences exert sexual selection on ornaments, which in turn generates sexual selection on any traits correlated with ornaments: preferences generate sexual selection on ornaments as well as themselves via their genetic correlation with the ornaments. This mechanism of ornament-preference coevolution was originally proposed by RA Fisher and is thus known as the Fisherian mechanism or the Fisher process (Fisher 1915; Fisher 1930; Andersson 1994).

Since the genesis of the idea nearly 100 years ago, the Fisherian mechanism has been modeled extensively using population and quantitative genetic models (O'Donald 1980; Arnold 1983; Arnold 1985; Mead and Arnold 2004). Because the vast majority of ornaments and preferences are quantitative traits controlled by many loci with continuous phenotypic distributions, we focus here primarily on the quantitative genetic models of the Fisher process (for a further justification see Arnold 1983 p. 68). In 1981 Russ Lande derived the first of these models, which has served as the foundation upon which nearly all subsequent quantitative genetic models of ornament-preference coevolution have been built (Mead & Arnold 2004). The standard quantitative genetic framework for the model is comprised of genetic, behavioral, selection, and equilibria components, which we will discuss in turn.

Genetic assumptions

Both ornament (z) and preference (y) are assumed to be sex-limited phenotypes, which vary phenotypically due to additive allelic effects at multiple autosomal loci as well as environmental effects. As a result, both ornaments and preferences are assumed to be normally distributed with means $\bar{z}$ and $\bar{y}$ and phenotypic variances σ^2 and τ^2, respectively. The additive genetic variances for the ornament (**G)** and for the preference (**H),** as well as the additive genetic covariance between the sexes (**B**) are assumed to be in mutation-selection balance, and thus are treated as constants. The **B**-matrix can be understood as the covariance of the additive effects of ornamental loci in males with the additive effects of preference loci in females, which is caused by either pleiotropy or linkage disequilibrium. In this particular model, **B** is assumed to be controlled by linkage disequilibrium caused by mate choice.

Behavioral assumptions

Females mate only once and males can mate multiply: the genetic mating system is polygynous where males provide no parental care. The probability that a given female in the population will accept a male with trait value z depends on that female's preference function $\psi(z|y)$, which may be expressed in three forms. One way to model female mating behavior is to assume that all females in the population most prefer males with the largest ornaments:

$$[1]\ (\psi(z|y) \propto e^{yz})$$

which Lande (1981) referred to as the psychophysical model of mating preference. In this model females vary in the slope of their preference function based on their phenotypic value (y), where females with large y values exhibit stronger discrimination among males, but all females in the population most prefer males with the largest ornaments.

Conversely, female mating behavior can be modeled using Gaussian functions, which come in two forms. Preferences are absolute when females assess each male independent of the distribution of males in the population:

$$[2]\ \psi(z|y) \propto\ e^{-(z-y)^2/2v^2}.$$

Preferences are relative when females assess males relative to the mean male phenotype after natural selection:

$$[3]\ \psi(z|y) \propto\ e^{-[z-(\bar{z}^*+y)]^2/2v^2}.$$

In both cases the female phenotype y represents the ornament value that a female most prefers and the probability of mating with males that deviate from the female's peak preference is described by what Lande termed tolerance (v^2).

Selection

Males experience stabilizing natural selection which is specified by a Gaussian function with an optimum θ and width ω^2, such that the non-reproductive fitness of a male with phenotype z is expressed as:

$$[4]\ w^*(z) =\ e^{-(z-\theta)^2/2\omega^2}.$$

After natural selection, males are subjected to sexual selection, the form of which is specified by the female preference function. Thus, the net relative fitness of a particular male is computed as the product of viability and mating success, which is averaged over the entire female population:

$$[5]\ W(z) = w^*(z) \int q(y)\psi^*(z|y)dy,$$

where $q(y)$ is the distribution of female phenotypes and $\psi^*(z|y)$ is the relative mating success of males that survived viability selection.

In Lande's original model there was no selection on females, so mean population fitness did not change (Lande 1981, eq [6]). Thus, the mean fitness in the population is

$$[6]\ \bar{W} = \int p(z)w^*(z)dz,$$

which can be used to calculate the total selection differential on the males:

$$[7]\ S = \bar{W}^{-1} \int zp(z)W(z)dz - \bar{z}$$

$$= \int q(y) \int zp^*(z)\psi^*(z|y)dzdy - \bar{z}.$$

The deterministic response to selection in males and the correlated response in females are given as

$$[8]\ \Delta\bar{z} = \frac{1}{2}GS/\sigma^2 \qquad \Delta\bar{y} = \frac{1}{2}BS/\sigma^2,$$

and it is from these that the evolutionary trajectory of the population can be shown to be

$$[9]\ \Delta\bar{y}/\Delta\bar{z} = B/G,$$

assuming that genetic variances and covariances are constant over time (Lande 1981).

Equilibria and stability

An equilibrium is reached when the two traits stop evolving ($\Delta\bar{z} = \Delta\bar{y} = 0$), and by using the deterministic response to selection [8] and selection differential on males [7] it can be shown that the population will equilibrate on all points of the line

$$[10]\ \bar{y} = (\alpha + \varepsilon)\bar{z} - \alpha\theta,$$

where the psychophysical model represents $\alpha = 1/\omega^2$ and $\varepsilon = 0$; the unimodal models represents $\alpha = v^2/\omega^2$ and $\varepsilon = 1$ for absolute or $\varepsilon = 0$ for relative preferences.

The next stage of the model analyzes the stability of the equilibria, which will determine whether or not the population can experience runaway sexual selection (Fisher 1930). Fisher imagined that under certain circumstances, sexual selection imposed on the males by the female mating preferences, as well as the preferences' correlated response to selection, would result in a positive feedback loop where the ornaments and preferences would increase at a geometric rate, essentially running away in value (Fisher 1930, Ch. 6). If the equilibrium is stable, then there is no possibility that the population would experience runaway sexual selection. Lande found that there is a stable and an unstable case, where the stability is determined by the relative slopes of the line of equilibria [10] and the evolutionary trajectory of the population [9]. If the evolutionary trajectory of the population is steeper than the line of equilibiria, then the population will never reach equilibrium, and thus runs away. Specifically, the system is unstable if $B/G > \alpha + \varepsilon$, which means that the population may experience runaway sexual selection if the additive genetic covariance between the ornament and the preference gets sufficiently large. The right side of the inequality represents the balance that is struck

between natural selection on the ornament and sexual selection driving the ornament away from its naturally selected optimum (Lande 1981, eq. [10]). Thus, if the genetic correlation between the ornament and preference is sufficiently strong and sexual selection is sufficiently strong relative to natural selection, then the population will experience runaway sexual selection until some of the assumptions of the model are broken (e.g., weak natural selection on males, etc.). Populations that do not experience runaway sexual selection are bound to a stable equilibrium: if the population drifts away from the line it will be attracted back to the line, though it will likely end up at a different point on the line (Lande 1981).

Lande's model has been modified to include spatial structure (Lande 1982; Day 2000), mutual ornamentation (Lande and Arnold 1985), direct selection on female preferences (Kirkpatrick 1985, 1986; Pomiankowski et al. 1991; Pomiankowski and Iwasa 1993; Iwasa and Pomiankowski 1995; Hall et al. 2000) and finite populations (Nichols and Butlin 1989; Uyeda et al. 2009). However, no models to date have tested the assumption that **G**, **H**, and **B** stay constant over time. Importantly, the entire Fisher process hinges on the genetic correlation between the ornament and preference: without it there is no way for preferences to evolve as a correlated response to the sexual selection that they generate on male ornaments. Can the Fisher process work without constant genetic variances and covariances? What magnitude of a genetic correlation will be required for ornament and preference elaboration? What happens under conditions that facilitate an unstable genetic correlation (e.g., small population size)? We

addressed these questions using individual-based simulation modeling, which allows us to relax many of the analytical assumptions.

Methods

We added sexual selection to previous individual-based simulations built by Jones et al. (2003). In these populations, every allele of every locus of every individual was explicitly modeled, so assumptions about infinite population size, infinite number of alleles, and infinite number of loci were naturally relaxed. Individuals were diploid and sexually reproduced by passing on a single allele at each of *n* loci to their offspring in non-overlapping generations. The allelic effects at each locus were strictly additive. Individual phenotypes were determined by summing the allelic effects to obtain the genetic (breeding) value of an individual, to which an environmental effect randomly selected from a bivariate normal distribution with a mean of zero and variances ϵ_z^2 and ϵ_y^2 (no environmental covariance) was added.

Starting conditions

Each run began with a genetically homogenous population where each individual had a value of zero for each locus, and thus a breeding value of zero. Genetic variation is introduced into the population by mutations at a rate μ, the effects of which are drawn from a bivariate normal distribution with a mean of zero and variance specified by α_z^2 and α_y^2. Mutational effects were added to the existing allelic values according to the continuum of alleles model (Crow and Kimura 1964). If the loci were pleiotropic, then the degree to which a mutation affecting one character corresponds to the mutational effects on the other character is determined by the mutational correlation r_μ. Populations

were allowed to reach mutation-selection-drift balance within 1,000 generations prior to the experimental runs. The life cycle consisted of random mating, progeny production, natural selection, drift, followed by random mating.

Life cycle

After the population reached mutation-selection-drift balance the experimental phase began. For mating, females randomly sampled individuals from the population. Each female mated once and was given a maximum of *m* encounters with other individuals, which could be either male or female. Once mate choice was added to the model, *m* imposed a cost to extreme female mating preferences because females with extreme preferences would be unlikely to mate if they were able to sample few individuals. The probability that a female would accept a mate was determined by a unimodal relative preference function (eqn. 3). Each female produced four offspring and these underwent mutation as described above. After mutations entered the population the offspring were subjected to sex-limited natural selection (eqn. 4) where ω_z^2 and ω_y^2 specified the width of the Gaussian function for males and females, respectively. At this point the population was regulated to its carrying capacity by randomly selecting *k* individuals that survived viability selection. If the population was below *k*, then all individuals made it to adult status and could potentially mate.

Exploring parameter space

It is useful as a first pass to understand the effects of each parameter in isolation, so accordingly, we varied each parameter while holding all others constant. Each parameter was assigned a standard value to be used when other parameters were varied

(Table 1.1). The degree of pleiotropy, *d*, refers to the proportion of pleiotropic loci compared to independent loci, where 0% indicates that all loci are independent and 100% indicates that all loci are pleiotropic. The number of loci was standardized to 50, which means that 50% pleiotropy resulted in 25 independent and 25 pleiotropic loci controlling each trait. Environmental variance values describe the width of the bivariate distribution of environmental effects with a mean 0. Maximum mating encounters, *m*, is described in the section titled "life cycle" above. Mutation rate is measured as μ mutations per locus per generation. For pleiotropic loci, a mutational correlation r_μ determines the probability that a mutational effect on an ornament will affect the preference in a predictable way. All runs with mutational correlation used a fully pleiotropic genetic architecture with 50 loci. The carrying capacity, *k*, determines the number of juveniles that survive viability selection that are allowed to enter the breeding pool. Because the population size can be lower than *k*, we have opted to not call this parameter "population size" as it may be misleading under certain circumstances. The strengths of selection describe the width of the Gaussian fitness surface, with 0 indicating no selection and smaller nonzero values representing strong selection (narrow fitness function). Finally, tolerance, v^2, describes the width of the individual-level preference functions with smaller values representing more discriminating females. See Jones et al. (2003) and Uyeda et al. (2009) for justifications of the values used.

Measurements

Appendix D summarizes the measures made each generation. For each run, we measured the mean breeding value and phenotypic value of the population at different

stages of the life cycle. From these means the phenotypic and genetic variance-covariance matrices (**P** and **G**, respectively) can be estimated, as well as the phenotypic and genetic correlations (r_p and r_g, respectively), the eigenvalues (λ_1 and λ_2) of the **G**-matrix, as well as various measures of selection. For example, immediately following natural selection the covariance between ornaments (or preferences) and its bearer's individual viability (probability of survival from the Gaussian fitness function) provides a measure of the natural selection differential on the traits. The opportunity for sexual selection was measured as the variance in relative mating success, while the mating differential was measured as the covariance between ornament (or preference) values and mating success (Jones 2009). The natural selection differential was measured in two ways: once as the covariance in trait values and viability (see above) and another as the change in the means from before to after selection. This latter measure takes into account the trait values of both sexes, though selection is sex-limited. The effect of genetic drift was computed as the change in the mean caused by the random selection of survivors of viability selection. Direct sexual selection was measured as the mating success-weighted phenotypic mean minus the mean after random selection of adults (drift) for both the ornament and preference. The total change in the phenotype in one generation was measured as the mating success-weighted mean minus the mean before natural selection.

Indirect selection occurs when the fitness of a trait is determined across a generation (Lande 1981). We measured the strength of indirect selection in all possible paths from progeny fitness to parent phenotypes. For example, males bearing certain ornament values may leave behind sons or daughters with higher viability and / or

mating success. Preferences can evolve in the same way. To be sure, the indirect fitness gained by preferences due to the mating success of sons is the definition of the Fisher process (Fisher 1915, 1930; Lande 1981). Therefore, for a complete picture of the source and intensity of indirect natural and sexual selection, we measured the covariances between parental phenotype values and aspects of their sex-specific offspring's fitness. The last eight rows of Appendix D identify these various sources of indirect selection.

For each combination of parameter values the simulations were replicated twenty times. From these twenty runs we calculated the mean and variance for each variable in Appendix D. To obtain an estimate of the mean of some variables it was necessary to use the absolute value to compute the mean because the variable took both positive and negative values, which cancel out to a value near zero. Thus, the magnitude of the variables is represented in the mean of the absolute values. Appendix D identifies which means were calculated with the absolute value.

Results

Despite the complexity of the model, we were able to identify several distinct patterns in the data (Tables 2.2-2.5). Based on the results from previous models where genetic variances and covariances were allowed to evolve (Jones et al. 2003) we did not expect to observe patterns that resemble the Fisherian mechanism, let alone runaway sexual selection. However, Fisherian sexual selection does operate in small populations, and thus, unstable genetic correlations. We found unique patterns similar to those found analytically (e.g., cyclical evolution of ornaments and preferences). Extremely similar patterns emerged by varying different parameter values (e.g., increasing the number of

loci has the same effect as increasing the mutational variance on the traits: both increase the probability of cyclical evolution). As the goal of this study is strictly phenomenological and descriptive, we will present the major effects of varying each parameter in turn. Finally, we will identify common patterns that we observed by varying different parameter values to gain insight into the mechanistic basis of what drives these patterns.

Degree of pleiotropy

As the genetic architecture of the two traits becomes more pleiotropic, three major patterns emerge. First, the degree of phenotypic elaboration of the ornament and preferences seems to be relatively insensitive to the degree of pleiotropy. In other words, when 0% of the genes are pleiotropic for the ornament and preference, the phenotypic values of the characters become as elaborated as when 100% of the loci are pleiotropic (Fig. 2.1A).

The genetic correlation between the ornament and preference is affected by the degree of pleiotropy, with more pleiotropy leading to an increase in the variation in r_g (Fig. 2.1B). When the genetic bases of the traits are independent, the only way that a genetic correlation may arise is through linkage disequilibrium caused by positive assortative mating with regard to ornament size and preference size (Lande 1981). Mutational correlation causes an automatic genetic correlation at a pleiotropic locus (Jones et al. 2007), but because r_μ was set to zero as a standard value (Table 2.1), the pleiotropic loci will not automatically lead to a genetic correlation. Interestingly, as the

degree of pleiotropy increases, r_g becomes less stable, which suggests that even mutationally independent pleiotropic loci will lead to a genetic correlation.

One intriguing result is that the level of genetic variation decreased with more pleiotropy (Fig. 2.1C-D), while the genetic correlation increased with increasing pleiotropy (Fig. 2.1B). Because correlations require variation in both parameters being correlated, this finding is surprising. When the genetic bases of the traits are shared it appears as though there is less overall genetic variation, though the variation present is arranged in such a way that the genetic correlation increases despite a mutational correlation of zero.

Maximum mating encounters

As the number of mating encounters increases, the chances that each individual female will find their optimal mate increases. The maximum number of mating encounters can also be seen as a cost to choice: females with large preference values will not likely mate when they can only sample a handful of males. As a consequence of this cost to extreme preferences, phenotypic elaboration decreases when females are allowed few encounters with males (Fig. 2.2A-B). The cost of choice decreases the probability that ornaments will become highly elaborated, which is well established within sexual selection research.

The genetic correlation tends to stay small and positive regardless of the number of mating encounters, which is likely attributable to the stable effective population size. Despite the small genetic correlation, the intensity of indirect sexual selection on the ornament and preference via son's mating success is relatively strong (Fig. 2.2C-D).

However, there is an asymmetry in the sexes with males exhibiting a more pronounced change due to the number of mating encounters (Fig. 2.2C), whereas indirect sexual selection acting on females increases slightly (Fig. 2.2D).

Mutation rate

The dynamics of the system were surprisingly robust to mutation rate. There were few detectable changes in phenotypic values, genetic correlations, levels of genetic variance, and selection intensities. On average, the phenotypic values became slightly larger as the mutation rate increased, though the magnitude of this effect is quite small.

Mutational correlation

We varied mutational correlation under a strictly pleiotropic genetic architecture (100%) and the effects were extreme. First, the probability of a population going extinct increases dramatically once r_{μ} increased above 0.1. Part of this is likely caused by the large intensity of indirect selection acting on the preference via son's mating success (i.e., the Fisherian mechanism). As each run begins, the preference drifts away from a value of zero and automatically imposes sexual selection on the ornament. The genetic correlation also starts out at a positive value that is approximately the same as the value for the mutational correlation. Thus, the preference evolves as a correlated response to sexual selection on the ornament and, because the genetic correlation is so high, the preference evolves very rapidly. Naturally, the effective population size drops dramatically because few males obtain all of the matings, genetic variation is lost, and eventually the populations go extinct if the mutational correlation is high enough (Fig. 2.3).

A surprising result from varying r_μ is that the genetic correlation can become strongly negative despite a strong positive mutational correlation. As mutations enter the population a pleiotropic loci, their effects on the two traits are governed by the mutation correlation: if it is positive and large, then the effects of the mutation on the two traits will be positively correlated and a genetic correlation is automatically formed. Oddly, as the effective population size decreases and drift causes the genetic correlation to become unstable, r_g will actually become negative and approaches a value of -1 despite mutations entering the population in a manner that would result in the exact opposite result (results not shown).

Mutational variances

As mutational variance increases, so too will phenotypic variation. However, our results show an asymmetry in the effects of varying mutational variance on the ornament and preference. Increasing ornamental mutational variance has very little effect on the system (Fig. 2.4A, C, D). The phenotypic values, levels of genetic variance, genetic correlation, and measures of selection resemble that of the standardized parameter runs: there is no clear effect. Conversely, increasing the mutational variance in the preference has several effects. First, when mutational variance on the preference is large, the populations tend to enter a cyclical pattern of evolutionary exaggeration and diminution of the ornaments and preferences (Fig. 2.4F). Moreover, the genetic correlation, while still relatively small (< 0.2), is larger than it is when ornament mutational variation is varied. Thus, by making the source of sexual selection more variable, the system becomes more evolvable (i.e., trait values change more rapidly through time).

Interestingly, the genetic variance in the ornament does not get depleted, thereby facilitating a response to selection.

Number of loci

The number of loci controlling the traits effectively changes the size of their mutational target, with more loci increasing the mutability, and thus, evolvability of the traits. When ten loci control each trait there is little phenotypic elaboration compared to when 100 loci control each trait. Moreover, when the number of loci per trait increases the phenotypes enter cyclical patterns of evolution: the cycle period decreases as the number of loci increases (Fig. 2.6). In other words, as the mutational target increases because of more loci, the ornament and preference have more genetic variation to respond to selection, and thus can evolve more rapidly. As a result, the length of time for each period of cyclical evolution decreases as the traits become more evolvable.

The genetic correlation becomes stronger as there are more loci. In runs with 100 loci, the genetic correlation slowly increases over time to a value of ~ 0.1; in runs with ten loci the genetic correlation stays very close to zero and does not change over time (Fig. 2.7). Intuitively, the levels of genetic variation increase as the number of loci increases, which likely causes of the buildup of the genetic correlation. As the genetic correlation builds up, the intensity of indirect selection increases for both phenotypes. Moreover, the variation in the intensity of selection over time decreases with increasing loci: selection becomes more consistent and strong as the mutational target of the two traits increases (Fig. 2.5).

Carrying capacity

Carrying capacity was varied to test for the effects of genetic drift on the system. With respect to phenotypic evolution, larger populations tended to evolve more extreme trait values and this was likely caused by the increase in genetic variance in the two traits. Small populations exhibited instability in their genetic correlations, and the magnitude of r_g was greater than in large populations, though it changed rapidly (Fig. 2.8). One consequence of a lack of genetic variance is that small populations would evolve an exaggerated ornament that would become "fixed", in that no genetic variance was available for a response to selection in either direction. Thus, the ornament value would plateau at a particular value and would not change (Fig. 2.9).

Strengths of selection

An interesting case arises when we eliminate natural selection on both traits because it shows the effects of sexual selection in isolation. When there is no natural selection on either trait the ornament evolves to values that are nearly two orders of magnitude larger than when there is weak stabilizing selection on the ornament (Fig. 2.10A). The female preference, however, does not change indefinitely: it plateaus at a value of ~ |6|, which imposes very strong sexual selection on the ornament (the mean female preference in the population is 6 phenotypic standard deviations from the mean male phenotype, meaning that few males receive all the matings). It is possible that the plateau in female preference is caused by sexual selection on females because females with extreme preferences do not end up mating. The results show that the intensity of direct sexual selection on the preference is relatively weak (Fig. 2.10B). However, when

indirect sexual selection on the preference is explored it becomes apparent that there is relatively strong indirect sexual selection on the preferences, but instead of it being mediated through the son's mating success (Fig. 2.10D), it is through the daughter's mating success (Fig. 2.10C). The daughters of mothers with extreme preferences are selected against because they do not end up mating with any males; their mating success decreases, and thus they are sexually selected against. An equilibrium is reached by the additive effects of selection for larger preference and selection for larger preferences, resulting in no net selection.

Another interesting pattern emerges when natural selection on the ornament was set to be very weak ($\omega^2 = 199$): many of the replicate populations went extinct. This means that when the force of natural selection is very weak compared to sexual selection, the ornament becomes so exaggerated and deviates so much from the naturally selected optimum that even weak natural selection will cause population extinction. In cases where the effective population size drops dramatically, the genetic correlation becomes erratic as described above.

Strong selection on either trait hinders the evolution of both traits. Because this is a coevolutionary dynamic, the consequences of selection on a single trait extend to the other trait. However, it appears as though the system is much more sensitive to selection on the preference. Even when selection on the preference is extremely weak ($\omega^2 = 499$), the ornament does not evolve to values greater than approximately 6 or 7, whereas the same level of selection on the ornament would lead to a trait value two orders of magnitude larger. Thus, the evolutionary dynamics are sensitive to selection on the

preferences, which is in line with results from analytical theory (Lande 1981; Kirkpatrick 1986; Pomiankowski et al. 1991; Hall et al. 2000).

Tolerance

Tolerance, or the width of the individual-level unimodal preference function (v^2), strongly affects the degree of elaboration of ornaments, but not preferences (Fig. 2.11A and B, respectively). First, when v^2 is small, individuals are highly selective about their choice of mate: males that deviate from each female's preference value have a small chance of mating. Consequentially, females with extreme preference values are unlikely to mate, so intolerance is a way to impose costs to preference in this model. Oddly, when v^2 is small, the population oscillates between phenotypic values that are fairly large relative to the phenotypes evolved in all other parameter combinations (Fig. 2.11A). Moreover, it isn't only the phenotypic values that oscillate: genetic variance and N_e oscillate, which causes r_g to become unstable at some points (Fig. 2.11B-D). Similarly, the different forms of selection oscillate according to the same pattern, though the cause of these universal oscillations remains a mystery.

As the level of tolerance increases, the intensity of sexual selection on the male ornament and, thus, indirect sexual selection on the preference decrease: females mate with males that may deviate largely from the female's peak preference, thereby decreasing the chances that their sons will resemble the female's optimal mate. This result, coupled with the decrease in genetic correlation in runs with few sampled males, further impedes the preference to evolve as a correlated response to selection.

Emergent properties

Several patterns occur across different parameter combinations and these may shed light on their mechanistic basis. First, despite evolving genetic correlations, we observed very rapid evolution of the ornament, but not the preference. This result is entirely a product of the preference function being *relative* instead of *absolute*. If preferences were absolute they would scale identically with the male ornament and would thus require much more phenotypic evolution. For constant directional sexual selection on the ornament relative preferences only need to evolve to a nonzero value. Regardless of the type of female preference function, our results show what appears to be runaway sexual selection under a broad range of conditions.

Second, multiple parameter combinations produced cyclical ornament-preference coevolution. Increasing the number of loci controlling the traits decreased the period of the cycles, which is the same pattern as decreasing tolerance or increasing the mutational variances of either trait (Figs. 2.4, 2.6, 2.12). In some cases it appears that an increase in genetic variance (either through larger mutational effects or simply a larger mutational target) is the cause of cyclical evolution.

Third, population extinction was observed in response to variation in carrying capacity, the number of mating encounters, mutational correlation, and the strength of natural selection on the ornament. One common feature of population extinction is that the effective population size dropped dramatically due to sexual selection acting strongly on males, where few males obtained all of the matings. Carrying capacity exacerbated this by starting with few individuals. Increasing the number of mating encounters

allowed females to be highly selective in choosing their mate. As the preferences evolved to larger values the females eventually ran out of males that were acceptable mates in the population, which caused immediate extinction. The mutational correlation created an automatic genetic correlation, which facilitated the evolution of the preference via the Fisherian mechanism, leading to runaway sexual selection. Finally, when natural selection was sufficiently weak on the male ornament, the population evolved extreme trait values that deviated from the naturally selected optimum so much that in a single generation no males survived to reproduce. This outcome is surprising because it is counterintuitive that weak selection would drive a population to extinction. However, in this case it appears as though sexual selection drove the population so far from the naturally selected optimum, which was allowed due to such weak natural selection, that the genetic load was so great that the population could not persist.

Fourth, in some cases the phenotypic values would become fixed over thousands of generations, which was caused by a loss of genetic variance for the traits (Fig. 2.9). This plateau in the phenotypic values was influenced by degree of pleiotropy, carrying capacity, and strength of selection. When the genetic architecture of the ornament and preference is 100% pleiotropic, The Fisherian mechanism leads to runaway sexual selection, which is halted when the male ornament runs out of genetic variance, at which point it is at a fixed value in the population. Small carrying capacities had low genetic variation to begin with, and thus were more likely to deplete the little amount of variance that was present. In both these cases we see that the ornament is more likely to plateau. However, an interesting exception arises when there is no selection on either trait. In

these runs the female preference evolves rapidly in concert with the male ornament but then becomes fixed at a value of ~ |6-7|. Oddly, this pattern does not seem to be driven by a loss of genetic variance, which is present and fairly large. Instead, it appears as though the covariance between a female's preference and her daughter's mating success selects against extreme preference values. While the intensity of this form of indirect selection is smaller than the intensity of direct selection, it is likely enough to stabilize the preference values.

Finally, and potentially the most influential result, is that preferences are highly unstable. From one generation to the next the preferences can change from a positive to a negative value and impose sexual selection in entirely different directions. It is likely that drift plays a major role in this: as preferences reach a state of quasi-equilibrium drift will change the mean preference value thereby changing the dynamics of the entire system and moving the preference to a new quasi-equilibrium. Because the preferences represent an evolving selection surface for the male ornaments, the instability of the preferences has the potential to drive most of the major patterns we discovered.

Discussion

The goal of our study was to explore the consequences of each parameter individually on the dynamics of the Fisherian mechanism. Because the Fisher process requires a genetic correlation between an ornament and a mating preference, and because under certain circumstances the genetic correlation becomes unstable, we expected that the Fisher process would not occur. However, our results indicate that indirect selection on the preference can be quite large, and that preferences respond to selection despite

having diminishingly small genetic correlations. Moreover, we found a set of emergent patterns that were caused by different parameters, and in some cases, for entirely different reasons.

Defining runaway sexual selection

One difficulty in our simulations was how to define runaway sexual selection. Using analytical solutions, Lande (1981) found that when the slope of the evolutionary trajectory of the population was steeper than the line of equilibria (the balance between natural and sexual selection on the ornament), then the population would evolve away from the equilibria at an ever increasing rate. However, Lande's solutions require very strict assumptions: variances and covariances do not evolve (required for the deterministic solution for the evolutionary trajectories) and selection on the ornament variances is weak (required for the solution of the line of equilibria). In our model we do not make these same assumptions, making Lande's definition of a runaway difficult to interpret. To be sure, in each run we calculated whether or not the slope of the evolutionary trajectory (**B**/**G**) was greater than the line of equilibria (ν^2/ω^2) and the vast majority of the time the system appeared to be stable, despite rapid evolution. Ultimately, when the major assumptions of Lande (1981) are relaxed, the analytical solution for the stability of the system are not applicable, which requires a new definition for runaway sexual selection that is more applicable to natural populations.

Cyclical evolution

Lande & Arnold (1985) found conditions that would facilitate evolutionary oscillations (p. 657), which was also present Lande (1981), though not explicitly

described. These conditions depend on levels of genetic variance and covariance, the relative strengths of natural and sexual selection, as well as the type of preference function. Iwasa & Pomiankowski (1995) modified one of their previous models (Pomiankowski et al. 1991; Pomiankowski & Iwasa 1993) by changing the shape of natural selection on the male ornament. They justified using a fourth-power function instead of a traditional second-power (Gaussian) function by arguing that in some cases selection is very weak near the optimum and then becomes very strong as phenotypes deviate beyond a certain point. By changing the shape of the natural selection fitness function, Iwasa & Pomiankowski found cyclical patterns of ornament exaggeration and diminution. We confirmed their results using a traditional second-power fitness surface and the pattern appears to be robust to a wide array of conditions.

The exact cause of the oscillations is puzzling, but we posit that it is caused by genetic drift in the preferences. When the preferences change direction, it appears as though the population reaches a mutation-selection balance, at which point drift causes the preference to change direction, often resulting in a runaway in the opposite direction. With respect to Iwasa & Pomiankowski's model, this pattern is akin to their fast and slow dynamics, though we did not opt to ignore certain parameters (mutation bias and the cost of choice) to obtain these results (Iwasa & Pomiankowski 1995: p. 420).

Cyclical evolution of ornaments and preferences would be difficult to detect in natural systems, especially considering that most of the periods of the cycle were approximately 1,000 generations. Even in a system like *Drosophila*, detecting a change over 1,000 generations would take about 25 years. Instead, empiricists would likely

observe a prolonged period of directional sexual selection, or, if they happened to catch the system at an inflection point, then they might conclude that intersexual selection was not taking place in their system. Ultimately, these results reveal how complex the coevolution between ornaments and preferences can be under realistic parameter values. Moreover, when phenotypic evolution is viewed on a finer time-scale, it shows that over the course of two generations preferences can switch from being for positive values to negative values. To be sure, studies in natural populations have found considerable temporal variation in the direction of sexual selection (Madsen and Shine 1993; Chaine and Lyon 2008; Gosden and Svensson 2008).

Given the level of analysis required just for the means, the variances in the means for each of the variables is also of importance. Tables 2.3 and 2.5 contain the average variance of the twenty replicate runs, which indicate how much the system changes through time. These mean variances were used to calculate the standard deviations used as error bars in all of the bar charts, though a strict analysis of the variances themselves will likely highlight some of the other mechanisms and patterns inherent in Fisherian sexual selection. However, due to space constraints the results from these variance means will be left in table form for the reader to study.

Our model has provided multiple interesting avenues for future research as well as confirmed aspects of previous theory under a smaller set of assumptions. We found that Fisherian sexual selection can operate in finite populations, which stands in the face of Nichols & Butlin (1989). Some of the parameter combinations lead to identical patterns of phenotypic evolution, which warrants empirical measures of these parameters

to ground the theory in data. One major result that surprised us is that the genetic correlation can be very small and still lead to preference evolution. Unfortunately, genetic correlations are difficult to measure, suggesting that non-detectable genetic correlations are present in natural systems. In future models we will explore the role of the specific type of preference function on the evolutionary dynamics as well as the combined effect of varying multiple parameters simultaneously.

CHAPTER III

A NOVEL GENETIC CORRELATION BETWEEN CHOOSINESS AND ATTRACTIVENESS

Introduction

Despite the tremendous amount of progress that has been made in the field of sexual selection over the last four decades, the evolutionary dynamics of mating preferences remain poorly understood and difficult to study. There have been numerous empirical attempts to test theoretical predictions regarding ornament-preference coevolution (Bakker 1993; Gray and Cade 1999; Brooks and Endler 2001; Iyengar et al. 2002; Arnqvist and Kirkpatrick 2005; Qvarnstrom et al. 2006; Shaw and Lesnick 2009; Wiley and Shaw 2010; Wiley et al. 2012). However, few clear empirical generalities have emerged with respect to the relative importance of various models of preference evolution. A major barrier to progress in this area stems from the problems associated with studying the genetic basis of preferences, which tend to be complex and can only be quantified from large, labor-intensive studies (Wagner 1998; Chenoweth and Blows 2006). Nevertheless, quantification of the genetic basis of preferences is a key requirement to test predictions of sexual selection theory, because genetic correlations between ornaments contributing to attractiveness and components of mating preferences play a central role in the most influential models of preference evolution (Lande 1981; Kirkpatrick 1987; Lande 1987; Mead and Arnold 2004).

Many of the problems associated with the study of mating preferences become

apparent from the seemingly simple exercise of defining the term. Even though models usually endeavor to describe mating preferences using a single variable, real mating preferences are best conceptualized as a continuous function, which probably cannot be fully described so simply. Preferences can be visualized as a function with a mating response on the y-axis and trait values that contribute to attractiveness on the x-axis (Fig. 3.1). Thus, in the case of female choice among males, preference functions specify the degree to which females are sexually attracted to males of different phenotypes and allow us to define several elements of female behavior. For instance, responsiveness is defined as the mean response of the female across all males, a value which provides a window into female motivation to mate (Reinhold et al. 2002; Bailey 2008). Choosiness is a measure of the variation in female responses to different phenotypes: choosier females are more variable in their responses to males differing in attractiveness (Fig. 3.1) (Gray and Cade 1999; Brooks and Endler 2001; Bailey 2008). Finally, if the preference function has an intermediate peak (Fig. 3.1B), then each individual female may have a peak preference to which she responds most readily. Importantly, each female can in principle have a different preference function, so females may show variation in responsiveness, choosiness, and peak preference (Fig. 3.1) (Gray and Cade 1999; Brooks and Endler 2001; Reinhold et al. 2002; Bailey 2008).

Most models of mating preference evolution use preference functions in one of two distinct ways. In one class of models, which we refer to as "open-ended preferences", all females are assumed to prefer males with the most extreme trait values, even though the strengths of preferences may vary among females. This model predicts

that all females will rank males in the same order but that some females will discriminate more strongly among males than other females, so the single variable describing female preference in these models is a measure of choosiness (Fig 3.1A) (Lande 1981). In the other main class of models, termed "unimodal preferences", each female is assumed to show a peak preference for a particular male phenotype, and the location of this peak may vary among females (Fig. 3.1B). This perspective on female preferences predicts that each female will most prefer a different male phenotype, so individual females will rank males differently in terms of attractiveness. However, unimodal models assume that choosiness is identical among females. Even though choosiness could be critically important in either model of preference functions, empirical demonstrations of intersexual genetic correlations between mating preferences and sexually selected traits have focused almost exclusively on peak preference rather than choosiness (Bakker 1993; Gray and Cade 1999; Brooks and Endler 2001; Iyengar et al. 2002; Arnqvist and Kirkpatrick 2005; Qvarnstrom et al. 2006; Shaw and Lesnick 2009; Wiley and Shaw 2010; Wiley et al. 2012).

Here we take advantage of isogenic lines of *Drosophila melanogaster* to address three fundamental questions related to the evolution of female mating preferences. First, we quantify the extent to which aspects of mating preferences and sexual attractiveness in both males and females show a genetic basis. Second, we use these data to distinguish between the open-ended and unimodal models of female preference functions, which result in distinct predictions regarding the nature of genetic variation in peak preference and choosiness. Finally, we test the widespread expectation from models of intersexual

selection that mate choice should produce a genetic correlation between male attractiveness and female preferences.

Methods

Inbred lines, controlled larval density, and general culturing procedures

Ten randomly chosen inbred lines from the Drosophila genetic reference panel (RAL-208, -304, -315, -360, -379, -437, -486, -517, -707, and -732) were obtained from the Bloomington Drosophila Stock Center (Bloomington, Indiana). The lines were cultured in 8-dram vials with approximately 10 ml of cornmeal-molasses-agar medium (1L water, 30 g nutritional yeast extract, 55 g cornmeal, 11 g Droso-agar, 72 ml molasses, 6 ml propionic acid, and 16 ml 15% tegosept in ethanol) seeded with 2 drops of yeast suspension (1 g of live yeast, 333 µl 1% acetic acid, 5 ml water) at 25° C (±2°), 60% relative humidity (±3%) on a 12:12h light cycle.

Lines were cultured under controlled larval density for at least two generations before testing in an effort to reduce environmentally induced phenotypic variation. If lines vary in fecundity, then larvae from high fecundity lines will experience higher competition for resources than larvae from low fecundity lines unless larval density is controlled by the experimenter. We controlled larval density for two generations to reduce maternal effects as much as possible. To control for larval density, we used juice-agar plates with a smear of yeast paste (1g active dry yeast, 1.3ml water) to collect eggs. Twenty individuals of each sex were left in laying pots (juice-agar plates and inverted plastic beakers) for 24h. Plates were changed each day for three days. First instar larvae were picked with toothpicks and placed in 10 ml of food at a density of 50 larvae per

vial. Eclosing adults were collected every 12 hours using CO_2 anesthesia. Adults from the F_2 generation and later were used in behavioral assays as well as for starting the next generation of controlled larval density. Individuals used for the behavioral assays were separated by sex into vials with 5 ml of food with 5 individuals per vial. All flies were 3-6 days old at the time of testing. Vials containing females were retained after the flies were assayed to assure virginity. Any trials containing a female from a non-virgin vial were discarded from the analysis.

Mating arrays

We designed a new type of mating apparatus for the behavioral assays. These mating arrays were designed to facilitate high-throughput testing and to allow males and females to be acclimated separately until each trial started. This method allows 20 no-choice tests to be conducted simultaneously. Each array consisted of 20 mating chambers (Fig. 3.S1) measuring 1 inch in diameter arranged in four rows of five columns. Arrays consist of four 12.5 x 6.5 x 0.125 inch pieces (layers) of PETG plastic (SABIC Polymershapes) and a track that held the four layers in place and allowed the pieces of plastic to slide back and forth. The outer layers act as physical barriers to keep the flies in the chambers. Each outer layer had 20 entry holes slightly larger than the tip of the aspirator for flies to be loaded into the chambers. The middle two pieces composed the chambers. The cylindrical chambers were 1 inch in diameter (identical to the vials in which the flies were housed) and 0.125 inches in height. When the two middle layers were aligned (Fig. 3.S1C, D), the depth of the mating chamber doubled to 0.25 inches.

Mating assays

Trials were run from 0-2 hours after the lights turned on each day. All trials were conducted in the same environmentally controlled room where the flies were cultured. Virgin males and females were aspirated singly into each chamber when the mating array was in the out of phase position (Fig. 3.S1A). Males and females were loaded on each layer separately, which was alternated for each array. For example, all males would be loaded on the upper layer first, followed by females on the lower layer. The next array would have females loaded first (upper layer) and males loaded second (lower layer). The loading of each array took approximately 20 minutes. We recorded the order in which each individual was loaded to statistically control for order effects if they were present, which they were not.

Fully loaded arrays were placed on a light box (Model 4 Slide Sorting Viewer, Graphic Technology, Inc. Newburgh, NY). After 10 minutes of acclimation, recording began from a video camera (JVC GZ-HD300BU) mounted directly above the light box and the chambers were aligned by sliding the two pieces of plastic until the two chambers became one. All trials were recorded for 1 hour. A light diffuser (8’ x 4’) was suspended above the cameras and light box to reduce reflections and inconsistent lighting from the fluorescent lights in the room.

Each video was scored for courtship latency, copulation latency, and copulation duration. Courtship latency was measured as the time from aligning the chambers to male orientation toward the female. Copulation latency was measured as the time from the onset of courtship to the male mounting the female. Copulation duration was

measured as the time from mounting to separation of the pair. Each pair was given a score depending on whether (1) or not (0) they courted and a score depending on whether (1) or not (0) they mated.

A fully factorial design was employed: there were 100 possible pairings between the 10 lines. Each pairing was replicated enough times to obtain 10 successful matings. However, some pairs had a low percentage of trials that resulted in mating, so the total sample size of tests varied. For example, the pairing between males from 208 and females from 304 has a total sample size of 11: one pair failed to mate. The sample sizes for each pairing range from 10-27, meaning that the proportions of successful matings range from 0.37 to 1.0. A total of 1322 trials were conducted in 71 arrays (not all arrays were full and some individuals were damaged during the experiment and discarded).

Statistical analysis

We recorded three scores for each pair: courtship latency, copulation latency, and copulation duration. Means and standard deviations were measured separately for each sex of each line. Courtship latency and copulation duration: an effect of male genotype indicates preference components, while female genotype indicates attractiveness. Conversely, for copulation latency, measures of female genotypes pertain to preference components, while male genotypes indicate attractiveness. Copulation duration calculations exclude data from non-mating pairs.

Following Brooks & Endler (2001), we analyzed three components of the choice data. For the following explanations, we are specifically describing data related to the choosing sex. For example, preference components were measured on males for latency

to court and copulation duration. Similarly, measures of female copulation latency were used to estimate female preference components.

For each line we estimated the least-squares mean and standard deviation using a general linear model including only the effect of interest (male line or female line). For example, when we calculated the values for each genotype's male courtship latency, we modeled ln(courtship latency) = male genotype. For females we ran separate analyses for females from each genotype with male genotype as the effect.

All statistics were performed in JMP 9 (SAS Institute). We used separate general linear models to analyze the courtship latency, copulation latency, and copulation duration data. Each response variable was transformed to the natural logarithm prior to the analysis. For copulation latency, pairs not mating in the allotted time were given a score for the maximum value of time, which is a conservative estimate of copulation latency. For each response variable, we built a model with male genotype, female genotype, male age, female age, and male genotype x female genotype interaction. This is our base model, which was the complete model for courtship latency. However, for copulation latency we wished to test the effects of courtship latency, which was added to the base model. Finally, copulation duration was tested for the effects in the base model, as well as courtship latency and copulation latency.

Results

Genetic variation in components of mating preferences

Our first goal was to test for the existence of genetic variation in components of mating preferences for both sexes in *D. melanogaster* (Lande 1981; Servedio and Lande

2006; Servedio 2007). Statistically significant differences in mean preference values among lines demonstrated that flies exhibit substantial genetic variation for all three types of mating preference as well as for male and female attractiveness (see significant male and female genotype effects in Tables 3.S1-3.S3). To investigate the variation in female preferences among lines in more detail, we ranked male lines according their mean attractiveness across all females (each genotype's mean male copulation latency) and plotted the responses of females from each genotype separately (Fig. 3.2). Several important observations emerge from this analysis. First, mean copulation latency for females varies substantially across lines, indicating genetic variation in responsiveness. Thus, females from some genotypes require more stimulation, prior to mating than others, regardless of the identity of potential mate. Second, we found considerable variation among lines in choosiness (calculated as the standard deviation in female copulation latency across males from all ten genotypes).

Finally, the shapes of the preference functions for overall attractiveness varied considerably among lines. By ordering males with respect to global attractiveness, we can visualize the degree to which preferences of specific genotypes resemble the population mean preference. If females from all of the genotypes exhibit identical open-ended preferences, then preference functions should increase monotonically. For example, line 732 shows a preference function that is similar to the population-level preference function: as male attractiveness increases, females allow males to copulate more quickly. Conversely, females from line 517 respond almost identically to the most and least globally attractive males. Hence, females from the different genotypes varied

considerably in their ranking of males (Fig. 3.2). The graphical representations of genotypic preference functions in addition to the significant interaction effect of male genotype x female genotype on copulation latency (Table 3.S2; $p = 0.005$) provide definitive evidence of genetic variation in preference functions.

Our results for male preferences are similar to those for females in the sense that we see evidence for significant genetic variance in both male courtship latency and copulation duration (Tables 3.S1 and 3.S3). Courtship latency can be interpreted as a measure of male eagerness to mate, and the significant effect of male genotype (Table 3.S1; $p < 0.0001$) shows that *D. melanogaster* populations harbor genetic variation for this trait. A significant effect of female genotype on courtship latency (Table 3.S1; $p < 0.0001$) further indicates that some female genotypes are more attractive to males than others. We found a similar pattern for copulation duration (Table 3.S3; male genotype: $p < 0.0001$, female genotype: $p = 0.0004$), which suggests that *D. melanogaster* populations are characterized by genetic variation in both male preferences and female attractiveness to males during both pre- and postcopulatory phases of sexual selection.

Identifying the appropriate preference function model

The second goal of our study was to test predictions of the open-ended and unimodal models of preference evolution. We can reject an open-ended model with respect to female preference, since females from each individual genotype rank male genotypes differently in terms of attractiveness (Fig. 3.2). These two models differ in their predictions in that the unimodal preference model predicts a significant male by

female genotype interaction effect, whereas the open-ended model does not. Interestingly, only female choice (i.e., copulation latency) results in such an interaction (Table 3.S2; $p = 0.005$). Thus, our data indicate that females from unique genotypes rank male genotypes differently in terms of attractiveness. These observations are at odds with the predictions of the open-ended preference model: if all genotypes agreed on the most attractive males, then our data would be consistent with an open-ended model for female preferences (Fig. 3.1A), and the interaction between male and female genotype would not be statistically significant. Rather, our data show that females vary in peak preference, a key aspect of the unimodal preference function hypothesis (Fig. 3.1B). However, our data also show that female choosiness varies among lines (Levene test: $F_{9,1312} = 2.6431$, $p = 0.0049$), so the female preference functions vary with respect to the location of the peak as well as the width of the preference function.

When we turn our attention to male preference functions, we find a pattern that contrasts with female preferences. Specifically, we find no evidence for a male genotype by female genotype interaction for either courtship latency or copulation duration (Table 3.S1, courtship latency interaction, $p = 0.416$; Table 3.S3, copulation duration interaction, $p = 0.777$). Males from all 10 genotypes tended to agree on which females were most attractive. Thus, an open-ended model of preferences may be more applicable than a unimodal model for male *D. melanogaster*. However, because sexual selection typically acts more strongly on males than on females in this species (Bateman 1948), female preference functions probably play a larger role in shaping the overall dynamics of the sexual selection process than male preference functions. Nevertheless, male

preferences are interesting in their own right, and we wished to compare pre- and postcopulatory male choice to see if these two phases of male choice were reinforcing or antagonistic. We found no genetic correlation between female pre- and postcopulatory attractiveness (Fig. 3.S2), raising the possibility that males evaluate independent sets of traits during pre- and postcopulatory mate choice.

Intersexual genetic correlations

The final goal of our study was to test the prediction from models of trait-preference coevolution that populations subject to intersexual selection should evolve a genetic correlation between male attractiveness and aspects of the female preference function (Lande 1981; Kirkpatrick 1987; Lande 1987; Mead and Arnold 2004). Perhaps our most striking result is the presence of a positive genetic correlation between male attractiveness and female choosiness (Fig. 3.3). Genotypes resulting in attractive males also result in females with heightened choosiness, whereas genotypes with indiscriminate females tend to have globally unattractive males. This result is predicted by verbal models exploring variation in choosiness (Widemo and Sæther 1999), because females that are more discriminating in their mating decisions are more likely to obtain their most-preferred mate. Conversely, non-choosy females that do not discriminate strongly among males are more likely to mate with globally unattractive males by chance. One possibility that could result in this genetic correlation is that females with heightened choosiness simply respond to males faster (i.e., exhibit high responsiveness), which would be indicated by a positive relationship between choosiness and responsiveness. We found no evidence to support this hypothesis (Fig. 3.S3), rendering

choosiness an independent attribute of female behavior that is genetically correlated with male attractiveness.

Discussion

Our study demonstrates two unique and major findings. First, *D. melanogaster* exhibits sexual dimorphism with respect to the shape of mating preference functions. In particular, males seem to be characterized by open-ended preferences with genetic variation in responsiveness, whereas females exhibit unimodal mating preference functions and display genetic variation for both peak preference and choosiness. Second, we found a previously undocumented genetic correlation between female choosiness and male attractiveness. These results carry multiple implications for the study of mating preference evolution and the sexual selection process, which we discuss below.

Turning to our first major result, males from different genotypes generally agreed on the attractiveness of females, while females varied in their rank-order preferences. There are two complementary reasons why we might expect to see this pattern. First, sexual selection is stronger in males than in females (Bateman 1948), which results in strong, persistent directional selection on overall male attractiveness. If females attend to several different aspects of the male phenotype in making mating decisions, then it may be possible for different males to achieve similar levels of attractiveness despite different underlying combinations of trait values (Blows et al. 2003). If females merely weight the male traits differently, we might expect the rank order of males to change dramatically across female genotypes. Of course, an alternative explanation for variation among females in their mating preference functions could involve non-adaptive

processes. For instance, if both directional and stabilizing selection on female mating preferences are weak, as might be expected from indirect benefits models of preference evolution, then the population may be expected to harbor substantial levels of additive genetic variance in preferences and genetic drift may also play a major role in the evolution of mean preferences.

Why different male genotypes appeared to rank females in the same order in terms of attractiveness is a separate but related question. One possibility is that males prefer traits tied to female fecundity, which could provide a direct benefit for male mate choice, especially under conditions where sperm limitation is a possibility. Thus, males preferring more fecund females would obtain direct fitness benefits, which would cause these preferences to increase in frequency in the population. Given a sufficiently large fitness benefit in terms of additional offspring, we might expect an open-ended preference for fecund females to come to predominate in the population. A second possibility is that our sample size or study design was simply not sufficient to demonstrate a statistically significant interaction between male and female genotypes for the aspects of male preferences that we measured. Regardless, our results do imply that males exhibit less genetic variation for mating preference functions, especially in terms of the rank order of prospective mates, compared to females in *D. melanogaster*.

Theory has collapsed mating preferences into a single dynamic parameter to describe a complex trait with multiple components. Thus, testing the predictions from sexual selection theory is limited because only one measurable attribute of choice is explicitly modeled for each type of preference. To be sure, the parameters for each of the

two forms of preferences describe completely different behavioral phenotypes, both of which result in positive genetic correlations between preference values (slope of open-ended function or peak preference of unimodal function) and ornament values (Lande 1981; Hall et al. 2000). We extended this robust result to a new behavioral phenotype, choosiness, which describes the degree of discrimination among potential mates (Gray and Cade 1999; Brooks and Endler 2001) and found a result similar to those obtained from analytical theory (Lande 1981; Hall et al. 2000). This genetic correlation provides the foundation for Fisherian sexual selection to operate, but is peculiar in that it combines two different attributes of the preference functions used to model mate choice. Specifically, females tend to display unimodal preference functions, as indicated by different peak preferences, but they also vary with respect to choosiness, which is the attribute of preference variation that is modeled in open-ended preference functions. This result suggests that existing single-parameter models of preference evolution may be inadequate to capture the complexity of trait-preference coevolution in natural populations. Taken together with the evidence for an intersexual genetic correlation, these results call for more explicit models of female mating behavior to investigate which specific attributes of mate choice are most likely to coevolve with male attractiveness.

One feature of our study that distinguishes it from many other studies of trait-preference coevolution is that we focus on a global measure of male attractiveness, as defined by the average response of females to the male genotype, rather than on specific measurable traits of males. For the present analysis, this approach is preferable to

targeting specific male traits for three reasons. First, studies aimed at finding the target of mating preferences demonstrate that attractiveness is a composite measure, which integrates many ornaments across multiple sensory modalities (Partan and Marler 2005). Thus, it is likely that many studies fail to find a causal relationship between male ornaments and female preferences simply because the specific traits to which females are attracted remain unmeasured. Second, by quantifying the effects of all phenotypic characters contributing to attractiveness we are able to assess if overall attractiveness of males has a genetic basis (Wedell and Tregenza 1999). This approach paves the way for future analyses that can characterize the genetic architecture of attractiveness and reveal constraints on sexually selected traits. Third, preferences result in sexual selection on mating success, which is quantified entirely by attractiveness, not individual traits (Wedell and Tregenza 1999). Ultimately, we have chosen to use overall attractiveness in place of individual traits because if attractiveness has no genetic basis, then there should be no expectation that sexual selection generated by mate choice will have evolutionary consequences for the individual traits that are the constituents of attractiveness.

In summary, this study reveals several novel and important aspects of the genetic basis of attractiveness and mating preferences in *D. melanogaster*, and many of our observations carry implications for the study of sexual selection in general. Importantly, we demonstrate that attractiveness and preferences of both males and females have a substantial genetic component. Even though previous studies have shown that attractiveness has a genetic basis, our investigation of female mating preferences provides unprecedented insights into the nature of preference functions. Our data show

definitively that female *D. melanogaster* show unimodal preference functions and that genotypes differ from one another with respect to both peak preference and choosiness. Thus, theoretical models that collapse mating decisions into a single parameter provide an inadequate description of female preferences. Future theoretical and empirical work should consider the possibility that both peak preferences and choosiness may simultaneously evolve in nature. In addition, our results show that female choosiness is genetically correlated with male attractiveness, indicating that the genetic architecture of sexually selected traits and preferences in *D. melanogaster* is compatible with a Fisherian process of runaway sexual selection. The particular traits that have been included in models of Fisherian sexual selection, however, do not include choosiness as a possible phenotype. We have found a new type of intersexual genetic correlation that would allow genes contributing to choosiness to experience indirect sexual selection through their genetic association with genes contributing to male attractiveness (i.e., Fisherian sexual selection). Thus, our results provide a new perspective on the nature of sexual selection in general, and mating preference functions in particular, whose complexity will have to be embraced in future studies of the genetic underpinnings of attractiveness and preferences.

CHAPTER IV

VARIATION IN INDIVIDUAL-LEVEL PREFERENCE FUNCTIONS AND THE NATURE OF INTERSEXUAL SELECTION ON MULTIVARIATE SONG TRAITS IN *DROSOPHILA MELANOGASTER*

Introduction

Intersexual selection, or mate choice, is often pinpointed as the major cause of many of the wildly extravagant ornamental traits and behaviors observed in animal communication systems (Kirkpatrick 1987; Kirkpatrick and Ryan 1991). Consequently, the role of mating preferences in secondary sexual trait evolution is one of the most highly studied phenomena in sexual selection research (Andersson 1994; Chenoweth and Blows 2006; Jones and Ratterman 2009). The coevolutionary dynamics of sex-limited traits have been thoroughly modeled, resulting in several competing hypotheses for the evolution of ornaments and preferences (Heisler et al. 1987; Mead and Arnold 2004). Considerable attention has focused on the direction and intensity of intersexual selection on ornamental traits, which are determined by population-level preference functions (Jones et al. 2012). One problem that hinders empirical research on intersexual selection and the evolution of mating preferences is the dearth of studies characterizing individual-level preference functions (Jennions and Petrie 1997; Widemo and Sæther 1999).

A useful way to understand intersexual selection is to recognize that each individual exhibiting a different preference function (hereafter “females”) imposes a

unique selection surface on the traits of interest in the opposite sex (hereafter "males"). Thus, each male will potentially encounter a distinct set of intersexual selection surfaces depending on which females he encounters during his lifetime. This perspective highlights the frequency-dependent nature of intersexual selection: the frequency distribution of preference functions in the population will largely determine the shape of selection on ornamental traits. Hence, it appears that knowledge of variation in individual-level preference functions will be necessary to achieve a complete understanding of the nature of intersexual selection on populations.

Measuring individual-level preference functions is a labor-intensive and time-consuming process because individuals must be tested with a range of stimuli, and replication is necessary at both the stimulus-level and the individual-level (Wagner 1998). This approach can create problems, however, because experience affects mating preferences (Bailey 2011). Individuals tend to habituate to the testing environment and either change their preference functions over time (Bailey 2011) or simply stop responding (Gerhardt and Huber 2002). A possible solution to this problem is to use genetically identical individuals to measure preference functions, resulting in genotype-level descriptions of mating preferences. Because each genotype is effectively a unique genetic individual, genotype-level traits can be interpreted as individual-level traits, though they are measured in a way that reduces the effects of repeated testing on single individuals (Chenoweth & Blows 2006). An added advantage of the use of isogenic lines is that any differences observed across lines can be attributed to heritable variation, and breeding values are easily estimated (Falconer et al. 1996; Lynch and Walsh 1998).

If breeding values for individual-level preference functions can be obtained, then direct tests of models of preference evolution can be made. The most basic model of intersexual selection, known as the Fisherian mechanism, requires genetic correlations between ornaments and preferences, and this genetic correlation is sufficient to drive the evolution of ornament and preference elaboration given appropriate starting conditions (Lande 1981). In this Fisherian model, as well as within increasingly elaborate quantitative genetic models, preference functions assume two basic forms. On the one hand, preferences can be monotonic increasing functions, which are termed open-ended preferences. The parameter describing this type of phenotype is analogous to a slope, with steeper slopes representing stronger preferences (psychophysical model: Lande 1981). Alternatively, preferences can assume a unimodal shape that is described by the value that maximizes the function, which is called the peak preference. Peak preferences can be thought of as the ornament value that maximizes the probability of a female accepting a male as a mate. Thus, the meaning of the variable used to quantify female preferences differs dramatically between the two types of preference functions, though either preference model can result in the coevolution of ornaments and preferences (Lande 1981, Hall et al. 2000). Ultimately, using isogenic lines to estimate preference functions is advantageous because (a) carryover effects of repeated testing of individuals is abolished, (b) breeding values for individual-level preference functions are obtained, and (c) estimates of a crucial assumption of Fisherian models of sexual selection can be tested directly.

Here we report the results from an experiment that employed isogenic lines of the fruit fly *Drosophila melanogaster* to measure mating preference functions at the level of the genotype, and thus, individual. We hypothesized that there would be genetic variation in multivariate preference functions for song characters. Moreover, we expected that preferences with high individual-level variability would exhibit reduced or nonexistent population-level preference functions, and thus weak or no intersexual selection on those trait combinations. We examined genetic correlations both within and between the sexes. Intersexual genetic correlations are predicted to exist between various behavioral attributes of the females and ornamental trait values of males. We tested the hypothesis that positive genetic correlations exist between ornaments and preferences. Within females, we used preference function values to examine whether genotypes that exhibited strong preferences for one trait exhibited strong preferences for other independent suites of traits, which would be indicated by genetic correlations. Finally, we evaluated the effect of the preference measure (copulation latency vs. mating success) on the patterns of mate choice and genetic correlations.

Methods

Fly lines and rearing conditions

We obtained ten fully inbred strains of *Drosophila melanogaster* from the Bloomington Stock Center. These lines originated in Raleigh, NC, and are a subset of the DGRP. To reduce environmentally-induced phenotypic variation, we controlled the larval density for at least two generations prior to behavioral trials. We used agar-juice-plates to collect larvae and seeded fresh vials of cornmeal-molasses-agar topped with a

yeast suspension with 50 larvae per 8-dram vial. Flies were housed and phenotyped at 25° C with 60% relative humidity and a 12 hour light cycle.

Behavioral phenotyping

All individuals used in behavioral trials were 3-6 day-old virgins aged in vials of five same-sex individuals. We used a fully-factorial design where both sexes from each genotype were phenotyped with the nine other genotypes, as well as their own, resulting in 100 male-female pair combinations. Each pairing was replicated at least 10 times resulting in a total of 1,322 tests, all of which occurred from 0-2 hours after lights-on.

We built novel mating chambers for high-throughput phenotyping. These "mating arrays" consisted of 20 circular mating chambers arranged in four rows and five columns (Fig. 4.S1). Each chamber was initially split into two separate chambers (Fig. 4.S1 A-B), into which flies were loaded and allowed to acclimate. The two sub-chambers were then aligned to allow male-female pairs to begin interacting. (Fig. 4.S1 C-D). The mating arrays were placed on top of a light source (Model 4 Slide Sorting Viewer, Graphic Technology, Inc. Newburgh, NY) and filmed from above using a high-definition digital video camera (JVC GZ-HD300BU). Each pairing was later analyzed and scored for copulation latency and whether or not the pair mated. Non-mating pairs received a value for the maximum amount of time allowed (3600 seconds).

Song recording

The materials and methods used to record the songs can be found in Turner & Miller (2012).

Song analysis

We used six traits to characterize male song. (1) Train length is the mean of all song trains for each recording. (2) Pulse length is the median pulse length for all songs greater than or equal to five pulses. (3) Number of cycles is the mean number of cycles per pulse for song bouts greater than or equal to five pulses. (4) Frequency is the median sound frequency for song bouts greater than or equal to five pulses. (5) The interpulse interval (IPI) is the time duration between the beginning of each pulse. (6) Effort was measured as the amount of pulse song per total time spent singing. See Turner & Miller (2012) for additional details.

Multiple recordings were made on each of the 192 isogenic lines from the Drosophila Genetic Reference Panel. We regressed each song trait on temperature, and for significant regressions we used the residuals as trait values to control for temperature effects. Next, we standardized song traits to have a mean of zero and a unit variance so that the traits were all expressed in units of phenotypic standard deviations. The six standardized song traits were used in a principal components analysis (PCA) and the scores for each individual recording were saved. We then computed the mean PC-score for each of the 192 lines, which are the breeding values for each principal component. We graphed the frequency-distribution of the 192 scores and plotted the values for the ten lines used in our analysis on the distribution of each principal component to visualize where our lines fall within the entire sample (Fig. 4.S2). The breeding values for each line were used to estimate the preference functions (see below).

Preference function analysis

We recorded two preference measures for all trials: mating success and copulation latency. Thus, two types of preference functions can be generated, each with a different behavioral proxy for preference. Mating success provides information on the influence of composite song values (PC scores) on whether or not a pair mates. Copulation latency provides information on how quickly pairs mate. Thus, each measure has a different biological interpretation. Copulation latency for each pair was log-transformed prior to analysis.

We tested for the effects of each principal component trait in a full model to examine the relative importance of linear and quadratic preferences. The full model was: preference $= PC(X) + PC(X)^2 +$ female genotype + (female genotype x $PC(X)$) + (female genotype x $PC(X)^2$), where preference was either mating success (binary) or copulation latency (continuous) and X represents the specific principal component we used (1 thru 4, accounting for 90% of the variance). To analyze mating success, we used a generalized linear model with a binomial distribution and logit link function. Copulation latency was analyzed with a general linear model. Interaction terms test for significant genetic variation in individual-level preference functions.

Following Lande & Arnold (1983), we ran two separate models to estimate linear and quadratic intersexual selection gradients (i.e., population-level preference functions), where each model included only the trait of interest, or in the case of quadratic preferences, the term of interest and its squared value. We discarded the linear term from

the quadratic model (Lande & Arnold 1983) and doubled the quadratic regression coefficients (Stinchcombe et al. 2008). Individual-level preference functions were obtained by estimating intersexual selection gradients individually for each genotype.

Estimation of genetic correlations

We tested for both intersexual and intrasexual genetic correlations. Intersexual correlations could occur between ornament values and preference values for both types of preference function. Intrasexual genetic correlations were between male attractiveness and PC scores as well as between female responsiveness (mean across all male genotypes), choosiness (SD in response to all males) and both types of preference function.

Within females, intrasexual genetic correlations provide a window into the degree to which components of preference are phenotypically integrated. For males, the intrasexual genetic correlation indicates the degree to which composite song traits predict attractiveness and can also estimate the extent to which unmeasured traits influence male attractiveness.

We tested the prediction from Lande (1981) that genetic correlations should build up between preference function and ornament values as a consequence of mate choice. For the open-ended preference function, we used linear individual-level preference functions. For peak-preference values, for each line we found the genotype with the shortest copulation latency (i.e., most preferred). We then used the PC scores for males from the most-preferred line as female peak-preference values.

Genetic correlations were estimated by least squares regression. Theory predicts that a genetic correlation arises from the process of mate choice itself, and that this relationship can occur between either the slopes of open-ended preference functions and ornament values, or between peak-preference and ornamental values (Lande 1981, Arnold 1983). For open-ended preferences, we regressed the slope of each genotype's linear preference function against the male PC scores for those genotypes. In other words, females from each genotype have a linear preference function for each principal component of male song traits. These values were regressed on the breeding values for the composite male song traits. Similarly, for peak-preference values, we regressed PC score that females from each line most preferred on male PC scores. Regressions were performed for PC1 to 4.

Intrasexual genetic correlations were estimated for males and females. For males, we tested whether genotypes that result in globally attractive males (lowest mean copulation latency across females from all genotypes) can be predicted from composite song traits by regressing attractiveness on each PC score. For females, to examine the degree to which female behavioral attributes were genetically integrated, we explored the relationships among and between preference functions, responsiveness (mean copulation latency for all males) and choosiness (standard deviation in responsiveness).

We corrected for simultaneously testing multiple hypotheses by calculating the false discovery rate and determining significance thresholds by the method of Benjamani & Hochberg (1995) at $\alpha = 0.05$. Resulting P-values below 0.05 are bolded if they are significant after accounting for the false discovery rate.

Results

Principal component analysis

Table 4.1 shows the results from the principal components analysis. We retained the first four principal components to evaluate preference functions. The ten lines we used to test preferences do not cover the entire range of phenotypic space for any of the principal components (Fig. 4.S1). Thus, we interpreted our analysis with respect to preferences near the mean male phenotype in the population.

Full model analysis and the measure of preference

The results of the full model for each of the principal components are summarized in Table 4.2. A major result that emerges from comparing the full models is that the measure of preference (i.e., copulation latency vs. mating success) has a considerable effect on the target of intersexual selection. Each composite male trait is subject to some form of selection, which varies depending on how fitness is measured. The full models also reveal that there is significant genetic variation in individual-level preference functions for three of the four traits (significant interactions between genotype and preference functions), directing us to characterize the differences in the shape of the preference functions (see below).

Population-level preference functions

Each PC was analyzed separately to estimate population-level preference functions. Linear population-level preferences were significant for all composite traits (Table 4.3). In addition, significant quadratic preferences were detected for PC3 (Table

4.3). These patterns were similar to those obtained from the full model (Table 4.2). However, when preference was estimated as mating success, both linear and quadratic preferences for all principal components exhibited significant population-level preference functions (Table 4.3), a result quite different from that of copulation latency, where only one quadratic preference for PC3 was significant (Table 4.2).

Individual-level preference functions

We analyzed individual-level preference functions for all traits (Table 4.4). PC3 did not appear to be a target of any significant individual-level preferences of linear or quadratic form, which is interesting because it is also the only trait for which significant linear and quadratic population-level preferences exist (Table 4.3). Figure 4.1 demonstrates the nature of genetic variation in the individual preferences. Quadratic preferences sometimes assume entirely opposite shapes (concave vs. convex) for different female genotypes, resulting in an absence of net preferences at the population level. Thus, extreme variation in individual-level preferences results a loss of intersexual selection at the population level for some traits (Fig. 4.1).

Genetic correlations

We identified significant intrasexual genetic correlations between multivariate song phenotypes and male global attractiveness for PC1 and PC2, but only when copulation latency was the fitness measure (Table 4.6). The various components of female choice behavior (responsiveness, choosiness, linear and quadratic preference functions) exhibited no genetic correlations, indicating genetic independence of responsiveness and choosiness from linear and quadratic preference functions (Table

4.6). However, female preference functions show some degree of genetic integration, where a strong preference for one PC resulted in strong preferences for other PCs (Table 4.5). The measure of preference had a substantial effect: nearly one half of the significant genetic correlations yielded from copulation latency were not significant when mating success was used as the preference measure (Table 4.5). Genetic correlations estimated with use of mating success data were only between linear terms or between quadratic terms, whereas correlations with copulation latency measures of preference occurred in all combinations (linear x linear, linear x quadratic, quadratic x quadratic) (Table 4.5).

We found no evidence for intersexual genetic correlations between any of the preference functions and ornament values when copulation latency was used as the measure of preference (Table 4.6).

Discussion

Our study resulted in four major results of considerable importance to the study of mating preference evolution and sexual selection. First, we found significant population-level linear preferences for all principal components of song traits, but only one significant quadratic preference (Fig. 4.1, Table 4.3). Second, we identified the cause for the non-significant quadratic population-level preferences as genetic variation in individual-level preference functions (Fig. 4.1, Table 4.2, 4.3). Third, we found significant intrasexual genetic correlations among preference functions, but not among other behavioral aspects of mate choice (preference functions and responsiveness or choosiness) (Table 4.5, 4.S1). Moreover, intersexual genetic correlations, which allow

preferences to evolve as a correlated response to selection on ornaments, were entirely absent (Table 4.6). Fourth, different measures of preference, either copulation latency or mating success, yielded qualitatively different patterns of mating preferences at the levels of both the population and the individual. However, the genetic correlations among preferences were largely robust to the different measures of preference (Table 4.5, 4.6, 4.S2).

Individual-level variation and the nature of sexual selection

The vast majority of studies characterizing mating preferences and intersexual selection do not measure individual-level preference functions (Ratterman et al. review; Wagner 1998; Jennions & Petrie 1997; Widemo & Sæther 1999). As a result, population-level preferences are measured first, and statistically significant preferences are preferentially used as the basis of future studies (Wagner 1998). However, we found that individual-level variation in preferences can result in different preferences cancelling out on average, resulting in no detectable population-level preferences. In our study, for instance, population-level linear preferences were significant for each of the composite song traits, and we found no significant variation among individuals in their linear preferences in these cases. In contrast, for three of the four quadratic preferences, we found significant individual-level variation but no significant population-level preference functions. The single significant quadratic population-level preference exhibited no individual-level variation. Thus, we can conclude that individual variation in preference functions, each of which may be strong and statistically significant, can result in the overall population-level preference being absent. This provides clear

evidence for the assertion that genetic variation in mating preferences may reduce the strength of sexual selection on traits involved in intrasexual selection (Chaine & Lyon 2006).

Wagner (1998) suggested that population-level preferences should only serve as a starting point when measuring mate choice. However, our results clearly indicate that an undetected population-level preference does not necessarily mean that individuals do not choose their mates based on the trait in question. Moreover, when natural populations are repeatedly sampled over time, the frequency distribution of preference functions may change as the population evolves. A major goal, which has yet to be achieved in any empirical system, should be to characterize the nature of preference function evolution by repeatedly measuring individual-level preferences through time in natural populations. Population-level preference functions are a natural outcome of preferences at the individual-level. However, the only way to completely understand the causes of population-level preference functions and their potential change over evolutionary time is to decompose them into the constituent individual-level preference functions. This goal is admittedly daunting from an empirical standpoint, but it seems to be a necessary next step in the study of mating preference evolution.

Genetic correlations, strong preferences, and attractiveness

Indirect selection on mating preferences results in evolutionary change only if ornaments and preferences are genetically correlated (Lande 1981; Hall et al. 2000). Theoretical models of the Fisherian mechanism usually handle mating preferences in one of two distinct ways. On the one hand, preferences can be treated as open-ended

functions, where each individual most favors males with the most extreme trait values (Lande 1981; Hall et al. 2000). On the other hand, preferences can be treated as unimodal preference functions, where each female's preferences are represented by a continuous function with a single peak (Lande 1981; Hall et al. 2000). In this case, the highest point on the peak represents the male phenotype most-preferred by the female, and her preference for males drops off as they depart in either direction from her peak preference. In theoretical models of this type of preference function, the width is usually held constant while the position of the peak is allowed to evolve (Lande 1981). The two approaches to modeling preferences (i.e., open-ended and unimodal) are quite different in their nature and meaning, as one is a scalar that indicates the relationship between a female's response and male ornament values (i.e., analogous to a slope), and the other is a preference value that equals an ornament value. Interestingly, both types of preferences are expected to coevolve with ornaments, though the rate of evolution differs between the preference functions (Hall et al. 2000). As these genetic correlations are expected to be generated by the process of assortative mating via mate choice, and exist merely as a consequence of the fact that individuals are choosing their mates, it is theoretically plausible that they should be found in all natural systems with any non-zero level of mate choice (Jones & Ratterman 2009). Surprisingly, we found no such intersexual genetic correlations between any ornaments and preferences in the present study.

One possible explanation for the lack of significant genetic correlations is that the ornaments themselves do not predict overall male attractiveness, which is what ultimately determines copulation latency and mating success (Wedell and Tregenza

1999; Hine et al. 2002). We tested this hypothesis and found that male principal component scores are not genetically correlated with attractiveness. We measured male attractiveness as mean copulation latency and the total proportion of pairings that resulted in mating, which was done by pooling female responses from all 10 genotypes. Courtship in *D. melanogaster* is multimodal, integrating chemical, acoustic, visual, and tactile sensory channels (Greenspan and Ferveur 2000). It is likely that unmeasured aspects of male courtship determine attractiveness, which would also explain why overall attractiveness is not correlated with composite song traits. Nevertheless, females do still express mating preferences for different multivariate song phenotypes, which is evidenced by the significant preference functions. It is possible that we detected no statistical effect of song traits on attractiveness because of the large amount of individual-level variability in preferences; while some genotypes may have deemed a male attractive based on his song components, other genotypes would likely disagree.

We unveiled significant genetic correlations between various combinations of female preference functions. We posit that these results are explained by an overall propensity for some genotypes to exhibit strong mating preferences, irrespective of the traits targeted by the preferences. Mate choice is an extremely complex behavioral phenotype, often integrating multiple sensory systems, neural processing modules, and physical responses (Guilford and Dawkins 1991; Candolin 2003; Partan and Marler 2005). Thus, it is plausible that a single component of mate choice determines intensity of response, which is genetically correlated with some, but not all, of the preference functions.

Sensitivity of results to the measure of preference

Given that preferences are best measured at the individual level, another important point is that the specific behavioral proxy for preference can produce qualitatively different results. Our main goal was to focus on the female preference functions rather than produce a detailed picture of the nature of selection acting on male traits, as we were constrained to phenotypic space near the population mean. In contrast, many studies of intersexual selection aim to generate selection surfaces for male traits using female preferences as proxies for fitness (Blows et al. 2003; Brooks et al. 2005). It is important to note, however, that few of these studies build selection surfaces using more than one preference measure. Our results indicate that these fitness surfaces are sensitive to the metric chosen to characterize preferences. Binary measures (i.e., mating success) are commonly used in studies where large sample sizes are needed, simply because they are easier to obtain than continuous measures. However, considering the difference in population-level preferences when using copulation latency and mating success as measures of preference (Table 4.3), it is likely that previous studies that used only one measure would result in entirely different fitness surfaces for the different measures of preferences. Future studies will benefit from contrasting measures of preference to gain a fuller understanding of the manner in which preferences can impose intersexual selection on ornamental traits.

Detection of genetic correlations was also sensitive to the measure of preference. When preference was measured as copulation latency, we found seven significant genetic correlations between various combinations of preference functions. However,

when mating success was used as a proxy for preference, only four genetic correlations were found, all of which were represented in the seven genetic correlations found for copulation latency. It is possible that the genetic architecture of mating preferences differs between the two measures. Alternatively, because there was total overlap among the genetic correlations (i.e., the four genetic correlations based on mating success are all included in the set of seven genetic correlations based on copulation latency), it is likely that mating success is not as sensitive a measure as copulation latency with respect to genetic correlations.

Conclusion

We found that genotypes vary considerably in their preference functions, which results in an inability to detect population-level preferences. Our results also highlight the importance of the specific measure of preference, where qualitatively different conclusions are reached depending on the behavioral assay used. Finally, we detected genetic correlations among the preference functions, though we did not find any intersexual genetic correlations, which are predicted to drive indirect preference evolution. We urge future studies of mating preferences to focus primarily on individual-level preference functions to estimate population-level measures of intersexual selection. Similarly, our results demonstrate the importance of measuring multiple response variables, which differ in their biological implications for choice.

CHAPTER V

CONCLUSIONS

This dissertation is focused on theoretical and empirical pursuits to studying mate choice evolution. The ultimate conclusion that can be made is that theory and empiricism are highly interdependent and, as such, cross-talk between the two approaches needs to be maximized. Theory will most accurately model natural systems by incorporating the specific measures made by empiricists. Similarly, empirical tests of models should focus on measuring the exact parameters in the models as opposed to surrogates for the parameters. I am confident that if this feedback is practiced, our understanding of the evolutionary process will benefit greatly.

REFERENCES

Andersson, M. B. 1994. Sexual selection. Princeton University Press, Princeton, New Jersey.

Arnold, S. J. 1983. Sexual selection: the interface of theory and empiricism. Mate choice:67-107.

Arnold, S. J. 1985. Quantitative genetic models of sexual selection. Experientia 41:1296-1310.

Arnqvist, G. and M. Kirkpatrick. 2005. The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. Am Nat 165:S26-S37.

Bailey, N. W. 2008. Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. Behav Ecol 19:960-966.

Bailey, N. W. 2011. Mate choice plasticity in the field cricket *Teleogryllus oceanicus*: effects of social experience in multiple modalities. Behav Ecol Sociobiol 65:2269-2278.

Bakker, T. C. M. 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. Nature 363:255-257.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349-368.

Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B:289-300.

Berglund, A., M. S. Widemo, and G. Rosenqvist. 2005. Sex-role reversal revisited: choosy females and ornamented, competitive males in a pipefish. Behav Ecol 16:649-655.

Blows, M. W., R. Brooks, and P. G. Kraft. 2003. Exploring complex fitness surfaces: Multiple ornamentation and polymorphism in male guppies. Evolution 57:1622-1630.

Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural-selection. Trends Ecol Evol 10:313-318.

Brooks, R. and J. A. Endler. 2001. Female guppies agree to differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. Evolution 55:1644-1655.

Brooks, R., J. Hunt, M. W. Blows, M. J. Smith, L. F. Bussiere, and M. D. Jennions. 2005. Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871-880.

Candolin, U. 2003. The use of multiple cues in mate choice. Biol Rev 78:575-595.

Chaine, A. S. and B. E. Lyon. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 319:459-462.

Chenoweth, S. F. and M. W. Blows. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. Am Nat 165:281-289.

Chenoweth, S. F. and M. W. Blows. 2006. Dissecting the complex genetic basis of mate choice. Nat Rev Genet 7:681-692.

Crow, J. F. and M. Kimura. 1964. The theory of genetic loads. Proc. 11th Int. Congr. Genet. 2:495-505

Darwin, C. 1871. The descent of man, and selection in relation to sex. John Murray, London, England.

Day, T. 2000. Sexual selection and the evolution of costly female preferences: spatial effects. Evolution 54:715-730.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics (4th edn). Pearson, Essex, England.

Fisher, R. 1930. The genetical theory of natural selection. Oxford University Press, Oxford, England.

Fisher, R. A. 1915. The evolution of sexual preference. The Eugenics Review 7:184.

Fowler-Finn, K. D. and R. L. Rodriguez. 2012. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. Evolution 66:459-468.

Gerhardt, H. C. and F. Huber. 2002. Acoustic communication in insects and anurans: Common problems and diverse solutions. University of Chicago Press, Chicago, Illinois.

Gosden, T. P. and E. I. Svensson. 2008. Spatial and temporal dynamics in a sexual selection mosaic. Evolution 62:845-856.

Gray, D. A. and W. H. Cade. 1999. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. Evolution 53:848-854.

Greenspan, R. J. and J. F. Ferveur. 2000. Courtship in *Drosophila*. Annu Rev Genet 34:205-232.

Guilford, T. and M. S. Dawkins. 1991. Receiver psychology and the evolution of animal signals. Anim Behav 42:1-14.

Hall, D. W., M. Kirkpatrick, and B. West. 2000. Runaway sexual selection when female preferences are directly selected. Evolution 54:1862-1869.

Hedrick, A. and T. Weber. 1998. Variance in female responses to the fine structure of male song in the field cricket, *Gryllus integer*. Behav Ecol 9:582-591.

Heisler, I., M. Andersson, S. Arnold, C. Boake, G. Borgia, G. Hausfater, M. Kirkpatrick, R. Lande, J. Maynard Smith, and P. O'Donald. 1987. The evolution of mating preferences and sexually selected traits: group report. In: Sexual selection: testing the alternatives. Bradbury, J.W, and Andersson, M. (eds.), p. 97-218. John Wiley and Sons, Berlin, Germany.

Hine, E., S. Lachish, M. Higgie, and M. W. Blows. 2002. Positive genetic correlation between female preference and offspring fitness. P Roy Soc Lond B Bio 269:2215-2219.

Iwasa, Y. and A. Pomiankowski. 1995. Continual change in mate preferences. Nature 377:420-422.

Iyengar, V. K., H. K. Reeve, and T. Eisner. 2002. Paternal inheritance of a female moth's mating preference. Nature 419:830-832.

Janetos, A. C. 1980. Strategies of female mate choice - a theoretical-analysis. Behav Ecol Sociobiol 7:107-112.

Janzen, F. J. and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564-1571.

Jennions, M. D. and M. Petrie. 1997. Variation in mate choice and mating preferences: A review of causes and consequences. Biol Rev Camb Philos 72:283-327.

Jones, A. G. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. Evolution 63:1673-1684.

Jones, A. G., S. J. Arnold, and R. Borger. 2003. Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. Evolution 57:1747-1760.

Jones, A. G., S. J. Arnold, and R. Burger. 2007. The mutation matrix and the evolution of evolvability. Evolution 61:727-745.

Jones, A. G. and N. L. Ratterman. 2009. Mate choice and sexual selection: What have we learned since Darwin? P Natl Acad Sci USA 106:10001-10008.

Jones, A. G., N. L. Ratterman, and K. A. Paczolt. 2012. The adaptive landscape in sexual selection research. The adaptive landscape in evolutionary biology. Oxford University Press, Oxford, England.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am Nat 157:245-261.

Kirkpatrick, M. 1985. Evolution of female choice and male parental investment in polygynous species: The demise of the" Sexy Son". Am Nat:788-810.

Kirkpatrick, M. 1986. The handicap mechanism of sexual selection does not work. Am Nat 127:222-240.

Kirkpatrick, M. 1987. Sexual selection by female choice in polygynous animals. Annu Rev Ecol Syst 18:43-70.

Kirkpatrick, M. and N. Heckman. 1989. A quantitative genetic model for growth, shape, reaction norms, and other infinite-dimensional characters. J Math Biol 27:429-450.

Kirkpatrick, M. and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33-38.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292-305.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. P Natl Acad Sci 78:3721-3725.

Lande, R. 1982. Rapid origin of sexual isolation and character divergence in a cline. Evolution 36:213-223.

Lande, R. 1987. Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. In: Sexual selection: testing the alternatives. Bradbury, J.W, and Andersson, M. (eds.), p. 83-94. John Wiley and Sons, Berlin, Germany.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210-1226.

Lande, R. and S. J. Arnold. 1985. Evolution of mating preference and sexual dimorphism. J Theor Biol 117:651-664.

Levine, M. W. and J. M. Shefner. 1991. Fundamentals of sensation and perception. Brooks/Cole, California.

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

Madsen, T. and R. Shine. 1993. Temporal variability in sexual selection acting on reproductive tactics and body size in male snakes. Am Nat 141:167-171.

McGuigan, K., A. Van Homrigh, and M. W. Blows. 2008. Genetic analysis of female preference functions as function-valued traits. Am Nat 172:194-202.

Mead, L. S. and S. J. Arnold. 2004. Quantitative genetic models of sexual selection. Trends Ecol Evol 19:264-271.

Mousseau, T. A. and D. A. Roff. 1987. Natural selection and the heritability of fitness components. Heredity 59:181-197.

Nichols, R. A. and R. K. Butlin. 1989. Does runaway sexual selection work in finite populations. J Evolution Biol 2:299-313.

O'Donald, P. 1980. Genetic models of sexual selection. Cambridge University Press, Cambridge, England.

Partan, S. R. and P. Marler. 2005. Issues in the classification of multimodal communication signals. Am Nat 166:231-245.

Pomiankowski, A. and Y. Iwasa. 1993. Evolution of multiple sexual preferences by Fisher's runaway process of sexual selection. P Roy Soc Lond B Bio 253:173-181.

Pomiankowski, A., Y. Iwasa, and S. Nee. 1991. The evolution of costly mate preferences 1. Fisher and biased mutation. Evolution 45:1422-1430.

Prum, R. O. 2012. Aesthetic evolution by mate choice: Darwin's really dangerous idea. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2253-2265.

Qvarnstrom, A., J. E. Brommer, and L. Gustafsson. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. Nature 441:84-86.

Real, L. 1990. Search theory and mate choice 1. Models of single-sex discrimination. Am Nat 136:376-405.

Reinhold, K., K. Reinhold, and K. J. Jacoby. 2002. Dissecting the repeatability of female choice in the grasshopper *Chorthippus biguttulus*. Anim Behav 64:245-250.

Ritchie, M. G. 1996. The shape of female mating preferences. P Natl Acad Sci USA 93:14628-14631.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution:849-861.

Servedio, M. R. 2007. Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. Evolution 61:2772-2789.

Servedio, M. R. and R. Lande. 2006. Population genetic models of male and mutual mate choice. Evolution 60:674-685.

Shaw, K. L. and S. C. Lesnick. 2009. Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. P Natl Acad Sci USA 106:9737-9742.

Shettleworth, S. J. 2009. Cognition, evolution, and behavior. Oxford University Press, Oxford, England.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: Double or nothing ? Evolution 62:2435-2440.

Turner, T. L. and P. M. Miller. 2012. Investigating natural variation in Drosophila courtship song by the evolve and resequence approach. Genetics 191:633-642.

Uyeda, J. C., S. J. Arnold, P. A. Hohenlohe, and L. S. Mead. 2009. Drift promotes speciation by sexual selection. Evolution 63:583-594.

Wagner, W. E. 1998. Measuring female mating preferences. Anim Behav 55:1029-1042.

Wedell, N. and T. Tregenza. 1999. Successful fathers sire successful sons. Evolution 53:620-625.

Widemo, F. and S. A. Sæther. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. Trends Ecol Evol 14:26-31.

Wiley, C., C. K. Ellison, and K. L. Shaw. 2012. Widespread genetic linkage of mating signals and preferences in the Hawaiian cricket. P Roy Soc B-Biol Sci 279:1203-1209.

Wiley, C. and K. L. Shaw. 2010. Multiple genetic linkages between female preference and male signal in rapidly speciating Hawaiian crickets. Evolution 64:2238-2245.

APPENDIX A

FIGURES

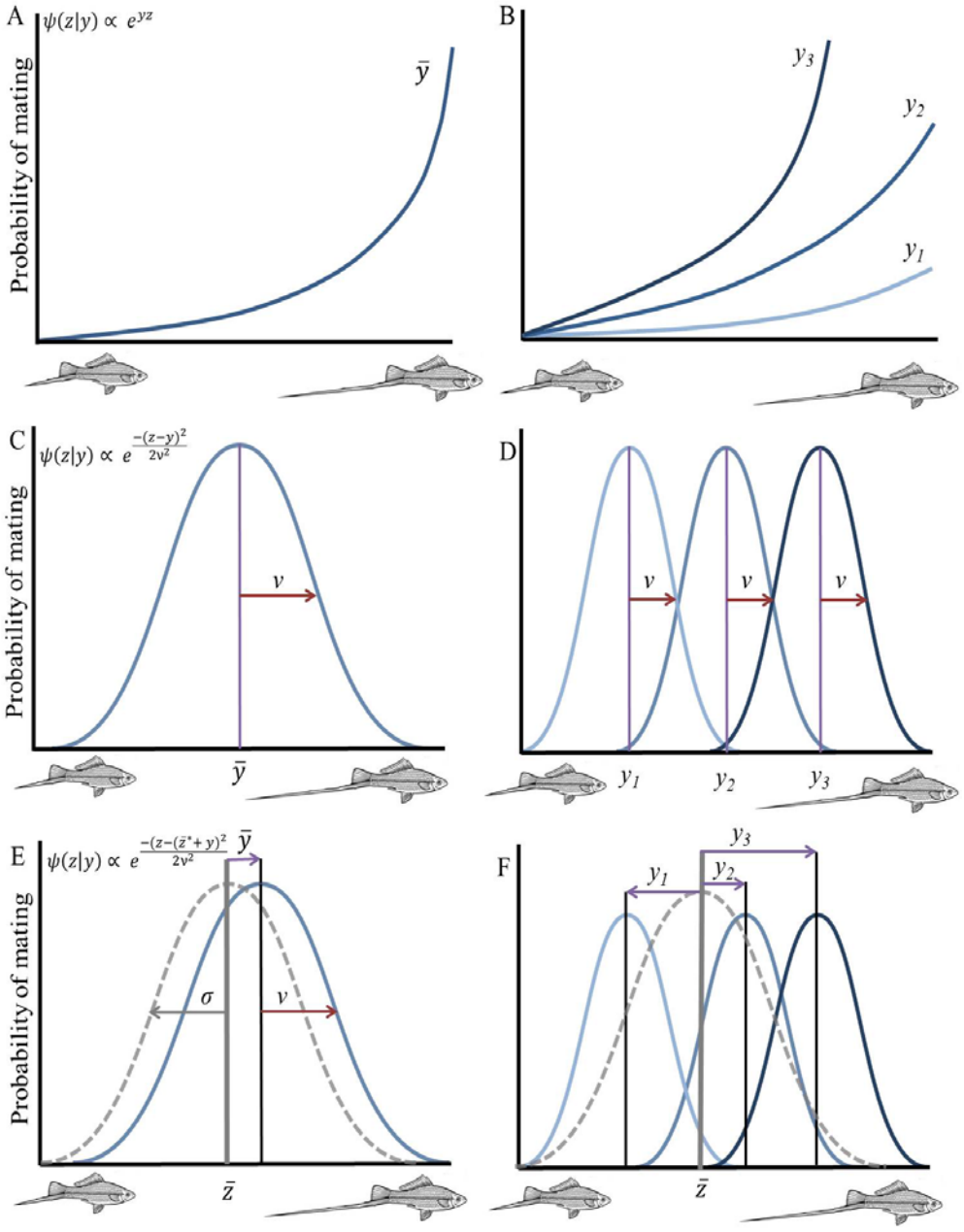

Figure 1.1. Theoretical preference functions. Each model of preference is shown at the population level (left) as well as the individual-level (right). The mathematical function for each model is indicated at the top left of each population-level preference function. Individual preference values are indicated as y_1-y_3, where individual 3 has the largest preference value in each model. Male ornament values (z) are indicated on the x-axis and the probability that a female will mate with a male ($\psi(z|y)$) is indicated on the y-axis. (A) The psychophysical model describes an open-ended preference function where the probability of mating increases exponentially with male ornament values. (B) Individuals vary in the psychophysical model in the slope of their exponential function. (C) The unimodal absolute model is described by a Gaussian function with a width parameter (v). (D) Individuals vary with respect to their peak preference. (E) The unimodal relative model differs from the absolute model in that female preferences are scaled relative to the mean male phenotype (gray bar) in the population. (F) Individual females may exhibit positive or negative preferences, depending on the relative distance of their peak preference from the mean male ornament value in the population. The unimodal relative model assumes that females assess the mean male phenotype in the population.

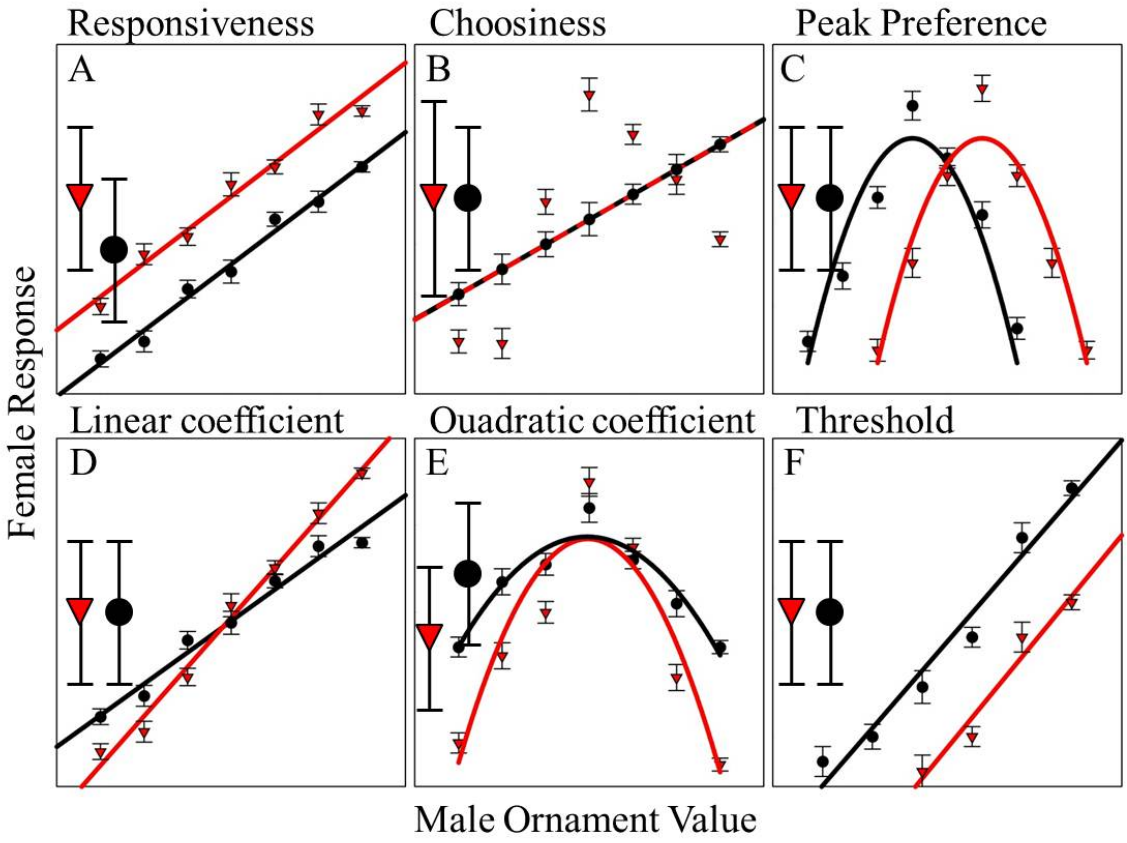

Figure 1.2. Empirical components of mate choice. Simulated data were used to generate responses (continuous scores) that differ in each attribute of choice behavior while holding all others constant (except in the case of the quadratic preference function, for which this is not possible). Two individuals are depicted, one by red triangles and one by black circles. The mean and standard deviation of each individual are computed with the pooled data of all trials for all stimuli, and are depicted by their respective symbols and bars on the left of each plot. (A) Responsiveness is the mean response to all stimuli. (B) Choosiness is estimated the standard deviation in response to all stimuli. Note the independence of choosiness from responsiveness, and, in this case, linear preference functions. (C) Peak preference is the ornament value that maximizes female response. (D) Linear preference functions are the linear regression coefficients of response on ornament values. (E) Quadratic preference functions are the quadratic regression coefficients of response on ornament values. (F) Thresholds are determined by x-intercepts.

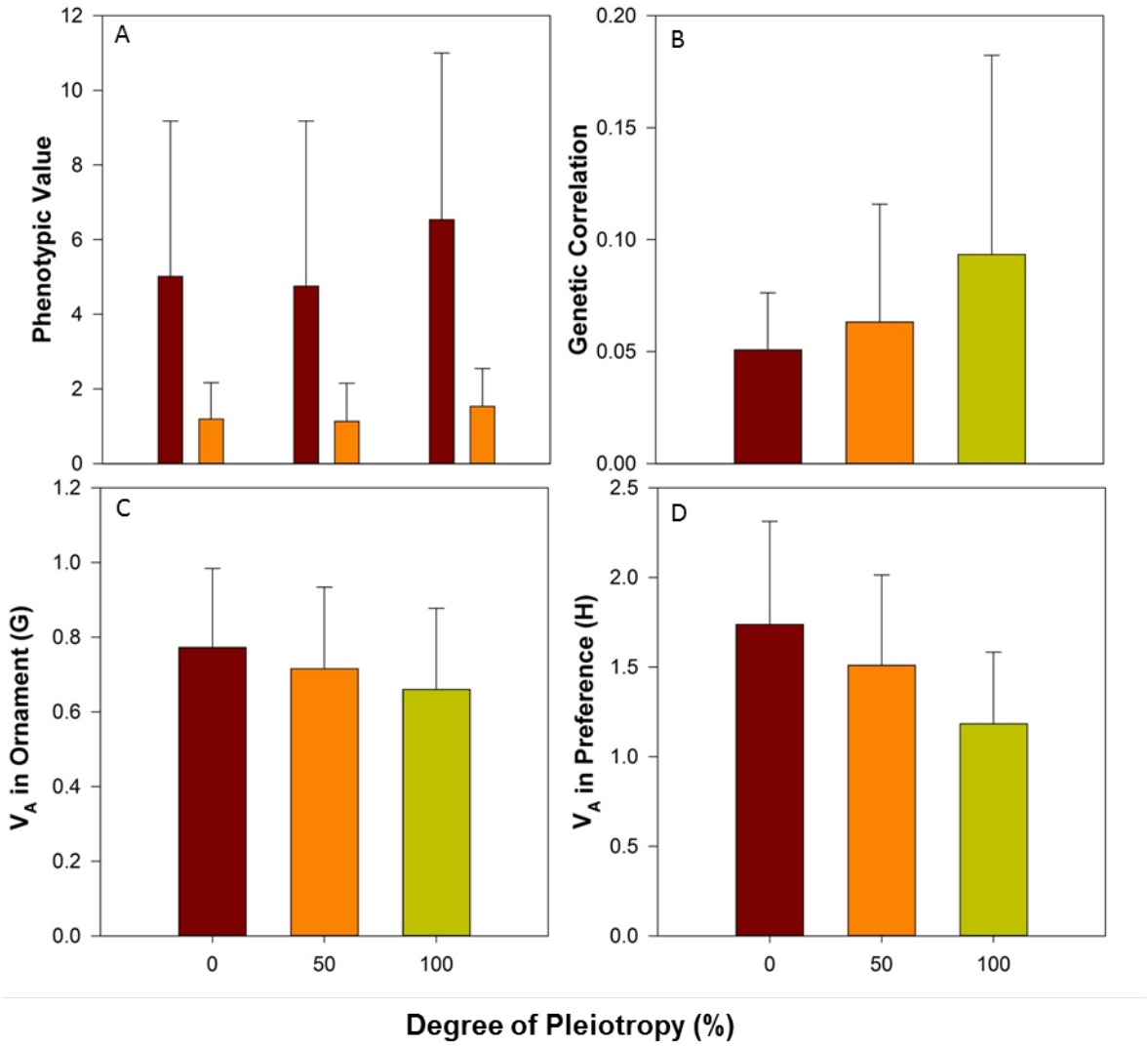

Figure 2.1. Pleiotropy. Degree of pleiotropy influences levels of additive genetic variance and the genetic correlation, but not phenotypic means. A. Ornament (maroon) and preference (orange) phenotypic means for 0, 50, and 100% pleiotropic genetic architectures. B. Despite a mutational correlation of zero, more pleiotropy leads to larger genetic correlations. C. Mean V_A in the ornament does not show as marked of a pattern as the preference, though it appears that the level of additive genetic variance also declines as the degree of pleiotropy increases. D. Mean V_A in the preference declines as the genetic architecture becomes more pleiotropic. Error bars are standard deviations calculated from the means of the twenty replicates.

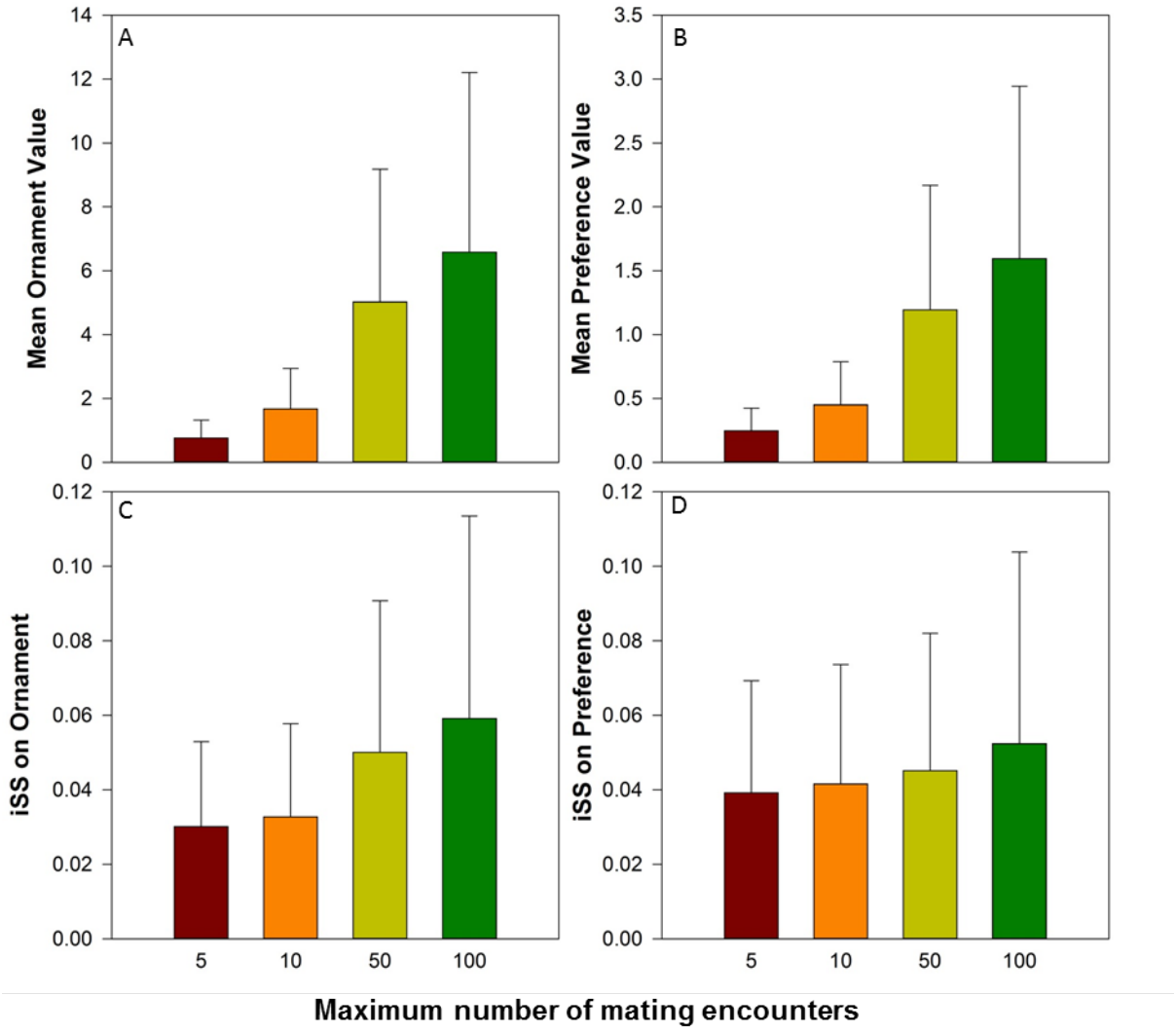

Figure 2.2. Mating encounters. The cost of extreme preference values was manipulated by changing the maximum number of individuals that each female was able to encounter while they searched for a mate, where individuals with extreme preference values are unlikely to find an acceptable mate, and thus will be faced with a cost to their preference value. A. As females are able to sample more males, the degree of elaboration of the ornament increases dramatically. B. Mean preference values also increase with more mating encounters. C & D show the intensities of indirect selection acting on the ornament (C) and the preference (D) for each number of encountered males. When few mates are sampled sexual selection operates with greater intensity which is translated to greater intensities of indirect sexual selection for both ornaments and preferences. Error bars are standard deviations calculated from the means of the twenty replicates.

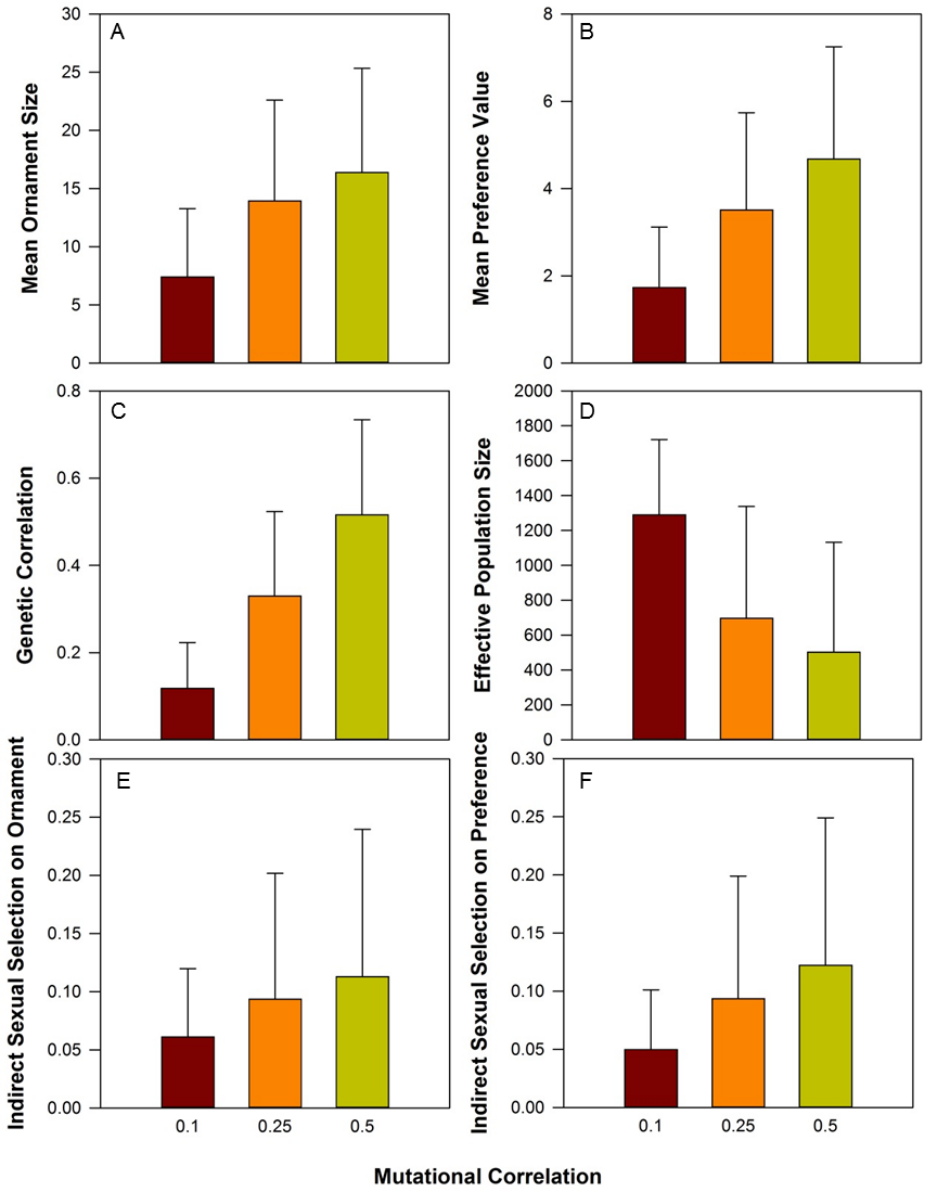

Figure 2.3. Mutational correlation. The mutational correlation has a substantial effect on multiple variables. A&B. Mean phenotype values for the ornament and preference increase with increasing mutational correlation. C. The mean genetic correlation across all twenty runs is equal to the mutational correlation, though there is variation in this as indicated by the error bars. D. The effective population size declines dramatically with increased mutational correlation, which is likely caused by many of the populations with $r_\mu = 0.5$ going extinct before the end of the simulation. E&F. The intensity of indirect sexual selection on the ornament and preference increase with an increase in mutational correlation, suggesting that the Fisher process is more likely to occur when the genetic architecture is pleiotropic with a large mutational correlation.

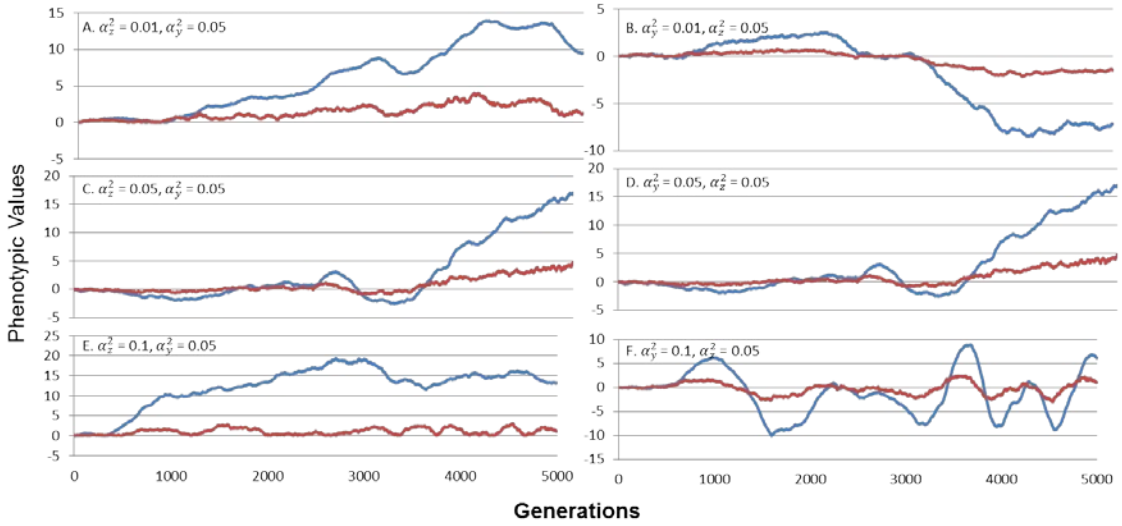

Figure 2.4. Mutational variances. Phenotypic values evolving through time with different mutational variances (blue = ornament; red = preference). On the left (A, C, & E) male mutational variance were changed while female mutational variance was held constant, and on the right (B, D, & F), female values were changed and male values were held constant. An asymmetry in the effects of sex-specific mutational variances can be inferred by comparing A-E with F: when female mutational variance is 0.1, the system enters cyclical evolution, whereas all other combinations fail to produce this pattern.

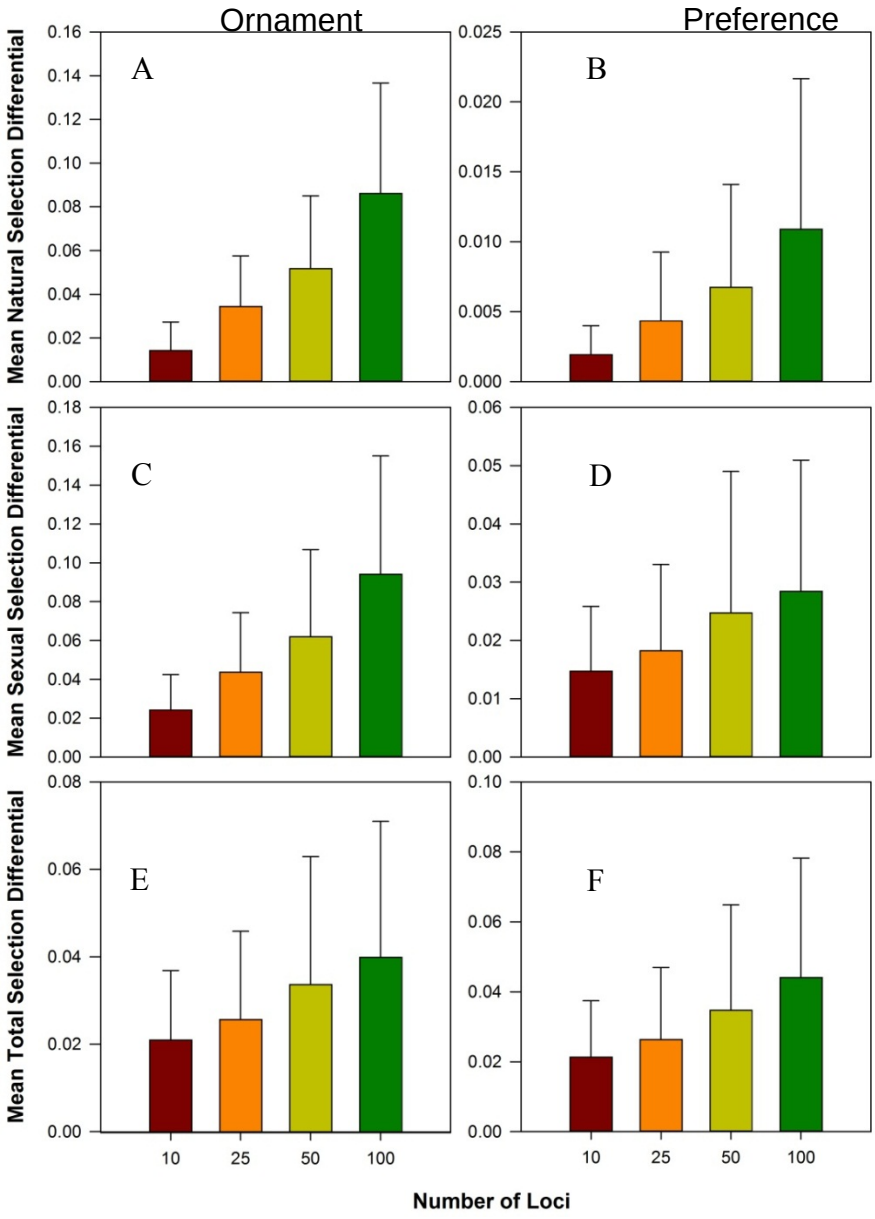

Figure 2.5. Number of loci: selection. The effects of number of loci on various selection differentials. In general, selection differentials increase with the number of loci. The graphs on the left are selection on the male ornament and the graphs on the right are selection on the preference. The means are calculated from the absolute value of each covariance (see Appendix 1): the total selection differential can be calculated as the sum of the natural and sexual selection differentials. Oddly, it appears as though natural and sexual selection act in the same direction for the preferences but not the ornaments.

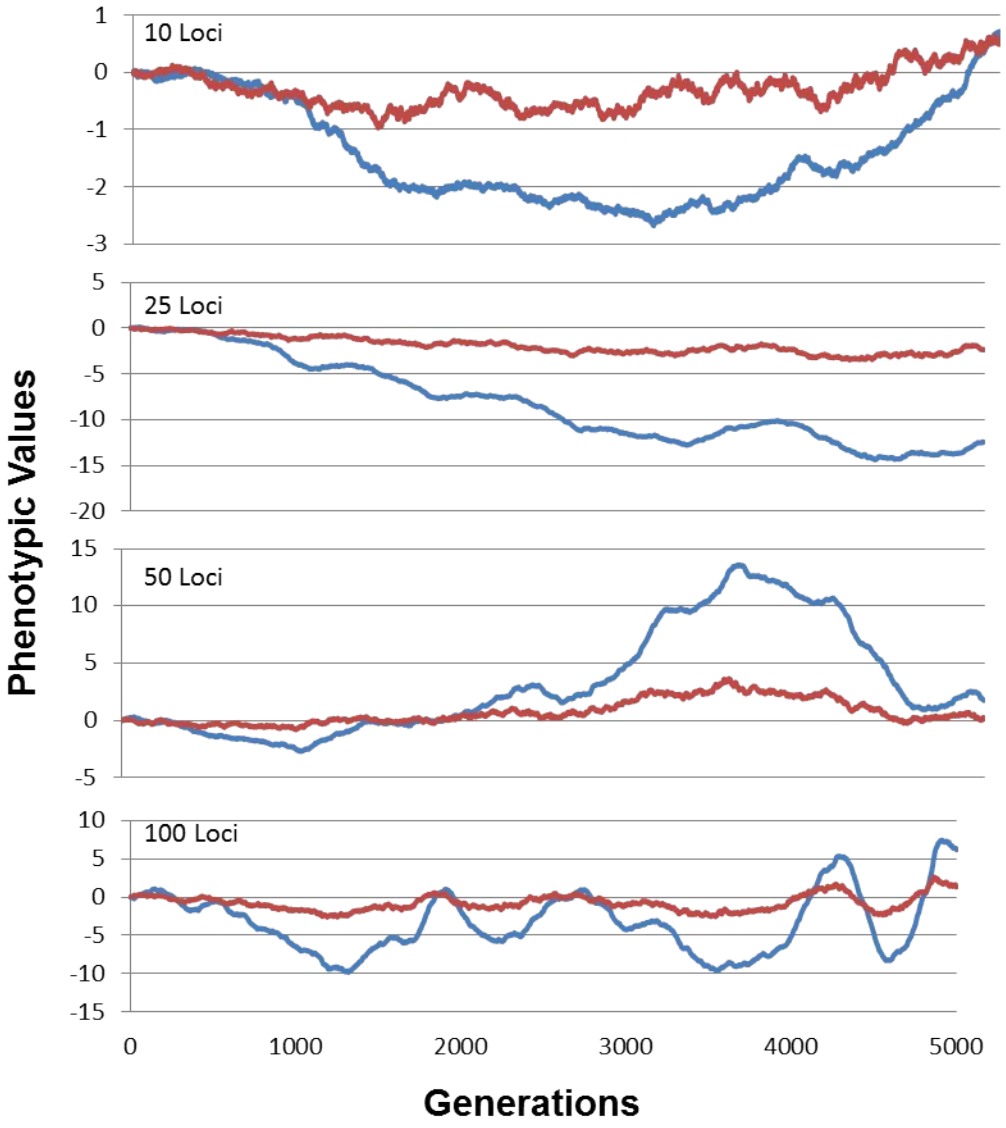

Figure 2.6. Number of loci: phenotypic evolution. Cyclical evolution of the ornament and preference is influenced by the number of loci controlling the traits. Fewer loci leads to a more stable, but less exaggerated evolutionary pattern. However, as the number of loci increases, the period of the cycles (time from peak to peak) decreases, likely because there is more genetic variation in the population. Male ornaments are in blue and female preferences are in red.

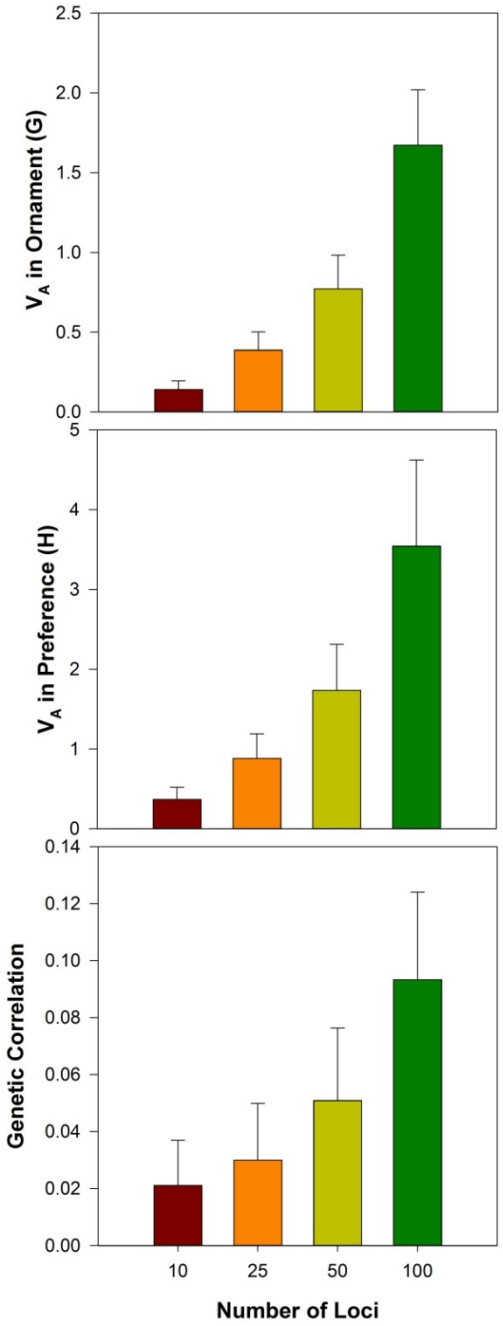

Figure 2.7. Number of loci: **G**, **H**, and r_g. Levels of genetic variation are affected by the number of loci controlling the traits. Because genetic correlations depend on the magnitude of **G** and **H**, an increase in genetic variation in the traits naturally leads to an increased genetic correlation. The increased genetic correlation is likely the cause of the cyclical patterns of phenotypic evolution seen in Figure 6.

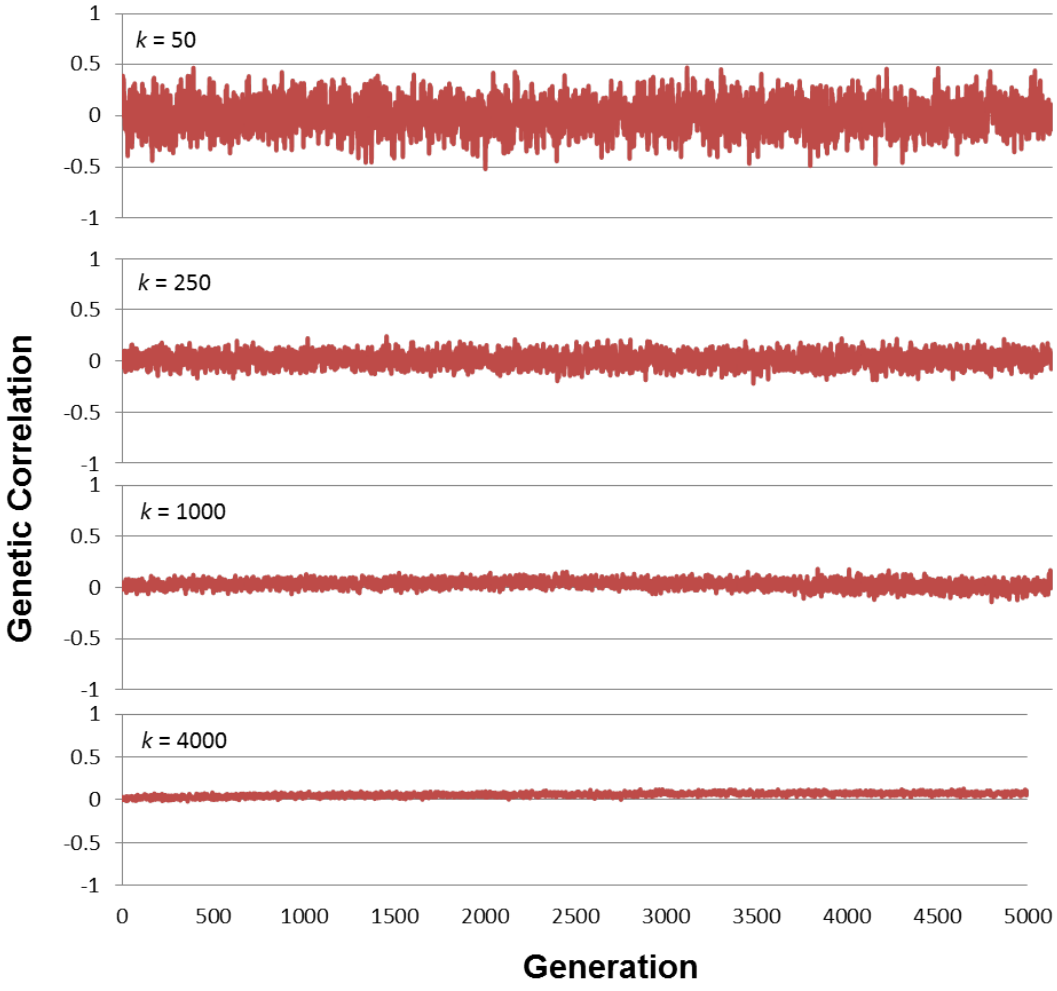

Figure 2.8. Population size. The intensity of genetic drift is manipulated by changing the carrying capacity (k) of the population, with smaller populations more susceptible to change due to drift. When populations are small (k = 50) the genetic correlation becomes unstable and changes dramatically through time compared to larger populations (k = 4,000). Similarly, the magnitude of the genetic correlation increases in small populations.

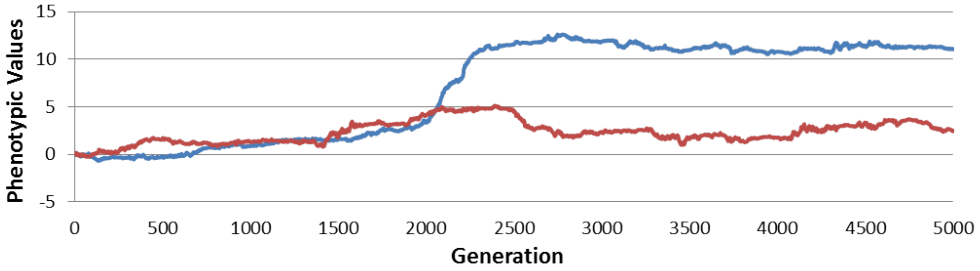

Figure 2.9. Getting stuck. The ornament (blue) increases in value until the genetic variation is depleted, at which point the ornament is stuck despite strong sexual selection from the female preference (red).

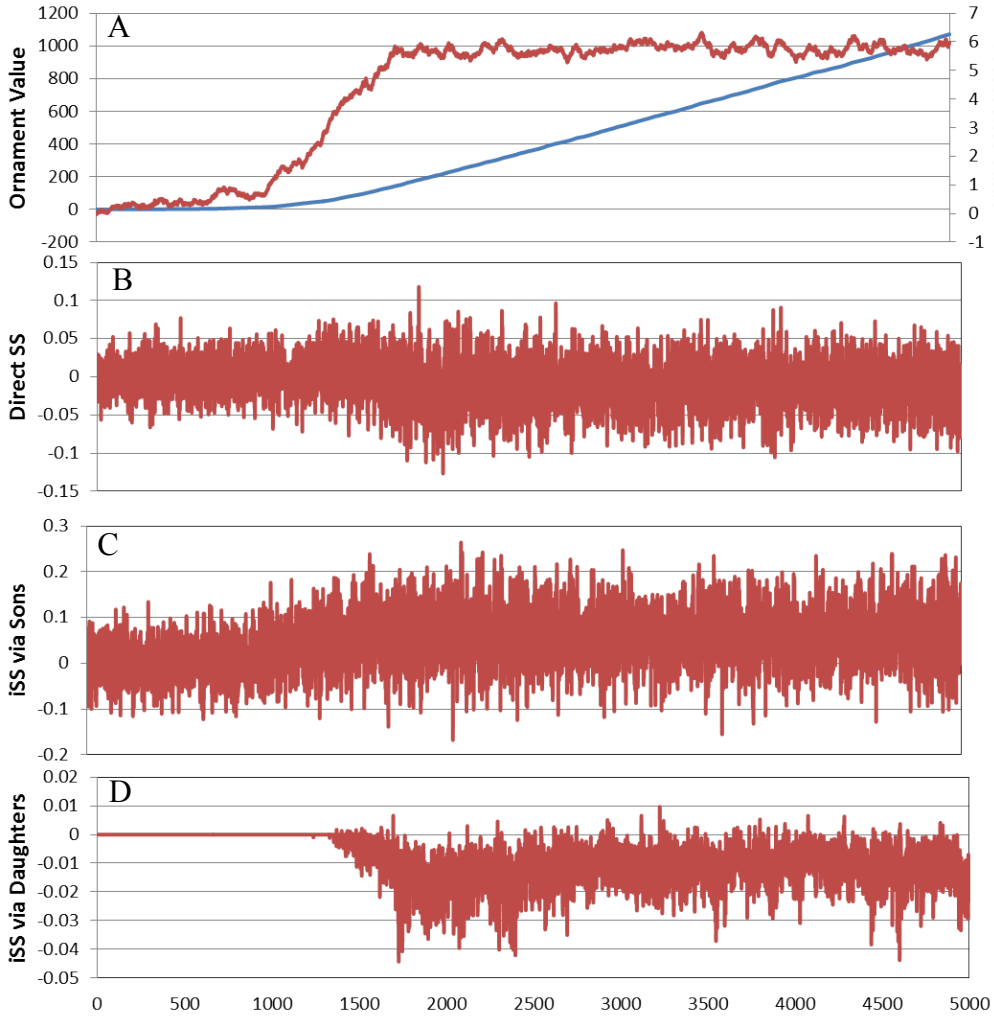

Figure 2.10. No selection on preferences. When there is no natural selection on males or females, the effects of sexual selection can be observed in isolation. A. Phenotypic values for the ornament (blue) and preference (red) show different patterns. The graph is scaled such that the preference values are indicated on the right side of the graph. The male ornament evolves to extreme values, though the preference plateaus. B. Direct sexual selection acting on the preference is variable and opposes further preference elaboration: females with extreme preference values simply do not mate and thus are sexually selected against. C. Indirect sexual selection via son's mating success (i.e., the Fisher process) is nearly twice as strong as direct sexual selection and is favoring females with larger preferences. D. Indirect sexual selection via daughter's mating success selects for less extreme preferences: daughters of females with extreme preferences are unlikely to mate and thus extreme preferences are selected against. Note the difference in y-axis scale for B, C, and D.

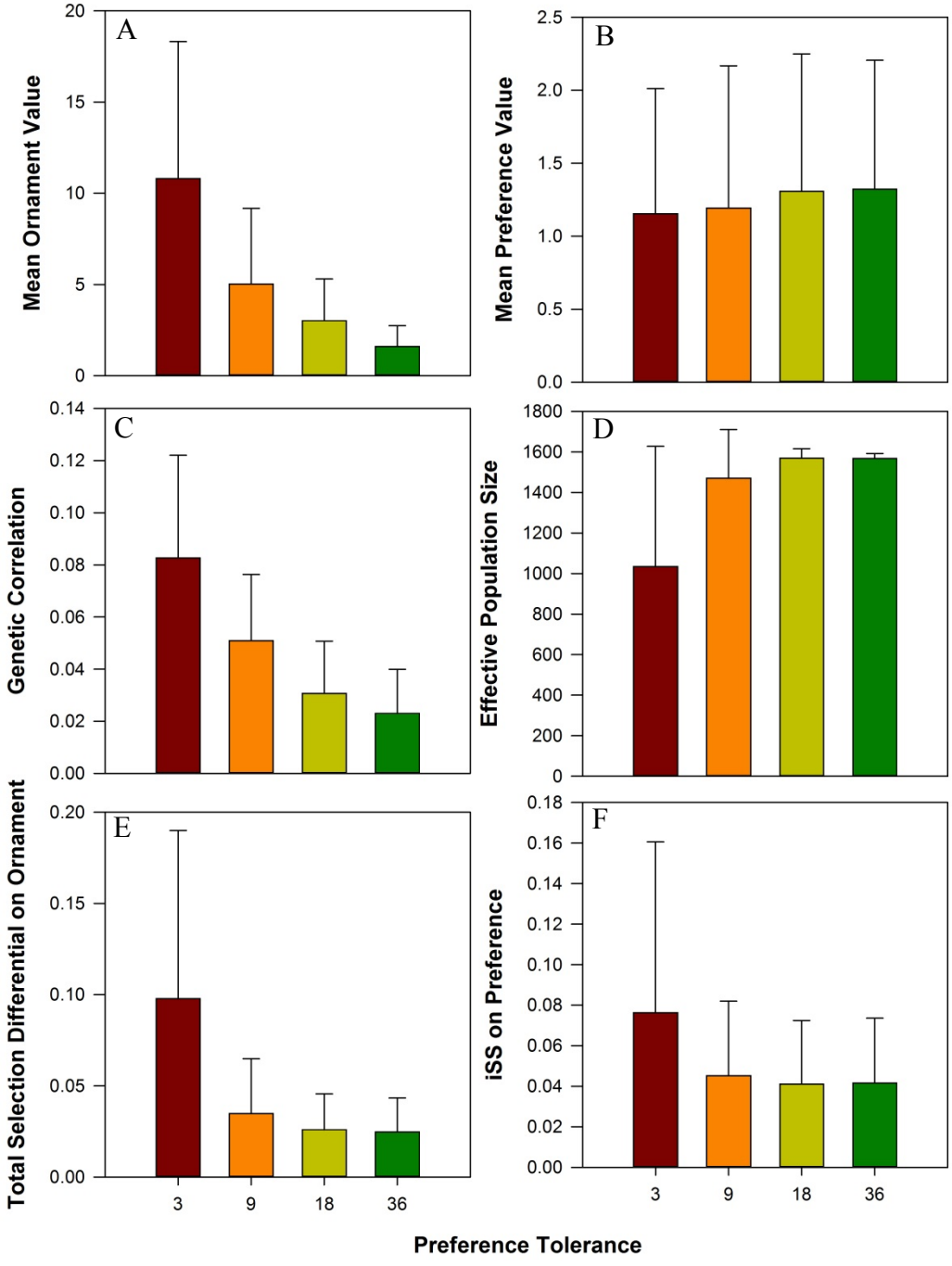

Figure 2.11. Tolerance. The width of the individual-level preference function is termed tolerance. Smaller numbers mean a narrower preference function, which translates into more selective females. A. Male ornaments become substantially more elaborate when

females are intolerant of males that deviate from their preference value. B. Female preferences do not seem to evolve as a response to variation in tolerance. C. Positive assortative mating causes genetic correlations in these runs, and when females are more strict about the identity of their mate, a stronger genetic correlation builds up. D. Effective population size only seems to be sensitive to small tolerances: larger values are much more similar and less variable. E. Total selection on the male ornament is much stronger when preference functions are narrow, which is likely the cause of the degree of elaboration in the ornaments. F. Indirect sexual selection on preferences via son's mating success (Fisherian mechanism) is dependent on tolerance, where stricter preferences experience stronger selection. It is odd that the preference values do not show the same pattern.

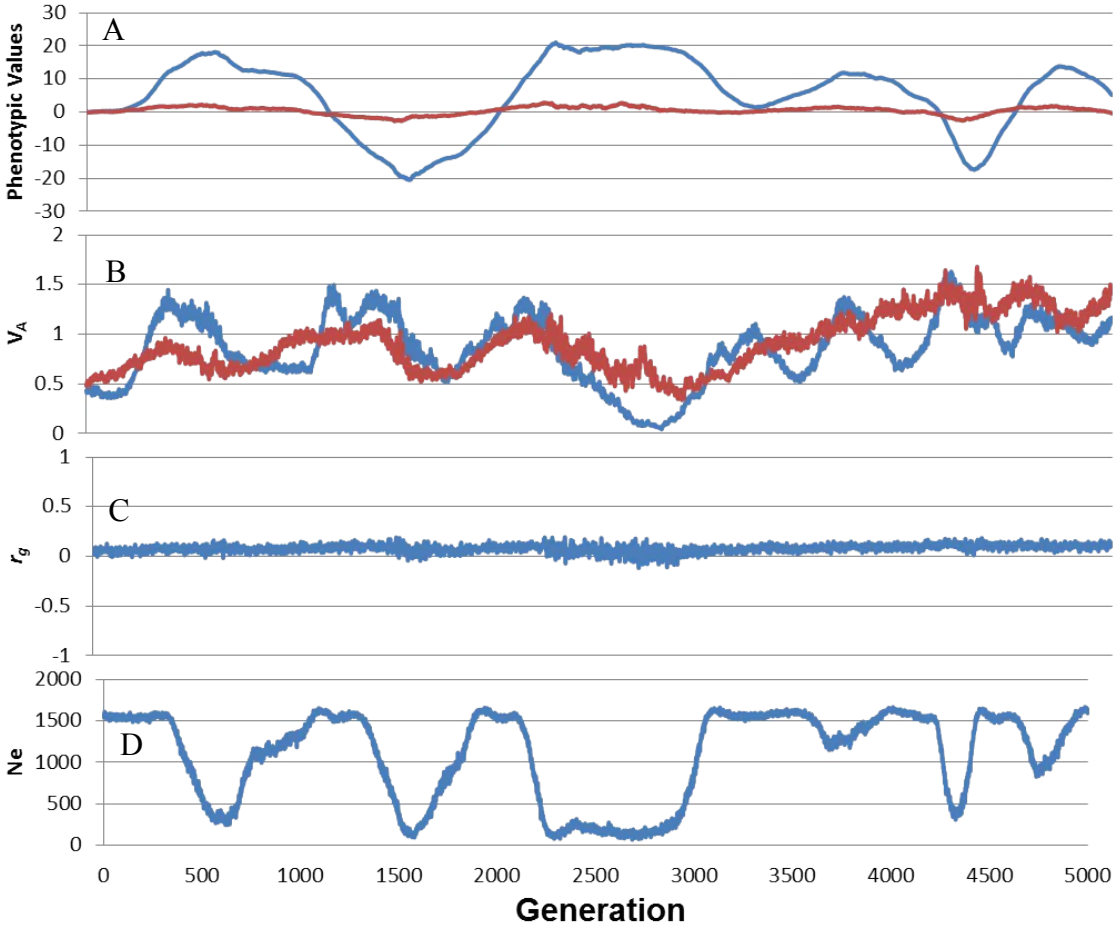

Figure 2.12. Intolerance. Narrow preference functions, caused by small values of v^2 (v^2 = 3 in this example) can lead to cyclical evolution. A. When female preference values deviate from zero they generate very strong sexual selection on the ornament, despite the preference values being very small. B. The levels of genetic variation fluctuate with the intensity of sexual selection. C. The genetic correlation does not increase to very large values, though the stability of the genetic correlation changes when the population size declines (D), which is a likely consequence of males with extreme ornament values not surviving natural selection.

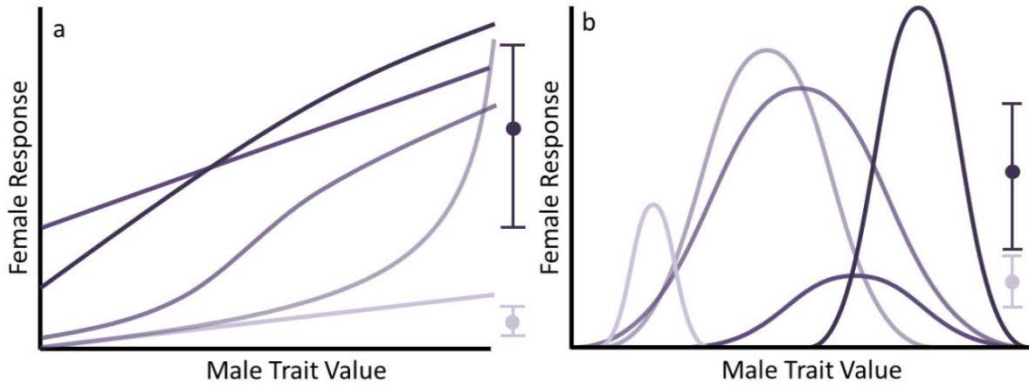

Figure 3.1. Hypothetical individual-level variation in preference functions. Preference function variation demonstrates the ways in which individuals may vary in their responsiveness, choosiness, and peak preference. The mean (responsiveness) and standard deviation (choosiness) of the largest and smallest female preference values are indicated by dots and bars, respectively. Though the two most extreme preference functions exhibit a positive relationship between responsiveness and choosiness, this is not a requirement, nor is it present in our data set. (a) Open-ended preference functions do not vary in peak preference because all females prefer the most extreme male trait values. However, females can vary in responsiveness and choosiness. (b) Unimodal preference functions can vary in the peak preference, responsiveness, and choosiness. Notice that the rank-order of male attractiveness changes for each function. Narrower functions represent choosier females and taller peaks represent females with higher responsiveness.

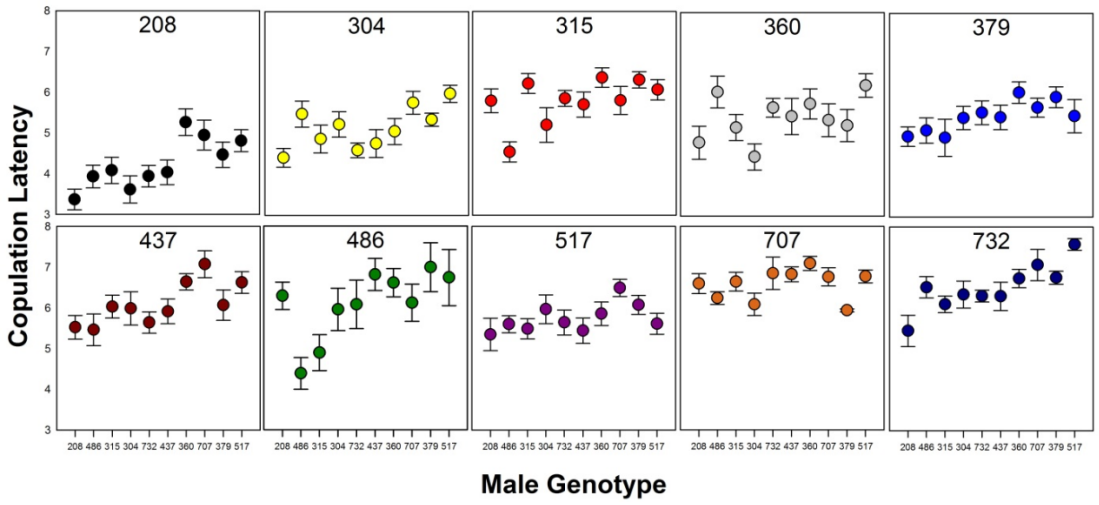

Figure 3.2. Individual female preference functions for attractiveness. Male genotypes have been ordered according to their global attractiveness along the x-axis and are identical in each graph, with the most attractive genotype (208) on the left and least attractive genotype (517) on the right. Copulation latency is measured in seconds on a log scale and the mean ± SEM is reported for each pairing between lines. Note that lower y-values indicate higher attractiveness because less time is needed for those males to attain copulations. Responsiveness is measured as the mean copulation latency of females from each genotype, and is evident in the variation among lines in the height of the means (e.g., 208 vs. 707). Choosiness is measured as the variance of the mean (responsiveness), with more variability in responses (e.g., 486) being considered more choosy than less variable responses (e.g., 379).

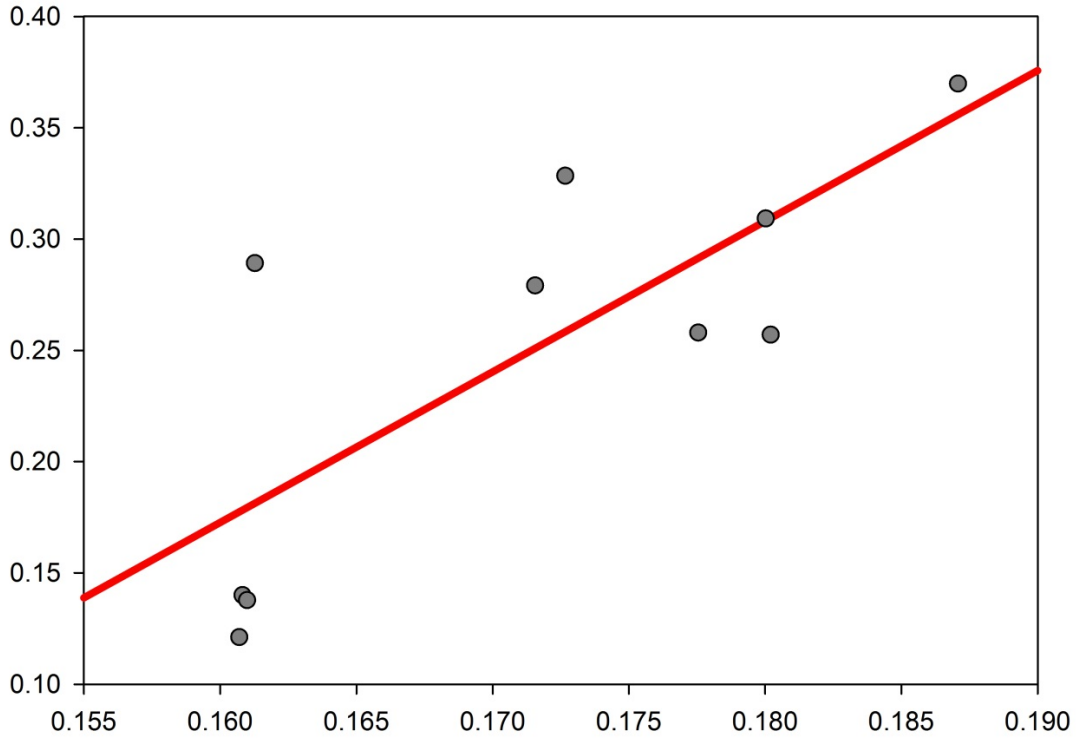

Figure 3.3. Genetic correlation between attractiveness and choosiness. A positive genetic correlation between male attractiveness and female choosiness. We estimated the genetic correlation by regressing the standard deviation in female copulation latency (choosiness) on mean male copulation latency (global attractiveness) for all ten isogenic lines. The relationship indicates that genotypes with heightened male attractiveness also have increased female choosiness ($r^2 = 0.597$; $n = 10$; $p = 0.008$). For the purpose of demonstrating the positive nature of the genetic correlation, we used inverse copulation latency so that larger values of male attractiveness corresponded to more attractive males.

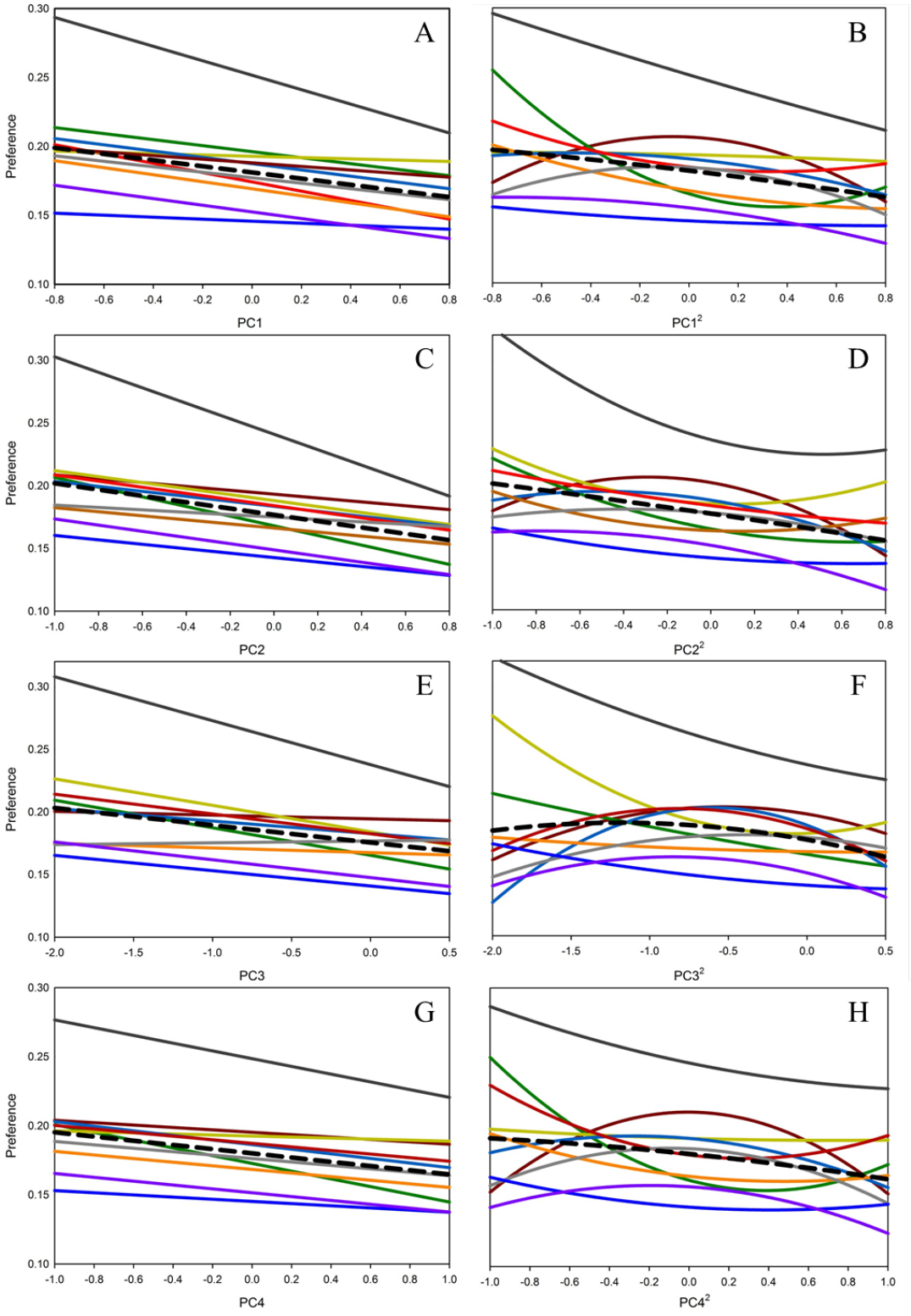
A
B
C
D
E
F
G
H
Preference
PC1
PC1²
PC2
PC2²
PC3
PC3²
PC4
PC4²

Figure 4.1. Individual-level preference functions for multivariate song traits. Individual-level preference functions were obtained by regressing copulation latency on linear or quadratic terms for each principal component. Preference (y-axis) was measured on a log scale from which the inverse was taken so that larger values represent larger preferences. The population-level preference function is indicated by the black dashed line. Linear and quadratic preference functions for each composite trait are shown on the same row: (A) PC1 b; (B) PC1 g; (C) PC2 b; (D) PC2 g; (E) PC3 b; (F) PC3 g; (G) PC4 b; (H) PC4 g. Regression coefficients for each individual-level preference function can be found in Table 2 (note that inverse values change the sign of the coefficients). Genotypes are represented by different colors, which are identical across the eight graphs: 208: dark gray; 304: green; 315: light red; 360: yellow; 379: light blue; 437: orange; 486: dark red; 517: light gray; 707: dark blue; 732: purple.

APPENDIX B

TABLES

Table 1.1. P definitions for behavioral components of mate choice.

Term	Conceptual Definition	Operational Definition	Measures	Synonym
Responsiveness	The overall propensity to respond to males	The mean value of response across all stimulus values	Mean of response variable	Motivation, Receptivity, Resistance
Choosiness	The degree of discrimination among male phenotypes	Measures of dispersion about the mean response indicating the degree to which individuals prefer some stimuli over others	Variability in response variable	Discrimination, strength of preference, search strategy
Peak Preference	The most attractive phenotype, whether or not it is represented by currently available males	Value of courter phenotype corresponding to maximum measured or predicted response across stimuli	Maximum response	Preference, Strength of preference
Linear preference function	The linear ranking of ornament values according to attractiveness	The linear regression coefficient of responses on stimulus values	β (linear regression coefficient)	Preference gradient, Strength of preference
Quadratic preference function	The nonlinear ranking of ornament values according to attractiveness	The quadratic regression coefficient of responses on stimulus values	γ (quadratic regression coefficient)	Nonlinear preference, nonlinear selection gradient / strength

Table 2.1. Details of the parameters varied in the model. Each parameter was varied individually while holding all others at their standard value. Twenty replicates runs were made for each variable value of each parameter.

Parameter Name	Symbol	Standard value	Variable values
Degree of pleiotropy	d	0%	0%, 50%, 100%
Maximum mating encounters	m	50	5, 10, 50, 100
Mutation rate	μ	0.0002	0.00005, 0.00009, 0.0001, 0.0002
Mutational correlation	r_μ	0	0.1, 0.25, 0.5
Mutational variance (ornament)	α_z^2	0.05	0.01, 0.05, 0.1
Mutational variance (preference)	α_y^2	0.05	0.01, 0.05, 0.1
Number of loci	n	50 per trait	10, 25, 50, 100 per trait
Carrying capacity	k	2000	50, 250, 500, 1000, 4000
Strength of selection (ornament)	ω_z^2	49	0, 9, 49, 99, 199
Strength of selection (preference)	ω_y^2	0	0, 9, 49, 99, 199
Tolerance	v^2	9	3, 9, 18, 36

Table 2.2. Mean values for twenty replicate runs for each treatment.

Treatment	$\lvert\bar{z}\rvert$	$\lvert\bar{y}\rvert$	$\bar{\mathbf{G}}$	$\bar{\mathbf{H}}$	$\lvert\overline{r_g}\rvert$	$\overline{N_e}$
d = 0	5.024	1.194	0.772	1.738	0.051	1470.566
d = 50	4.764	1.138	0.716	1.510	0.063	1447.582
d = 100	6.543	1.543	0.660	1.184	0.094	1340.344
m = 5	0.761	0.246	0.617	1.496	0.042	1551.608
m = 10	1.676	0.451	0.703	1.757	0.049	1564.786
m = 50	5.024	1.194	0.772	1.738	0.051	1470.566
m = 100	6.582	1.596	0.767	1.718	0.052	1365.099
μ = 0.00005	1.821	0.511	0.228	0.474	0.023	1563.201
μ = 0.00009	3.435	0.866	0.375	0.831	0.029	1539.969
μ = 0.0001	3.512	0.859	0.413	0.953	0.032	1533.314
μ = 0.0002	5.024	1.194	0.772	1.738	0.051	1470.566
r_μ = 0.1	7.387	1.733	0.704	1.148	0.118	1289.271
r_μ = 0.25	13.937	3.513	0.443	0.527	0.330	696.079
r_μ = 0.5	16.383	4.678	0.302	0.318	0.516	502.597
α_z^2 = 0.01	4.352	1.077	0.293	1.833	0.036	1528.613
α_z^2 = 0.05	5.024	1.194	0.772	1.738	0.051	1470.566
α_z^2 = 0.1	5.918	1.396	1.023	1.741	0.056	1445.677
α_y^2 = 0.01	1.908	0.467	0.610	0.376	0.026	1567.055
α_y^2 = 0.05	5.024	1.194	0.772	1.738	0.051	1470.566
α_y^2 = 0.1	4.586	1.123	0.977	3.234	0.074	1537.383
n = 10	1.290	0.375	0.141	0.368	0.021	1562.281
n = 25	3.590	0.867	0.388	0.883	0.030	1527.617
n = 50	5.024	1.194	0.772	1.738	0.051	1470.566
n = 50	4.438	1.122	1.672	3.545	0.093	1555.545
k = 50	3.570	1.436	0.067	0.073	0.112	37.537
k = 250	5.539	1.458	0.252	0.353	0.054	178.610
k = 500	6.393	1.504	0.401	0.619	0.044	340.972
k = 1000	5.996	1.408	0.602	1.131	0.042	705.372
k = 4000	3.609	0.875	0.903	2.390	0.062	3134.342
ω_z^2= 0	423.685	4.344	1.083	1.204	0.047	1430.013
ω_z^2= 9	0.720	0.849	0.427	1.901	0.037	1580.171
ω_z^2= 49	5.024	1.194	0.772	1.738	0.051	1470.566
ω_z^2= 99	12.206	1.614	0.757	1.307	0.052	1162.964
ω_z^2= 199	24.785	2.044	0.633	0.825	0.050	849.506
ω_y^2 = 0	5.024	1.194	0.772	1.738	0.051	1470.566
ω_y^2 = 9	0.170	0.067	0.530	0.548	0.025	1521.190
ω_y^2 = 49	0.383	0.145	0.585	1.149	0.036	1545.304
ω_y^2 = 99	0.616	0.213	0.594	1.430	0.041	1550.049
ω_y^2 = 199	0.942	0.294	0.632	1.507	0.043	1554.810
ω_y^2 = 499	1.918	0.505	0.719	1.829	0.050	1566.647
v^2 = 3	10.807	1.155	0.704	0.908	0.083	1033.902
v^2 = 9	5.024	1.194	0.772	1.738	0.051	1470.566
v^2 = 18	3.021	1.309	0.800	1.739	0.031	1569.407
v^2 = 36	1.608	1.323	0.877	1.825	0.023	1567.667

Table 2.3. Variances of twenty replicate runs for each treatment.

Treatment	$\overline{Var(\lvert\bar{z}\rvert)}$	$\overline{Var(\lvert\bar{y}\rvert)}$	$\overline{Var(\bar{\mathbf{G}})}$	$\overline{Var(\bar{\mathbf{H}})}$	$\overline{Var(\lvert\overline{r_g}\rvert)}$	$\overline{Var(\overline{N_e})}$
d = 0	17.271	0.949	0.045	0.331	0.001	57673.500
d = 50	19.494	1.043	0.048	0.254	0.003	83128.338
d = 100	19.829	1.013	0.047	0.159	0.008	101766.197
m = 5	0.311	0.032	0.009	0.167	0.001	504.591
m = 10	1.608	0.112	0.020	0.308	0.001	677.707
m = 50	17.271	0.949	0.045	0.331	0.001	57673.500
m = 100	31.604	1.818	0.066	0.424	0.001	145515.200
μ = 0.00005	3.452	0.194	0.010	0.038	0.000	1132.768
μ = 0.00009	10.274	0.532	0.020	0.097	0.000	16383.709
μ = 0.0001	11.368	0.580	0.021	0.134	0.000	25985.752
μ = 0.0002	17.271	0.949	0.045	0.331	0.001	57673.500
r_μ = 0.1	34.619	1.912	0.082	0.210	0.011	186537.746
r_μ = 0.25	75.146	4.967	0.147	0.183	0.038	412009.087
r_μ = 0.5	80.208	6.631	0.120	0.122	0.048	396739.038
α_z^2 = 0.01	12.292	0.641	0.005	0.359	0.000	18275.226
α_z^2 = 0.05	17.271	0.949	0.045	0.331	0.001	57673.500
α_z^2 = 0.1	16.120	0.831	0.092	0.421	0.001	63298.178
α_y^2 = 0.01	2.387	0.126	0.011	0.012	0.000	742.360
α_y^2 = 0.05	17.271	0.949	0.045	0.331	0.001	57673.500
α_y^2 = 0.1	12.303	0.686	0.073	1.152	0.001	17425.628
n = 10	1.530	0.085	0.003	0.023	0.000	703.871
n = 25	8.787	0.428	0.013	0.094	0.000	18582.423
n = 50	17.271	0.949	0.045	0.331	0.001	57673.500
n = 50	9.414	0.594	0.121	1.167	0.001	9995.738
k = 50	10.495	1.119	0.003	0.004	0.007	22.166
k = 250	17.931	1.079	0.012	0.025	0.002	1012.263
k = 500	20.098	1.011	0.023	0.050	0.001	5934.381
k = 1000	24.749	1.363	0.030	0.146	0.001	30243.304
k = 4000	7.969	0.439	0.073	0.873	0.001	16303.886
ω_z^2= 0	124164.593	4.473	0.083	0.079	0.001	4873.683
ω_z^2= 9	0.235	0.326	0.004	0.446	0.000	456.784
ω_z^2= 49	17.271	0.949	0.045	0.331	0.001	57673.500
ω_z^2= 99	113.594	1.988	0.133	0.351	0.001	299802.722
ω_z^2= 199	302.783	2.806	0.221	0.322	0.001	434446.107
ω_y^2 = 0	17.271	0.949	0.045	0.331	0.001	57673.500
ω_y^2 = 9	0.016	0.003	0.005	0.006	0.000	559.189
ω_y^2 = 49	0.084	0.012	0.006	0.067	0.000	505.605
ω_y^2 = 99	0.230	0.026	0.008	0.155	0.001	504.404
ω_y^2 = 199	0.532	0.049	0.010	0.215	0.001	521.741
ω_y^2 = 499	2.451	0.151	0.029	0.403	0.001	782.579
v^2 = 3	56.417	0.733	0.149	0.226	0.002	352919.423
v^2 = 9	17.271	0.949	0.045	0.331	0.001	57673.500
v^2 = 18	5.206	0.884	0.024	0.313	0.000	2199.885
v^2 = 36	1.278	0.781	0.028	0.389	0.000	624.129

Table 2.4 Mean values of sources of selection across twenty runs.

Treatment	$\overline{\lvert S_{NS_z} \rvert}$	$\overline{\lvert D_z \rvert}$	$\overline{\lvert S_{SS_z} \rvert}$	$\overline{\lvert S_z \rvert}$	$\overline{\lvert S_{NS_y} \rvert}$	$\overline{\lvert D_y \rvert}$	$\overline{\lvert S_{SS_y} \rvert}$	$\overline{\lvert S_y \rvert}$	$\lvert \overline{\imath SS_{z_m}} \rvert$	$\lvert \overline{\imath SS_{y_m}} \rvert$
$d = 0$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03364	0.05032	0.04533
$d = 50$	0.04477	0.01637	0.05887	0.03580	0.00593	0.01981	0.02606	0.03422	0.04657	0.04487
$d = 100$	0.04601	0.01613	0.06616	0.04012	0.00669	0.01846	0.03099	0.03836	0.05222	0.04651
$m = 5$	0.01236	0.01589	0.02292	0.02504	0.00218	0.01977	0.02011	0.02824	0.03015	0.03923
$m = 10$	0.02762	0.01629	0.03693	0.02849	0.00339	0.02071	0.02066	0.02951	0.03275	0.04159
$m = 50$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$m = 100$	0.05309	0.01663	0.07256	0.04265	0.00771	0.02050	0.03230	0.04065	0.05915	0.05238
$\mu = 0.00005$	0.02067	0.01389	0.03123	0.02293	0.00246	0.01531	0.01523	0.02172	0.02694	0.03031
$\mu = 0.00009$	0.03480	0.01474	0.04557	0.02616	0.00423	0.01698	0.01776	0.02520	0.03259	0.03382
$\mu = 0.0001$	0.03595	0.01485	0.04599	0.02714	0.00440	0.01753	0.01901	0.02655	0.03346	0.03530
$\mu = 0.0002$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$r_\mu = 0.1$	0.04893	0.01637	0.07255	0.04785	0.00764	0.01823	0.03428	0.04099	0.06094	0.04978
$r_\mu = 0.25$	0.02449	0.01482	0.11966	0.11024	0.00883	0.01524	0.08703	0.08967	0.09351	0.09359
$r_\mu = 0.5$	0.01704	0.01388	0.15199	0.14619	0.00934	0.01397	0.11817	0.11958	0.11287	0.12219
$\alpha_z^2 = 0.01$	0.03781	0.01427	0.05008	0.03286	0.00623	0.02107	0.02237	0.03168	0.03000	0.04115
$\alpha_z^2 = 0.05$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$\alpha_z^2 = 0.1$	0.06635	0.01779	0.07574	0.03618	0.00781	0.02067	0.02552	0.03422	0.06358	0.04704
$\alpha_y^2 = 0.01$	0.02809	0.01586	0.03542	0.02360	0.00253	0.01476	0.01470	0.02105	0.03182	0.02923
$\alpha_y^2 = 0.05$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$\alpha_y^2 = 0.1$	0.06329	0.01756	0.07338	0.04194	0.00902	0.02574	0.02735	0.03885	0.05627	0.05722
$n = 10$	0.01423	0.01336	0.02419	0.02129	0.00193	0.01470	0.01470	0.02094	0.02527	0.02916
$n = 25$	0.03445	0.01473	0.04362	0.02635	0.00435	0.01722	0.01824	0.02567	0.03239	0.03413
$n = 50$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$n = 50$	0.08616	0.02038	0.09415	0.04401	0.01090	0.02673	0.02843	0.03986	0.07733	0.06696
$k = 50$	0.02990	0.08103	0.10221	0.12766	0.01880	0.08168	0.08767	0.12318	0.14000	0.15518
$k = 250$	0.03727	0.03972	0.07072	0.07084	0.01327	0.04129	0.04999	0.06745	0.07421	0.08183
$k = 500$	0.04137	0.02961	0.06667	0.05836	0.01108	0.03182	0.04295	0.05603	0.06015	0.06569
$k = 1000$	0.04885	0.02240	0.06621	0.04694	0.00889	0.02586	0.03385	0.04476	0.05394	0.05465
$k = 4000$	0.05555	0.01223	0.06147	0.02510	0.00524	0.01624	0.01675	0.02372	0.04183	0.03678
$\omega_z^2 = 0$	0.00000	0.01804	0.41163	0.41233	0.00000	0.01864	0.02482	0.03081	0.18870	0.06542
$\omega_z^2 = 9$	0.04592	0.01458	0.04916	0.02235	0.00500	0.02123	0.02101	0.03023	0.02915	0.04240
$\omega_z^2 = 49$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$\omega_z^2 = 99$	0.03309	0.01658	0.07671	0.06397	0.00729	0.01888	0.04484	0.05190	0.06062	0.05404
$\omega_z^2 = 199$	0.02030	0.01587	0.10696	0.09924	0.00768	0.01672	0.06924	0.07428	0.06642	0.06188
$\omega_y^2 = 0$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$\omega_y^2 = 9$	0.00480	0.01486	0.01525	0.02172	0.00718	0.01454	0.01603	0.02285	0.02920	0.02936
$\omega_y^2 = 49$	0.00681	0.01558	0.01792	0.02327	0.00488	0.01808	0.01850	0.02639	0.02932	0.03623
$\omega_y^2 = 99$	0.01026	0.01567	0.02122	0.02456	0.00431	0.01927	0.01963	0.02788	0.02990	0.03878
$\omega_y^2 = 199$	0.01547	0.01589	0.02605	0.02610	0.00375	0.01970	0.01985	0.02818	0.03045	0.03949
$\omega_y^2 = 499$	0.03116	0.01629	0.04002	0.02872	0.00462	0.02103	0.02108	0.03002	0.03394	0.04195
$v^2 = 3$	0.04906	0.01635	0.11041	0.09770	0.00846	0.01722	0.05296	0.05890	0.08061	0.07619
$v^2 = 9$	0.05171	0.01667	0.06189	0.03474	0.00675	0.02074	0.02474	0.03365	0.05006	0.04513
$v^2 = 18$	0.04491	0.01674	0.05088	0.02588	0.00496	0.02078	0.02086	0.02996	0.04066	0.04104
$v^2 = 36$	0.02890	0.01712	0.03488	0.02465	0.00341	0.02091	0.02085	0.02975	0.03593	0.04157

Table 3.1. ANOVA table for courtship latency. A model explaining courtship latency was built that included sex-specific genetic effects and age effects. Both male and female genotypes contribute to variation in courtship latency, demonstrating genetic variation in male courtship propensity and female precopulatory attractiveness.

Source	df	Sum of Squares	Mean Square	F Ratio	p-value
Model	106	292.4800	2.75924	3.0056	<.0001
Error	1215	1115.4092	0.91803		
Total	1321	1407.8892			
Effect	**df**	**Sum of Squares**	**Mean Square**	**F Ratio**	**p-value**
Male Genotype	9	160.58391	17.84266	19.4358	< 0.0001
Female Genotype	9	40.85546	4.53950	4.9448	< 0.0001
Male Age	4	2.12172	0.53043	0.5778	0.6788
Female Age	3	0.79306	0.26435	0.2880	0.8341
Male x Female Genotype	81	76.37103	0.94285	1.0270	0.4160

Table 3.2. ANOVA table for copulation latency. A model explaining copulation latency was built that included sex-specific genetic effects, age-effects, and the effect of courtship latency. Genetic variation exists for both males and females for precopulatory attractiveness (males) and precopulatory preferences (females). The significant interaction indicates that combinations of genotypes contribute to variation in copulation latency due to different rank-ordering of males by females.

Source	df	Sum of Squares	Mean Square	F Ratio	p-value
Model	107	1182.2083	11.0487	5.9966	<.0001
Error	1214	2236.7775	1.8425		
Total	1321	3418.9858			
Effect	df	Sum of Squares	Mean Square	F Ratio	p-value
Male Genotype	9	139.13850	15.45983	8.3907	< 0.0001
Female Genotype	9	715.04046	79.44894	43.1205	< 0.0001
Male Age	4	14.72219	3.68055	1.9976	0.0927
Female Age	3	29.84374	9.94791	5.3992	0.0011
Male x Female Genotype	81	219.84374	2.71415	1.4731	0.0050
ln(Courtship Latency)	1	1.18870	1.18870	0.6452	0.4220

Table 3.3. ANOVA table for copulation duration. We built a model to explain variation in copulation duration due to sex-specific genetic effects, age-effects, courtship latency, and copulation latency. Genetic variation exists for female postcopulatory attractiveness and male copulatory duration. The other three significant terms were included as covariates, as our goal was to account for their variation as opposed to explain it.

Source	df	Sum of Squares	Mean Square	F Ratio	p-value
Model	108	28.64996	0.265277	2.1777	< 0.0001
Error	860	104.75965	0.121814		
Total	968	133.40961			
Effect	**df**	**Sum of Squares**	**Mean Square**	**F Ratio**	**p-value**
Male Genotype	9	8.3303874	0.925599	7.5985	< 0.0001
Female Genotype	9	3.7106852	0.412298	3.3847	0.0004
Male Age	4	0.9902199	0.247555	2.0322	0.0880
Female Age	3	1.4366960	0.478899	3.9314	0.0084
Male x Female Genotype	81	8.6166811	0.106379	0.8733	0.7772
ln (Courtship Latency)	1	4.9015976	4.901598	40.2385	< 0.0001
ln (Copulation Latency)	1	0.5613253	0.561325	4.6081	0.0321

Table 4.1. Principal components analysis of male song traits. Six traits were measured for multiple individuals from 192 isogenic lines (left column). These traits were standardized (mean = 0, variance = 1) and a principal components analysis was performed. To obtain breeding values for each line, we computed the mean PC score among males from each line. These breeding values were used as trait values in our analyses.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Explained variation (%)	**30.542**	**25.548**	**17.978**	**15.366**	**8.349**	**2.217**
Cumulative variation (%)	**30.542**	**56.089**	**74.067**	**89.434**	**97.783**	**100**
Standardized Train Length	0.71432	-0.07961	0.48183	-0.02476	0.50030	-0.01846
Standardized # Cycles per Pulse	-0.77513	0.41849	0.27165	-0.23602	0.22520	0.20934
Standardized Effort	0.36462	0.48366	0.66082	-0.15262	-0.41610	0.00257
Standardized Residual Pulse	-0.35610	0.07002	0.33361	0.86963	0.01347	-0.02363
Standardized Residual Frequency	-0.11264	0.93021	-0.22489	-0.03832	0.16275	-0.20852
Standardized Residual IPI	0.67004	0.49726	-0.41735	0.29094	-0.01175	0.21167

Table 4.2. Full model results. Results from full models for each of the principal components using both copulation latency and mating success as measures of preference. Identical models were run for each principal component of song traits. Copulation latency was analyzed with a general linear model and mating success analyzed with a generalized linear model with a binomial distribution and a logit link function. Bolded terms are significant after correction for multiple comparisons.

		Copulation latency				**Mating success**	
Source	DF	Sum of Squares	Mean Square	*F*	*P*	L-R χ^2	*P*
PC1	**1**	**1.349079**	**1.349079**	**24.8761**	**<.0001**	1.69616938	0.1928
$PC1^2$	1	0.062667	0.062667	1.1555	0.2826	4.48047753	0.0343
Female Genotype	**9**	**12.493476**	**1.388164**	**25.5968**	**<.0001**	**102.517035**	**<.0001**
PC1*Female Genotype	9	0.826064	0.091785	1.6925	0.086	13.8595476	0.1274
$PC1^2$*Female Genotype	**9**	**1.122635**	**0.124737**	**2.3001**	**0.0146**	14.523678	0.1049
PC2	**1**	**1.963828**	**1.963828**	**36.469**	**<.0001**	**4.89111172**	**0.027**
$PC2^2$	1	0.024472	0.024472	0.4545	0.5003	0.85318884	0.3557
Female Genotype	**9**	**12.807256**	**1.423028**	**26.4262**	**<.0001**	**81.4791332**	**<.0001**
PC2*Female Genotype	9	0.578572	0.064286	1.1938	0.2948	17.0961397	0.0472
$PC2^2$ *Female Genotype	**9**	**1.135177**	**0.126131**	**2.3423**	**0.0128**	**21.8302949**	**0.0094**
PC3	**1**	**1.4554069**	**1.455407**	**26.6759**	**<.0001**	5.00632244	0.0253
$PC3^2$	**1**	**0.2570966**	**0.257097**	**4.7123**	**0.0301**	1.00686288	0.3157
Female Genotype	**9**	**8.4773921**	**0.941932**	**17.2645**	**<.0001**	**41.4780546**	**<.0001**
PC3*Female Genotype	9	0.69041	0.076712	1.406	0.1802	15.5213333	0.0776
$PC3^2$*Female Genotype	9	0.734318	0.081591	1.4955	0.1443	9.97199396	0.3528
PC4	**1**	**0.768972**	**0.768972**	**14.1687**	**0.0002**	2.13009319	0.1444
$PC4^2$	1	0.087398	0.087398	1.6103	0.2047	1.5560487	0.2122
Female Genotype	**9**	**11.818748**	**1.313194**	**24.1962**	**<.0001**	**90.8246409**	**<.0001**
PC4*Female Genotype	9	0.95877	0.10653	1.9629	0.0402	14.9105659	0.0934
$PC4^2$*Female Genotype	**9**	**1.743344**	**0.193705**	**3.5691**	**0.0002**	**21.1212395**	**0.0121**

Table 4.3. Population-level preference functions

	Copulation Latency				Mating success			
Term	Estimate	Std Error	t Ratio	p	Estimate	Std Error	L-R χ^2	p
PC1	**0.0973766**	**0.018862**	**5.16**	**<.0001**	**0.4683523**	**0.1616588**	**8.3604549**	**0.0038**
$PC1^2$	0.0984174	0.045294	1.09	0.2775	**1.7561132**	**0.3911959**	**4.9916602**	**0.0255**
PC2	**0.109609**	**0.018301**	**5.99**	**<.0001**	**0.5932096**	**0.1622795**	**13.612994**	**0.0002**
$PC2^2$	0.084649	0.03612	1.17	0.2415	**1.4614576**	**0.3045219**	**5.6582516**	**0.0174**
PC3	**0.0577094**	**0.012476**	**4.63**	**<.0001**	**0.2912699**	**0.1105266**	**7.0769869**	**0.0078**
$PC3^2$	**0.121674**	**0.025597**	**2.38**	**0.0176**	**1.2746888**	**0.2215281**	**8.1054599**	**0.0044**
PC4	**0.0658753**	**0.015168**	**4.34**	**<.0001**	**0.3951443**	**0.1296771**	**9.2527801**	**0.0024**
$PC4^2$	0.0868362	0.031105	1.4	0.163	**1.3025352**	**0.2680474**	**5.8295296**	**0.0158**

Table 4.4. Individual-level preference functions.

Copulation Latency

Line	PC1 b	PC1 g	PC2 b	PC2 g	PC3 b	PC3 g	PC4 b	PC4 g
208	**0.146*** **	-0.026	**0.161** **	-0.290	**0.088* **	-0.039	0.069	-0.083
304	0.063	**0.754** **	0.040	**0.677** **	-0.004	0.240	0.015	**0.684*** **
315	**0.170** **	**-0.715** **	**0.195*** **	-0.179	**0.108** **	0.008	**0.142** **	**-0.465** **
360	-0.003	0.228	0.047	-0.304	0.055	-0.209	-0.015	0.117
379	**0.124* **	0.111	0.108	0.351	0.051	0.387*	0.089*	0.231
437	**0.131** **	0.079	0.075	-0.177	0.008	0.002	0.064	-0.012
486	0.038	-0.166	0.111*	0.002	0.080*	0.226	0.043	-0.231
517	0.094*	0.489*	0.039	0.182	-0.017	0.127	0.061	0.330*
707	0.0434	-0.115	**0.109** **	-0.082	**0.078** **	0.007	0.050	-0.156
732	**0.150** **	0.227	**0.161*** **	0.257	**0.089** **	0.245	**0.093* **	0.287

Mating Success

Line	PC1 b	PC1 g	PC2 b	PC2 g	PC3 b	PC3 g	PC4 b	PC4 g
208	0.697	0.946	1.185	-80.080*	0.673	-2.037	0.345	-3.824
304	-0.22	7.809**	0.004	**8.597*** **	-0.034	2.647	-0.129	**8.127*** **
315	0.824	-3.937	1.096*	-0.419	0.491	-0.092	0.861	-3.663
360	0.137	2.064	-0.424	-2.803	-0.293	-0.842	-0.181	1.599
379	0.966	0.765	0.805	3.983*	0.267	3.793*	0.756	2.410
437	0.673	4.93	0.004	-0.350	-0.365	0.040	0.387	2.365
486	-0.534	0.826	0.205	-0.077	0.413	0.883	-0.306	-0.611
517	0.34	7.395*	0.282	0.615	-0.121	-0.289	0.313	3.014
707	0.291	-0.918	0.881	1.201	**0.654* **	1.512	0.431	-0.632
732	**1.46*** **	3.326	**1.64*** **	3.409	**0.793** **	2.777*	**1.070** **	2.557

Table 4.5. Genetic correlation matrix for preference functions.

	PC1 b	PC1 g	PC2 b	PC2 g	PC3 b	PC3 g	PC4 b	PC4 g
PC1 b	1	-0.3328	0.6644	0.01029	0.2287	0.2430	**0.8765**	-0.1740
PC1 g	-0.2102	1	**-0.7702**	0.6372	-0.7792	0.2622	-0.6245	**0.9580**
PC2 b	0.7176	-0.4536	1	-0.3179	**0.8701**	0.0393	**0.7867**	-0.6386
PC2 g	-0.1357	0.1828	-0.3072	1	-0.4630	**0.8187**	-0.0582	**0.7782**
PC3 b	0.3551	-0.5768	**0.8864**	-0.3297	1	-0.1476	0.4439	-0.7019
PC3 g	0.1598	0.1237	0.1988	0.6302	0.1791	1	0.2822	0.4148
PC4 b	**0.9335**	-0.3294	**0.8146**	0.01873	0.4923	0.2908	1	-0.4525
PC4 g	-0.1981	**0.8265**	-0.4530	0.5659	-0.5329	0.5652	-0.2411	1

Table 4.6. Intersexual genetic correlation matrix.

	Copulation Latency					**Mating Success**			
Term	Estimate	Std Error	t Ratio	*P*	Term	Estimate	Std Error	t Ratio	*P*
PC1 x PC1 b	0.0060	0.4098	0.01	0.9886	PC1 x PC1 b	0.2707	0.2235	1.21	0.2604
PC2 x PC2 b	0.0330	0.4409	0.07	0.9422	PC2 x PC2 b	0.1376	0.2191	0.63	0.5474
PC3 x PC3 b	-0.2160	0.8245	-0.26	0.7999	PC3 x PC3 b	-0.4198	0.4919	-0.85	0.4183
PC4 x PC4 b	0.8048	0.6252	1.29	0.234	PC4 x PC4 b	0.7946	0.2671	2.97	0.0177
PC1 x PC1 Min	-0.0990	0.1495	-0.66	0.5267	Peak preference was not able to be calculated due to a				
PC2 x PC2 Min	-0.2001	0.1865	-1.07	0.3147	large number of ties for the proportion of pairs that				
PC3 x PC3 Min	-0.1686	0.2158	-0.78	0.4572	mated in each cross. For example, females from line 208				
PC4 x PC4 Min	-0.2525	0.1887	-1.34	0.2177	had values of 1.0 for 5 lines.				

APPENDIX C

SUPPLEMENTARY MATERIAL

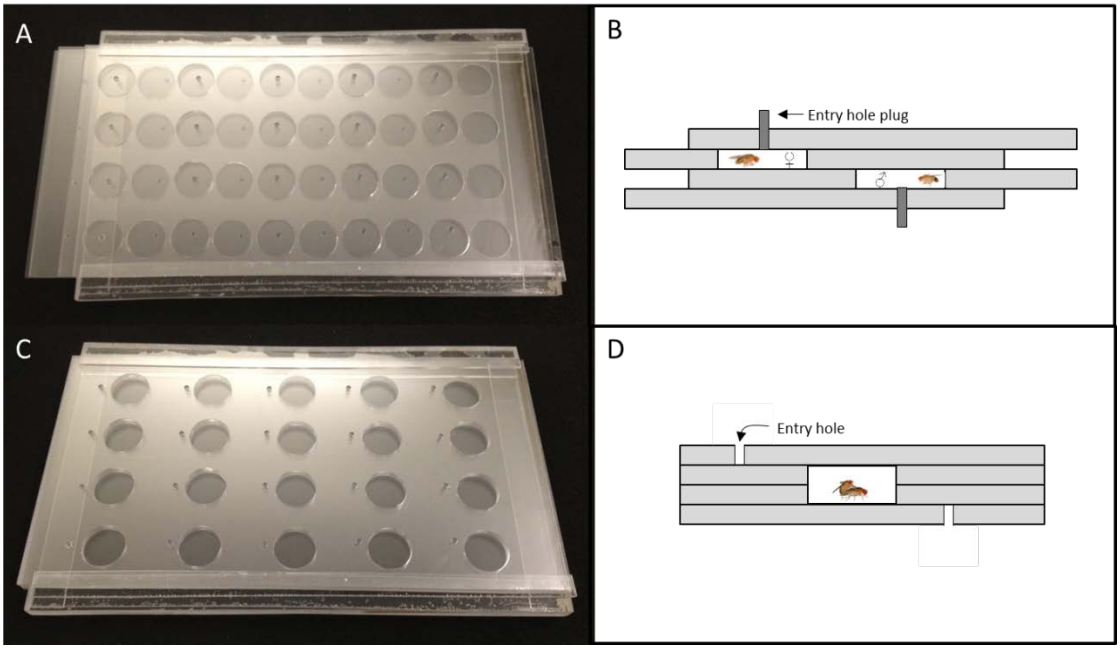

Figure 3.S1. High-throughput mating arrays. Each array consists of four layers of plastic: a top and bottom sheet keep the flies in the mating chamber, and two inner sheets to house the flies. (A) Out-of-phase position. Individuals were aspirated into each half of a chamber, one sex per side, alternated throughout the experiment. Thus, twenty males were contained in chambers separated from twenty females housed in separate chambers. (B) Cross-section of a single chamber while out-of-phase. Plugs were used to keep flies from escaping after they were aspirated into their individual chambers. After the final individual was loaded, the mating array was moved on top of a light source, underneath a video camera. Five minutes of acclimation were allowed, at which point the chambers were aligned by gently sliding the pieces of plastic so that each male and female were simultaneously introduced into one single chamber. (C) In-phase position. (D) Cross-section of a single chamber while in-phase. When the chambers were aligned, the flies from each pair were mixed and video recorded for later analysis. Pairs were given one hour to mate. If, at the end of an hour, any pairs were observed to be copulating, the video was allowed to continue recording until the male dismounted.

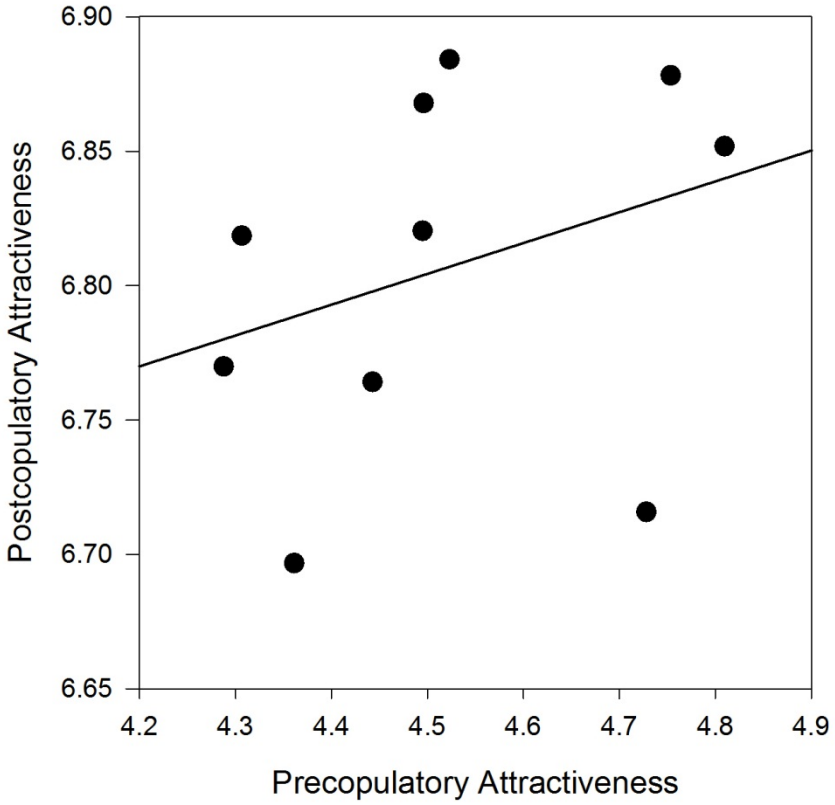

Figure 3.S2. Comparison of pre- and postcopulatory male mate choice. A genetic correlation between attractiveness in pre- and postcopulatory stages of mate choice would be indicated by a positive regression of female attractiveness in each stage. No relationship was found: females that were courted sooner were not mated longer (ANOVA: $F_{1,8} = 0.9035$, $p = 0.370$). Units on both axes are measured on a log scale (ln(seconds)).

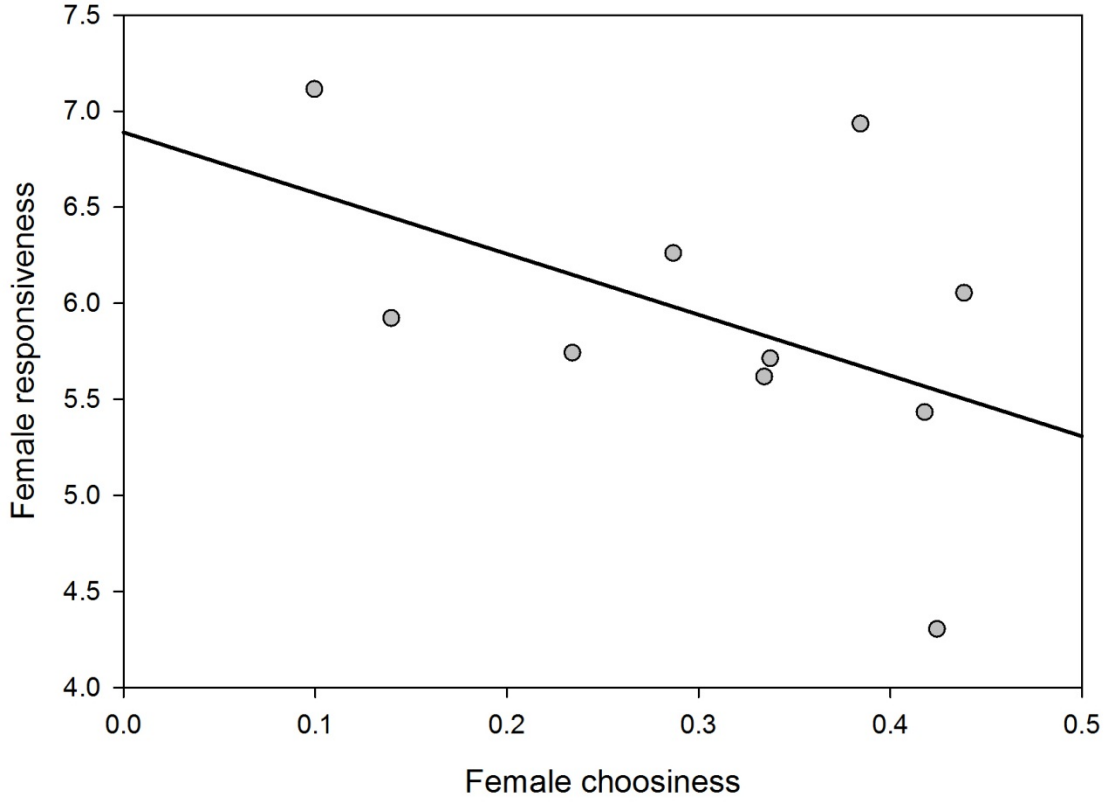

Figure 3.S3. Test for a correlation between female responsiveness and choosiness. Least squares means of copulation latency were obtained for females in each line, yielding the mean copulation latency for all 100 pairwise combinations of isogenic lines. To calculate female responsiveness to all males, we averaged the least squares means for each male genotype. Female choosiness was measured in the same way, but instead of the mean we computed the standard deviation. There is no significant relationship between the two components of female choice (ANOVA: $F_{1,8} = 2.3657$, $p = 0.163$).

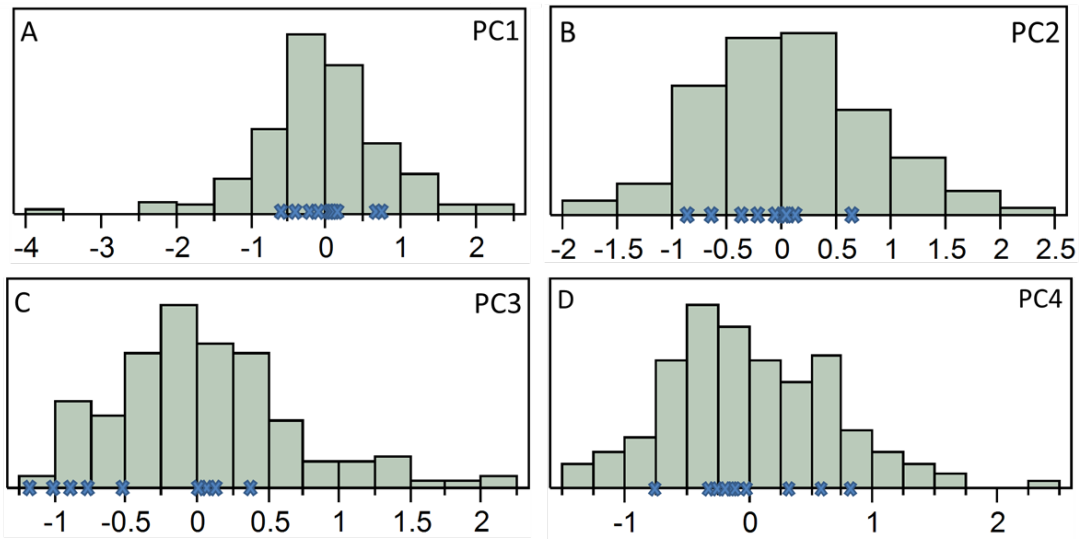

Figure 4.S4. Frequency distributions of mean principal component scores for all 192 lines are shown for PC1-4 (A-D, respectively). The x-axis is PC-score and the y-axis is frequency of genotypes with each PC-score. For each principal component, we plotted the breeding value for each of the ten lines used in our experiment to see where the breeding values used in this experiment reside with respect to the entire sample of 192 songs. As we randomly sampled the ten lines with no prior knowledge of their breeding values for the various song traits, we were not able to cover the entire phenotypic distribution for the principal components.

Table 4.S1. We tested for intrasexual genetic correlations between preference functions (linear: b; quadratic: g) and other attributes of female reproductive behavior. Choosiness is the standard deviation of each line's response to all males. Genotypes with a large standard deviation are choosier than ones with smaller standard deviations because they exhibit strong preferences for some genotypes and strong preferences against others. Responsiveness is the mean copulation latency for females from each genotype across males from all 10 lines. Note that "Mating Success" only refers to the calculation of the regression coefficients, and not responsiveness or choosiness. We additionally tested for intrasexual genetic correlations between male PC scores and their global attractiveness, which is calculated as mean copulation latency for males across all female lines. Males with larger copulation latency values, averaged across females from all 10 lines, are globally unattractive. The genetic correlation between PC2 g and responsiveness is largely due to an outlier (208: -80.080), and is considered to be an artifact.

	Copulation Latency					**Mating Success**			
Term	Estimate	Std Error	t Ratio	p	Term	Estimate	Std Error	t Ratio	p
PC1 b x choosy	0.6582	0.6960	0.95	0.372	PC1 b x choosy	0.4338	1.7228	0.25	0.8075
PC2 b x choosy	0.8323	0.7142	1.17	0.2774	PC2 b x choosy	0.5508	1.9065	0.29	0.78
PC3 b x choosy	0.8723	0.9216	0.95	0.3716	PC3 b x choosy	0.3611	1.2424	0.29	0.7787
PC4 b x choosy	0.3344	0.9788	0.34	0.7414	PC4 b x choosy	-0.1028	1.3532	-0.08	0.9413
PC1 g x choosy	0.5948	0.7551	0.79	0.4536	PC1 g x choosy	-2.8026	10.7250	-0.26	0.8005
PC2 g x choosy	0.6053	0.4963	1.22	0.2573	PC2 g x choosy	-63.3240	73.4060	-0.86	0.4134
PC3 g x choosy	0.1248	0.3883	0.32	0.7561	PC3 g x choosy	-2.1631	5.3386	-0.41	0.696
PC4 g x choosy	0.4645	0.5606	0.83	0.4314	PC4 g x choosy	-2.4864	10.3507	-0.24	0.8162
PC1 b x responsive	-0.0030	0.0262	-0.12	0.9103	PC1 b x responsive	0.2063	0.2560	0.81	0.4436
PC2 b x responsive	0.0033	0.0249	0.13	0.8991	PC2 b x responsive	0.1667	0.2890	0.58	0.5798
PC3 b x responsive	0.0045	0.0197	0.23	0.8266	PC3 b x responsive	0.0733	0.1905	0.38	0.7105
PC4 b x responsive	0.0108	0.0191	0.56	0.588	PC4 b x responsive	0.2509	0.1885	1.33	0.2198
PC1 g x responsive	0.0056	0.1206	0.05	0.9639	PC1 g x responsive	-0.4721	1.6491	-0.29	0.7819
PC2 g x responsive	-0.0812	0.0781	-1.04	0.3287	PC2 g x responsive	23.6703	8.3334	2.84	0.0218
PC3 g x responsive	-0.0137	0.0599	-0.23	0.8248	PC3 g x responsive	1.1020	0.7328	1.5	0.1711
PC4 g x responsive	0.0005	0.0899	0.01	0.9958	PC4 g x responsive	0.7146	1.5785	0.45	0.6628
PC1 x attractive	0.0983	0.0386	2.55	0.0344	PC1 x attractive	-0.0966	0.0659	-1.47	0.1809
PC2 x attractive	0.1036	0.0352	2.94	0.0186	PC2 x attractive	-0.1234	0.0580	-2.13	0.066
PC3 x attractive	0.0502	0.0290	1.73	0.1222	PC3 x attractive	-0.0558	0.0445	-1.25	0.2452
PC4 x attractive	0.0607	0.0357	1.7	0.1268	PC4 x attractive	-0.0815	0.0521	-1.56	0.1562

APPENDIX D

Table 3.S1. Measures of selection and drift were taken each generation for 5,000 generations of simulated evolution. For a more detailed description of each measure please refer to the main text.

Symbol	Measure	Calculation
S_{NS_z}	Natural selection differential on ornament	$\bar{z}^* - \bar{z}$; $\bar{z}^*$ denotes mean after NS
S_{NS_y}	Natural selection differential on preference	$\bar{y}^* - \bar{y}$; $\bar{y}^*$ denotes mean after NS
D_z	Genetic drift of the mean ornament	$\bar{z}^{**} - \bar{z}^*$; $\bar{z}^{**}$ denotes mean after random selection of adults
D_y	Genetic drift of the mean preference	$\bar{y}^{**} - \bar{y}^*$; $\bar{y}^{**}$ denotes mean after random selection of adults
S_{SS_z}	Sexual selection differential on ornament	$[(\sum_i^{N_m} z_i * MS_i) \,/\, N_m] - \bar{z}^{**}$; N_m denotes number of males in the population
S_{SS_y}	Sexual selection differential on preference	$[(\sum_i^{N_f} y_i * MS_i)/N_f] - \bar{y}^{**}$; N_f denotes number of females in the population
S_z	Total selection on ornament	$\bar{z}^{***} - z$; $\bar{z}^{***}$ denotes mean after sexual selection
S_y	Total selection on preference	$\bar{y}^{***} - y$; y^{***} denotes mean after sexual selection
$\Delta\bar{z}$	Generational change in mean ornament	$[(\sum_i^{N_m} z_i * MS_i)/N_m] - \bar{z}$
$\Delta\bar{y}$	Generational change in mean preference	$[(\sum_i^{N_f} y_i * MS_i)/N_f] - \bar{y}$
iSS_{z_m}	Indirect SS on ornament from son's mating success	COV(z, sons' mating success)
iSS_{y_m}	Indirect SS on preference from son's mating success	COV(y, sons' mating success)