Genetic Analysis of Female Preference Functions as Function-Valued Traits

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ABSTRACT: The genetic analysis of female preferences has been seen as a particularly challenging empirical endeavor because of difficulties in generating suitable preference metrics in experiments large enough to adequately characterize variation. In this article, we take an alternative approach, treating female preference as a function-valued trait and exploiting random-coefficient models to characterize the genetic basis of female preference without measuring preference functions in each individual. Applying this approach to *Drosophila bunnanda*, in which females assess males through a multivariate contact pheromone system, we gain three valuable insights into the genetic basis of female preference functions. First, most genetic variation was attributable to one eigenfunction, suggesting shared genetic control of preferences for nine male pheromones. Second, genetic variance in female preference functions was not associated with genetic variance in the pheromones, implying that genetic variation in female preference did not maintain genetic variation in male traits. Finally, breeding values for female preference functions were skewed away from the direction of selection on the male traits, suggesting directional selection on female preferences. The genetic analysis of female preference functions as function-valued traits offers a robust statistical framework for investigations of female preference, in addition to alleviating some experimental difficulties associated with estimating variation in preference functions.

Keywords: mate choice, random regression, *Drosophila bunnanda*, random-coefficient models, genetic covariance function, sexual selection.

Female preferences for male display traits are responsible for some of the most spectacular phenotypic diversity found in the natural world. Although the phenotypic consequences of female preference are well described (Jennions and Petrie 1997), much less is known about the genetic basis of female preferences and, subsequently, how female preferences evolve. A number of studies have demonstrated that female preference is heritable (Bakker and Pomiankowski 1995; Jennions and Petrie 1997; Chenoweth and Blows 2006), but the inherent difficulties in quantifying mating preferences (Wagner 1998; Chenoweth and Blows 2006) have limited the application and scope of quantitative genetic experiments on such traits.

Female preferences can be measured at two levels (Wagner 1998). First, by determining which males in a population do and which do not gain mates, a population-level preference can be determined. This type of measure of female preferences is particularly useful in estimating the strength of sexual selection operating on male display traits (Kingsolver et al. 2001*a*; Chenoweth and Blows 2006). However, such measures are not informative about variation among females in their preferences for male traits (Wagner 1998) and are therefore not amenable to genetic analyses.

Second, measuring individual female preference functions, which describe a quantitative relationship between levels of a male trait and a female's preference for it, allows the direct assessment of variation among females in their mate preferences (Ritchie 1996). Measuring female preference functions is empirically very challenging, and the estimation of among-female variation in preference functions has been considered impossible in the many species in which individual females cannot be experimentally presented with a number of alternative male trait levels in a suitably controlled way (Wagner 1998). For example, this situation arises in species in which female preference can be inferred only by allowing pairs to copulate, thereby changing the motivation of females in subsequent choice tests. As a consequence of these experimental difficulties, genetic analyses of female preference functions are rare (Chenoweth and Blows 2006) and have tended to rely on approximate analyses to demonstrate genetic variance in preference functions, such as family mean approaches (Ritchie et al. 2005), segregation among wide crosses (Ritchie

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2000), or the genetic analysis of regression slopes (Brooks and Endler 2001*b*).

Female preference functions are an example of a class of traits known as function-valued characters, which are best described as a continuous function rather than as scalar values (Kirkpatrick and Heckman 1989; de Jong 1990; Gomulkiewicz and Kirkpatrick 1992; Kingsolver et al. 2001*b*; Meyer and Kirkpatrick 2005). Although phenotypic characterization of female preferences has utilized a number of ways to represent preferences as functions (Ritchie 1996; Ritchie et al. 2005), genetic analysis of female preference functions has yet to take advantage of the methods applicable to function-valued traits, particularly those based on random-coefficient (sometimes called random-regression) mixed models (Meyer and Kirkpatrick 2005). Random-coefficient models allow the direct fitting of continuous functions at the appropriate (genetic) level of the experimental design within a single analysis. Consequently, the full power of hypothesis testing within the restricted maximum likelihood framework of the mixed model can be applied to determining the genetic basis of the female preference functions. A convenient attribute of such models is the ability to consider multiple male traits simultaneously when testing the distribution of genetic variance in female preferences for those traits. This may be particularly useful, given that males typically employ multiple sexual signals (Candolin 2003; Chenoweth and Blows 2006)

In this article, we detail how the random-coefficient approach to the analysis of functional-valued traits can be applied to the genetic analysis of female preference functions in an experimental design that does not require the estimation of the preference functions of individual females. We present a genetic experiment based on simple two-stimulus choice tests (Wagner 1998; a female makes a choice between two males), which are typically used only to estimate population-level measures of the strength of preference and sexual selection (Chenoweth and Blows 2006). Our approach provides two advances in the genetic analysis of female preference: the application of random-regression techniques, required for the genetic analysis of such function-valued traits (Meyer and Kirkpatrick 2005), and a way of estimating the genetic basis of female preference functions in species in which it is not possible to sequentially present females with a number of males (Wagner 1998; Chenoweth and Blows 2006).

The basic premise of our approach centers on modeling the variation in a female preference function that is not estimated for each individual female but is estimated for groups of related females. Only a single observation of any one female's mating preference is made (a choice between two males), but many such observations are made on groups of related females. Random-coefficient models can then be applied to estimate the variation in the preference function among the related groups of females, resulting in an estimate of the genetic variance for that preference function. The implementation of a genetic analysis of a function-valued trait is in many ways analogous to standard quantitative genetic practice. Once the appropriate form of the function has been decided on (e.g., linear, polynomial, or a more complex curve), the additive genetic covariance function is estimated at the appropriate level in the experimental design (e.g., the sire level in a paternal half-sib experiment). For more than one trait, a multivariate genetic covariance function that describes the genetic covariance among the functions for the different traits can be estimated.

Utilizing a half-sib breeding design, we estimate the genetic variance in female preference functions for nine male traits of *Drosophila bunnanda*, a species native to the northeastern rainforests of Australia (Schiffer and McEvey 2006). Female *D. bunnanda* exert sexual selection on nine male cuticular hydrocarbons (CHCs), which act as a contact pheromone system (Van Homrigh et al. 2007). We first estimate the genetic (co)variance of the female preference functions for these nine CHCs, assuming a linear preference function for each of the nine male traits. Simultaneous estimation of the genetic variance in the nine preference functions allowed factor analytic modeling of the genetic covariance among the preference functions to determine the presence of multivariate additive genetic variance in female preferences in this population. Second, we compare the distribution of genetic variance in female preference functions with the distribution of genetic variance for the male signal traits to infer that variation in female preference does not play a major role in the maintenance of genetic variation in the male signaling traits. Finally, we estimate the level of skew in the breeding values of the female preference functions to investigate a possible association between the strength of selection on male phenotypes and the strength of selection on female preference itself.

Methods

This experiment was first reported by Van Homrigh et al. (2007), where we were interested in the genetic basis of the male CHCs and the population-level female preference for them. Here, we take advantage of the fact that the females in the mate choice tests also came from the half-sib breeding design (fig. 1; as they were not subject to any analyses, females were described as coming from the stock population in Van Homrigh et al. 2007). Briefly, a standard half-sib breeding design was employed, with 125 sires each mated to four dams. The mating preferences of two virgin females from each half-sib family were determined in a

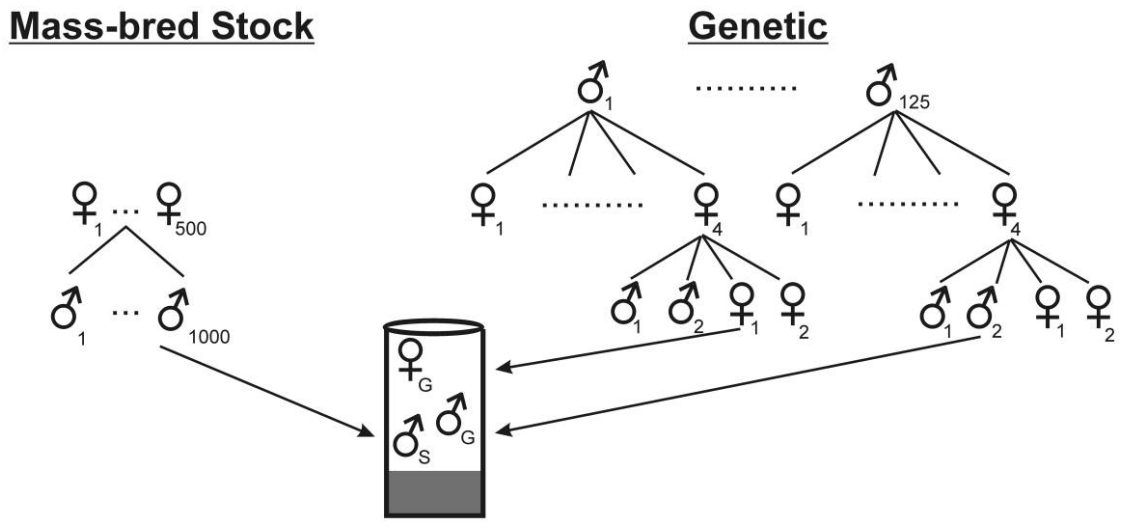

Figure 1: Schematic of the experimental design. Half-sib families were generated by mating each of 125 sires with four dams. Inseminated females from the same mass-bred population were also placed individually to lay. Female preference was determined in two-stimulus choice trials where one male came from the mass-bred stock (unknown parents; *S*) and the other male came from the genetic breeding design (*G*) but was unrelated to the choosing female, who also came from the genetic breeding design.

binomial mate choice trial in which one male came from the breeding design (but was unrelated to the female) and one male came from the same mass-bred population and was raised under the same conditions as the genetic animals but was of unknown parentage. All flies were the same age during the trials (6–8 days). To allow identification, males from the breeding design had a small piece clipped from their left wing, and males from the stock were clipped on the right. After intromission was achieved by one of the males, the male from the half-sib family was recorded as being successful or unsuccessful in gaining a mating, immediately removed, and prepared for gas chromatography (see Van Homrigh et al. 2007 for methods). The right wing was then collected from each female and male from the genetic design, and wing size was estimated as centroid size following Van Homrigh et al. (2007). The male from the stock population was not considered further. As a consequence of incomplete records for some females, the data set analyzed here consists of 638 females from 111 sires. All analyses were conducted using the Mixed procedure in SAS (ver. 9.1). Given the large sample and the equal numbers of chosen and rejected males in this sample (321 vs. 317), we assume a normal distribution for hypothesis testing throughout (Van Homrigh et al. 2007).

Our application of the random-coefficient approach to modeling the variation in female preference differs in two important respects from how random regression has been previously applied in evolutionary studies. First, evolutionary studies have usually been concerned with modeling a number of phenotypic (response) traits that vary with a single independent variable, such as age (Wilson et al. 2005), or an environmental variable, such as temperature (Kingsolver et al. 2004). In contrast, our female preference functions are characterized by a single response variable (the choice that a female made resulting in male mating success) and multiple independent variables (the nine male traits under sexual selection). The modeling of multiple independent variables in random-coefficient models could potentially present difficulties (Meyer and Kirkpatrick 2005), particularly if the independent variables have different scales or qualitatively different relationships with the response variable. However, these issues do not affect our analyses because all our traits are measured on the same scale and have similar (linear; see below) relationships with female preference (the response variable).

Second, random-coefficient models offer the opportunity to fit more complex functions to the data than simple linear relationships can, a feature that is often exploited in genetic studies (e.g., Kingsolver et al. 2004; Wilson et al. 2005). In such analyses, a limit on the complexity of the genetic covariance function that can be applied is determined by the number of character states of the independent variable (e.g., number of age classes or number of temperatures). The genetic (co)variance for the parameters that describe the function (first-, second-, or higher-order polynomials) is then determined and is called the "genetic covariance function." In our case, it is the genetic

covariance among the coefficients for the same function type (e.g., first-order polynomial) for different independent (male) traits that is of interest. We retain the usage of the term "genetic covariance function" to describe the genetic covariance among linear preferences for multiple male traits.

Detailed analyses of population-level preference in the closely related species *Drosophila serrata* have indicated that female preferences for male CHCs are linear and open ended (Chenoweth and Blows 2005). We found similar patterns of population-level preference in the current data set for *Drosophila bunnanda*, using cubic splines (data not presented). Although these population-level preferences are linear, it is still possible that variation among individuals existed in preferences that were nonlinear. Preliminary genetic analyses returned zero genetic variance components for second-order (quadratic) coefficients for all individual traits. We therefore chose to model only linear preference functions in this study.

We applied a multivariate random-coefficient model to the male phenotype (CHC) data, using

$$\mathbf{y}_{jk} = \alpha + \mathbf{X}_{jk}\mathbf{b} + \mathbf{Z}^{(\mathrm{d})}_{jk}\boldsymbol{\delta}^{(\mathrm{d})}_{jk} + \mathbf{Z}^{(\mathrm{s})}_{jk}\boldsymbol{\delta}^{(\mathrm{s})}_{k} + \boldsymbol{\varepsilon}_{jk}, \qquad (1)$$

where for each female from the *j*th dam within the *k*th sire the response variable is the binomial male mating success score (y_{ijk}), represented as the vector of observations $\mathbf{y}_{jk}$, so that $\mathbf{y}_{jk} = \{y_{ijk}\}_i \otimes \mathbf{1}_{n,jk}$, where n is the number of offspring of the *j*th dam (Longford 1993).

Model (1) contains two fixed effects: the intercept (α) and the population-wide regression slopes ($\mathbf{b}$) for the set of continuous variables (the nine male CHC trait values and female wing size) that are represented in the design matrix ($\mathbf{X}$). Female wing size (a surrogate measure of body size; Partridge et al. 1987) was included as a covariate in the models because female size and condition can affect mating behavior (Hunt et al. 2005). Specifically, female size influences male mating behavior in a closely related species, *D. serrata* (Chenoweth et al. 2007). The vector of slopes $\mathbf{b}$ is equivalent to the vector of directional selection gradients ($\boldsymbol{\beta}$) commonly used to measure the strength of selection (Lande and Arnold 1983). The estimates of the fixed effects for each CHC were very close, but not identical, to the elements of the vector of sexual selection gradients ($\boldsymbol{\beta}$) reported by Van Homrigh et al. (2007; table 1). The small deviations between the elements of $\boldsymbol{\beta}$ and the fixed effect slopes $\mathbf{b}$ reported here are attributable to the reduced sample used here because only records for which female genetics and male CHC data were available (976 vs. 638) were considered and because of the inclusion of female size as a covariate.

The genetic basis of female preference was estimated through the random-effects part of model (1), represented by the linear combinations $\mathbf{Z}^{(\mathrm{d})}_{jk}\boldsymbol{\delta}^{(\mathrm{d})}_{jk}$ and $\mathbf{Z}^{(\mathrm{s})}_{jk}\boldsymbol{\delta}^{(\mathrm{s})}_{k}$, which give the departure of the regression slope for the *j*th dam within each *k*th sire and of each *k*th sire from the population regression $\mathbf{X}_{jk}\mathbf{b}$, respectively. Here, $\mathbf{Z}^{(\mathrm{d})}_{jk}$ and $\mathbf{Z}^{(\mathrm{s})}_{jk}$ are the design matrices at the dam and sire levels, respectively, and the variances $\boldsymbol{\delta}^{(\mathrm{d})}_{jk}$ and $\boldsymbol{\delta}^{(\mathrm{s})}_{k}$ are assumed to have distributions $\sim N(\mathbf{0}, \boldsymbol{\Sigma}_{\mathrm{d}})$ and $\sim N(\mathbf{0}, \boldsymbol{\Sigma}_{\mathrm{s}})$, respectively. At the residual (error) level, it was not possible to estimate the residual (co)variance among female preference functions because we took only a single measure for each female. We therefore reduced the vector of parameters to be estimated at this level (Meyer 1991), an approach that is required whenever traits are measured on different individuals, such as when estimating intersex genetic correlations (Chenoweth et al. 2008). We estimated a separate error for groups of sires that had different numbers of daughters included in the experiment to account for the heterogeneity expected as a consequence of family sample size.

Initial analyses that attempted to fit a nine-dimensional unconstrained covariance structure for $\boldsymbol{\Sigma}_{\mathrm{d}}$ and $\boldsymbol{\Sigma}_{\mathrm{s}}$ failed to converge. This problem with fitting a full model to the data is expected when the higher nested levels (sires and dams) have small numbers of observations for each subject (i.e., maxima of four dams within each sire and two individuals within each dam; Longford 1993, p. 168). We determined that a factor analytic covariance structure (Kirkpatrick and Meyer 2004; Meyer and Kirkpatrick 2005; Hine and Blows 2006) with ranks of 3 at the sire level and 1 at the dam level was the model with the most complex covariance structure that would converge. The reduced-

Table 1: Parameters estimated on the female preference functions for nine male cuticular hydrocarbons (CHCs)

Trait	$\boldsymbol{\beta}$	$\mathbf{b}$	$\boldsymbol{g}_{\max}$	Skew
2-Me-C_{24}	.135	.227	−.253	.472*
$C_{25:1}$ (A)	−.876	−.995	−.226	.436
$C_{25:1}$ (B)	−.163	−.308	−.101	.278
$C_{25}H_{48}$ (B)	.265	.337	.184	−.174
7,11-$C_{27:2}$	.827	.925	.136	−.294
$C_{27:1}$	.156	.310	.222	−.172
$C_{27}H_{50}$ (A)	−.478	−.514	−.001	.058
2-Me-C_{28}	.294	.228	.658	−.161
2-Me-C_{30}	−.115	−.119	−.583	.293

Note: The vector of directional selection gradients, $\boldsymbol{\beta}$, was estimated from the population regression (Van Homrigh et al. 2007). The random regression model (1) was run to estimate the fixed effect (b), which describes the population regression slopes for the nine CHCs, and the first eigenfunction of the additive genetic covariance function ($\boldsymbol{g}_{\max}$). Model (1) was also used to estimate the sire breeding values for the female preference functions (as best linear unbiased predictors), and their distribution was examined to determine the skew in the breeding values for each CHC (standard error of skew for each trait was 0.229).

* $P < .05$.

rank (three-dimensional) estimate of $\mathbf{\Sigma}_s$ is then the additive genetic variance-covariance function among the slopes at the sire level, which we refer to hereafter as $\mathcal{G}$.

Results

Genetic Variance in Female Preference Functions

The genetic variance-covariance ($\mathcal{G}$) function of female preference for the nine male CHCs is displayed in table 2. Genetic variance in female preference functions appears to exist for each of the nine male traits, but the genetic basis of preference is complex, with both positive and negative genetic covariation among CHCs. We used factor analytic modeling of the covariance at the sire level to identify statistically robust trait covariation within the $\mathcal{G}$ function. Only one dimension of the sire covariance matrix was statistically supported (log-likelihood ratio test for the first factor of $\mathcal{G}$: $\chi^2 = 17.6$, df $= 9$, $P = .0401$). This first eigenfunction ($\boldsymbol{g}_{max}$) accounted for 64.4% of the estimated genetic variance in female preference functions, indicating that much of the genetic variance resided in a single eigenfunction. The trait loadings for $\boldsymbol{g}_{max}$ (table 1) showed that opposing contributions from two methylalkanes (2-Me-C_{28} and 2-Me-C_{30}) made the strongest contributions to this vector.

The genetic variance in the preferred combination of male traits represented by $\boldsymbol{\beta}$ was estimated in a univariate fashion by first constructing $\boldsymbol{\beta}$ as a univariate male trait (by applying the vector $\boldsymbol{\beta}$ [table 1; Van Homrigh et al. 2007] to each male's CHC measures). This variable was then placed into the same random-regression model used for the nine CHCs. The genetic variance in the female preference function for $\boldsymbol{\beta}$ was 0.625, and it accounted for 9.3% of the genetic variance in female preference functions, as estimated by dividing the genetic variance in $\boldsymbol{\beta}$ by the trace of the female preference $\mathcal{G}$ function ($\Sigma\lambda_i = 6.74$). In males, this univariate attractiveness trait was associated with a very small amount of genetic variation (0.021), accounting for less than 1% of the genetic variance in the suite of nine CHC traits (Van Homrigh et al. 2007).

We looked for an association between the genetic variance in female preference and the genetic variance in the male CHC traits by comparing the orientation of the three-dimensional subspace of female preference (table 2) with the three-dimensional subspace of the male CHC $\mathcal{G}$ matrix (reported in Van Homrigh et al. 2007). These three eigenvectors accounted for 89% of the genetic variance in the nine male CHCs and are statistically supported (Van Homrigh et al. 2007). We used the subspace comparison methodology detailed by Blows et al. (2004). Briefly, the two three-dimensional subspaces were represented by two matrices **A** and **B**, and a bounded measure of the similarity of the two subspaces was given by the sum of the eigenvalues of **S** ($\mathbf{S} = \mathbf{A}^T\mathbf{B}\mathbf{B}^T\mathbf{A}$). The two subspaces were virtually unrelated, with a value of the sum of the eigenvalues of **S** of 0.102, where 0 indicates orthogonal subspaces and 3 indicates completely shared subspace. This result suggests the level of genetic variance in female preference is unrelated to the level of genetic variance in the male traits.

Genetic Skew in Female Preference Functions

Best linear unbiased predictors of the breeding values for the female preference functions for the nine male traits were estimated from the random-regression model and are presented as histograms in figure 2. Female preference breeding values were opposite in sign to the linear selection gradients in $\boldsymbol{\beta}$ (with the exception of that for 2-Me-C_{24}; table 1), suggesting fewer than expected breeding values for female preference functions in the direction of sexual selection on the male traits. An analysis of the female preference function for the univariate combination of traits preferred by females ($\boldsymbol{\beta}$) reinforced this interpretation. Breeding values of the preference function for $\boldsymbol{\beta}$ were substantially skewed (fig. 3; skew $= -1.353$, $t = 5.908$,

Table 2: Genetic variance-covariance ($\mathcal{G}$) function of linear female preferences for the nine male cuticular hydrocarbons, estimated from the sire covariance matrix in a three-dimensional factor analytic model

	2-Me-C_{24}	$C_{25:1}$ (A)	$C_{25:1}$ (B)	$C_{25}H_{48}$ (B)	7,11-$C_{27:2}$	$C_{27:1}$	$C_{27}H_{50}$ (A)	2-Me-C_{28}	2-Me-C_{30}
2-Me-C_{24}	.464	**1.082**	**−.229**	**−.348**	**−.751**	**−.479**	**−.698**	**−.644**	**.938**
$C_{25:1}$ (A)	.511	.593	**−.335**	**−.074**	**−.447**	**−.293**	**−.637**	**−.304**	**.562**
$C_{25:1}$ (B)	−.065	−.149	.216	**−.689**	**−.084**	**−.312**	**.697**	**−.531**	**.266**
$C_{25}H_{48}$ (B)	−.097	−.032	−.182	.207	**.426**	**.487**	**−.467**	**.831**	**−.667**
7,11-$C_{27:2}$	−.179	−.166	−.019	.086	.152	**−.061**	**.062**	**.374**	**−.430**
$C_{27:1}$	−.229	−.219	−.142	.198	−.035	.611	**.022**	**.447**	**−.429**
$C_{27}H_{50}$ (A)	−.418	−.595	.397	−.237	.044	.020	.957	**−.257**	**−.096**
2-Me-C_{28}	−.562	−.413	−.441	.615	.386	.590	−.378	2.029	**−.797**
2-Me-C_{30}	.707	.660	.190	−.426	−.383	−.489	−.133	−1.620	1.512

Note: Genetic variances are along the diagonal, covariances are below the diagonal, and genetic correlations are above the diagonal and in boldface.

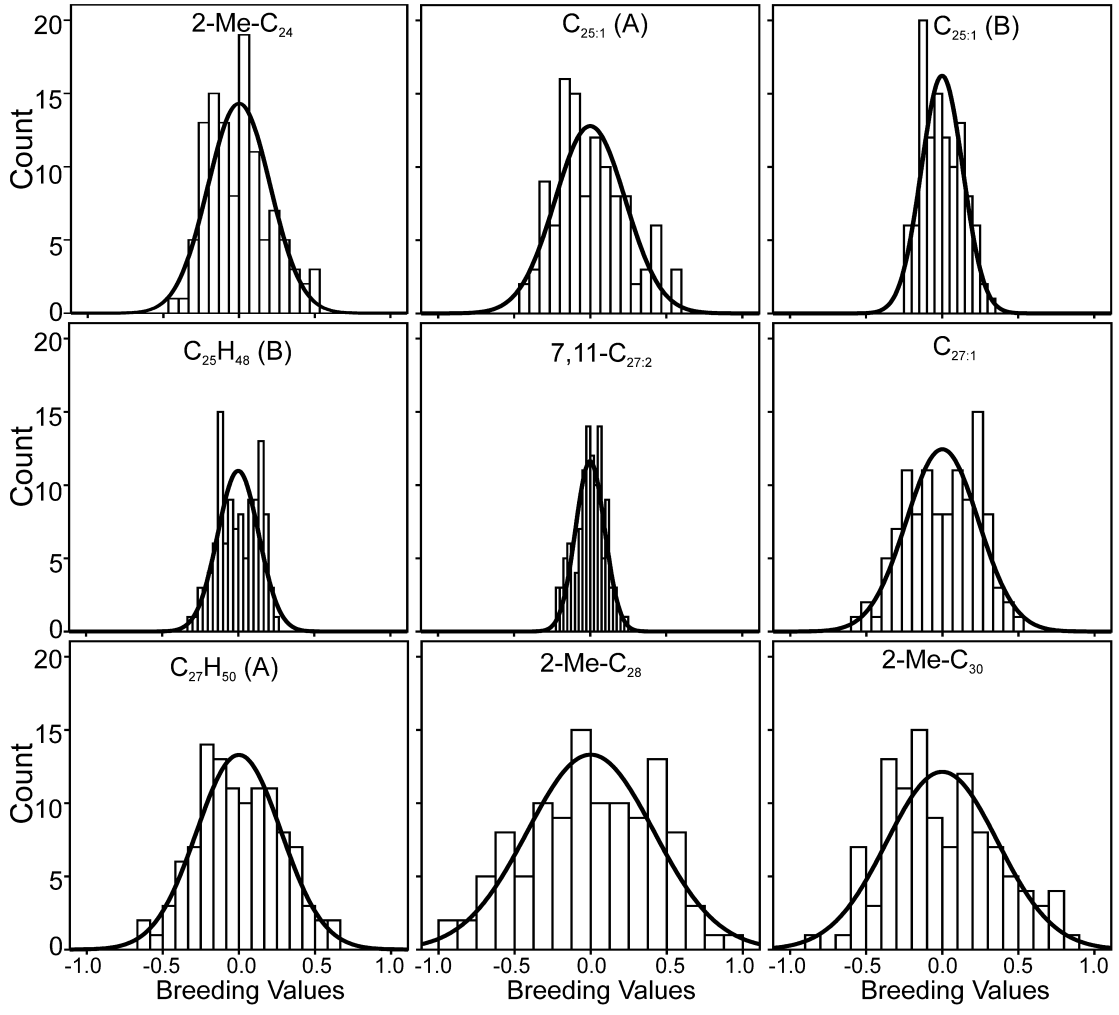

Figure 2: Best linear unbiased predictor estimates of the breeding values for female preference functions for nine male cuticular hydrocarbons.

df = 111, $P < .001$) and were at least three times more skewed than preference for any individual male CHC. The presence of skew in the breeding values of $\boldsymbol{\beta}$ was robust to the normality assumptions used to estimate the breeding values, as it was also present ($t = 2.865$, df = 111, $P = .005$) when the breeding values were estimated using a binomial error structure and logit link function applied in the Glimmix procedure in SAS. The genetic skew in the preference function for $\boldsymbol{\beta}$ indicated reduced genetic variance for an increase in the slope of the female preference function in the direction that would further strengthen female preference for this trait combination.

Discussion

The genetic analysis of female preference functions has been perceived as a particularly difficult empirical endeavor (Chenoweth and Blows 2006). Here we have shown that the problem may be addressed using the function-valued approach that has been advocated for use with any trait that is best described as a continuous function rather than a scalar measure (Kirkpatrick and Heckman 1989; de Jong 1990; Gomulkiewicz and Kirkpatrick 1992; Kingsolver et al. 2001*b*; Meyer and Kirkpatrick 2005). The application of this approach to the estimation of genetic variance in female preference functions does not require the estimation of preference functions for individuals, opening the way for empirical investigations of the genetics of preference in the many species for which individual preference functions are unmeasurable.

The function-valued approach provided three valuable insights into the genetic basis of female preference functions in *Drosophila bunnanda*. First, females of many spe-

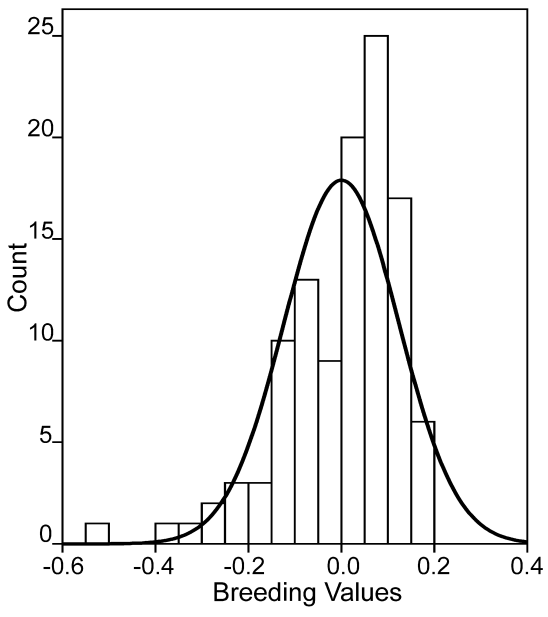

Figure 3: Best linear unbiased predictor estimates of the breeding values for the female preference function for the population regression represented by the vector of linear selection gradients ($\boldsymbol{\beta}$).

cies often have preferences for multiple male traits (Brooks and Endler 2001*a*; Coleman et al. 2004). Here, a single trait ($\boldsymbol{g}_{\max}$) explained 64% of the genetic variance in female preference functions for nine male traits. This suggests that female preference for multiple male traits may not necessarily be based on genetically independent preferences for each male trait. This result contrasts with the finding in guppies, in which preference for orange and black coloration responded to selection as independent traits (Brooks and Couldridge 1999). Further studies on visual, acoustic, and other chemical signaling systems will be required to determine whether the genetic basis of preference for multiple male traits is typically highly correlated (as in this study) or independent (Brooks and Couldridge 1999) and whether the extent of preference function covariation is related to the sensory system involved.

Second, variation in female preference functions within populations may be one mechanism that maintains genetic variance in the male traits under sexual selection (Kokko 1997; Brooks and Endler 2001*b*). Our results did not provide support for this hypothesis because the inherited variation among females in their preferences functions was not associated with genetic variation among males for CHC trait expression. Consequently, genetic variance in female preference functions does not appear to be a mechanism for maintaining genetic variance in male traits in *D. bunnanda*.

Finally, the breeding values for female preference functions tended to be skewed away from the direction in which preference operated on the male trait. That is, there are fewer breeding values, and consequently less genetic variance, for increasing the slope of the preference function in the direction that preference is already selecting on the male trait. Many applications of quantitative genetics depend on the assumption that breeding values of continuous traits are normally distributed (Bulmer 1971; Lande 1980). However, with a finite number of loci and alleles, the distribution of allelic effects may not be Gaussian (Turelli 1984; Barton and Turelli 1987). When a finite number of alleles underlie the response to selection, the additive genetic variance itself is predicted to respond to directional selection, particularly if some alleles have greater phenotypic effects than others (Barton and Turelli 1987). For example, if favorable alleles are rare before selection, allelic skew is generated at those loci responding to selection because alleles increase in frequency toward fixation (Barton and Turelli 1987; Reeve 2000). Therefore, in the presence of directional selection, the distribution of breeding values is predicted to become skewed.

The observed skew in female preference function breeding values, particularly for the combination of traits ($\boldsymbol{\beta}$) preferred by females in this population, suggests that female preference functions themselves are under strong directional selection. There are two potential major sources of selection on female preference (Kirkpatrick 1996; Kokko et al. 2006). Direct selection on female preferences can arise when females receive a material benefit from choosing a particular male (e.g., nuptial gifts, access to resources). Indirect (genetic) benefits may be gained by females through the performance of their offspring as a consequence of choosing males with "good genes." There are no known direct benefits to mate choice in *D. bunnanda*. However, male CHCs of *D. bunnanda* are genetically correlated with condition (Van Homrigh et al. 2007), consistent with the hypothesis of indirect selection on female preference. There is also evidence consistent with good-genes mate choice in a closely related species, *Drosophila serrata* (Hine et al. 2002). Consequently, indirect selection may be responsible for the genetic skew in these female preference functions. It follows that female preference may be maintained in this population as a consequence of indirect selection.

While adopting the random-coefficient modeling approach advocated here may open the way for empirically dissecting the genetics of preference in many taxa, it is not without limitations. The sample size required to detect significant levels of genetic variance in female preference functions appears to be greater than that required for scalar traits, perhaps as a consequence of different traits (or parts thereof) being measured on different individuals (Meyer

1991). With measurements on 638 females from 111 sires, we were still able to gain statistical support for only 64% of the estimated additive genetic variance in preference functions. In addition, our approach requires the use of more individuals than does measuring focal females multiple times, and this may be a limiting factor for some species (Kingsolver et al. 2004).

In conclusion, the genetic analysis of female preference has represented an empirical challenge due principally to difficulties in generating suitable metrics of preference in experiments large enough to allow the appropriate characterization of variation among females (Wagner 1998; Chenoweth and Blows 2006). The genetic analysis of female preference functions as function-valued traits offers a robust statistical framework in which to place investigations of female preference and at the same time alleviates some of the experimental difficulty in estimating variation in preference functions. Our analysis of female preference functions in *D. bunnanda* suggests that preferences for multiple traits are genetically correlated and that preferences themselves may be under strong selection.

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Left, Mating *Drosophila bunnanda* female has accepted a male (photograph by A. Van Homrigh). *Right*, rainforest habitat of *D. bunnanda* (photograph by K. McGuigan).