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QUANTITATIVE GENETICS OF FEMALE MATE PREFERENCES IN AN ANCESTRAL AND A NOVEL ENVIRONMENT

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A female's mate preference is a potentially complex function relating variation in multiple male phenotypes with her probability of accepting individual males as a mate. Estimating the quantitative genetic basis preference functions within a population is empirically challenging yet key to understanding preference evolution. We employed a recently described approach that uses random-coefficient mixed models in the analysis of function-valued traits. Using a half-sibling breeding design in a laboratory-adapted *Drosophila serrata* population, we estimated the genetic (co)variance function of female preference for male sexual displays composed of nine contact pheromones. The breeding design was performed across two environments: the food to which the population was well adapted and a novel food that reduced average female productivity by 35%. Significant genetic variance in female preference was detected and the majority (64.2%) was attributable to a single genetic dimension (eigenfunction), suggesting that preferences for different pheromones are not genetically independent. The second eigenfunction, accounting for 24% of the total genetic variance, approached significance in a conservative test, suggesting the existence of a second, independent genetic dimension. There was no evidence that the genetic basis of female preference differed between the two environments, suggesting the absence of genotype-by-environment interactions and hence a lack of condition-dependent preference expression.

KEY WORDS: **Adaptation, condition dependence, female choice, function-valued trait, sexual selection.**

Female mate preferences describe the relationship between the phenotype of males and their probability of being accepted as a mate. Females often assess multiple traits when choosing mates, including various sexual signals and displays (Candolin 2003; Chenoweth and Blows 2006), and many of these traits vary continuously among males. Therefore, in contrast to scalar-valued traits that take on a single value for individuals, mate preferences constitute potentially complex functions describing the quantitative relationship between multiple male traits and a female's acceptance.

Since Darwin (1871) first recognized that female mate preferences could be responsible for the evolution of the extravagant male sexual displays that characterize many species, the evolutionary consequences of mate preferences have received much attention (Andersson 1994). The typical approach involves measuring the mating success of a range of male phenotypes across a group of females, providing information on the average female preference within the population (Wagner 1998; Kingsolver et al. 2001; Chenoweth and Blows 2006). Such population-level mate preferences are useful because they quantify sexual selection on male traits arising from female mate choice in a currency appropriate to the microevolutionary equations for predicting the short-term response to selection (Lande 1979; Lande and Arnold

1983). This approach has contributed to our understanding of the evolution of extravagant male displays that cannot be explained by natural selection alone. However, mate preferences are predicted to evolve via a coevolutionary process between males and females that involves both direct and indirect selection; knowledge of the genetic basis of preferences is therefore key to understanding this process. Population-level preferences obscure individual preference variation and therefore provide little insight into its genetic basis (Wagner 1998; Chenoweth and Blows 2006).

Estimating individual mate preferences can present a significant empirical challenge because it is often difficult to evaluate the responses of individual females to a range of male phenotypes under controlled conditions (Wagner 1998; Chenoweth and Blows 2006). In many species for example, male display traits cannot be synthesized artificially and measuring preferences requires copulation, an outcome that may alter a female's preference with respect to subsequent males. These difficulties were overcome recently by the application by McGuigan et al. (2008) of quantitative genetic methods for function-valued traits (Kirkpatrick and Heckman 1989; De Jong 1990; Gomulkiewicz and Kirkpatrick 1992; Kingsolver et al. 2001; Kirkpatrick and Meyer 2004; Meyer and Kirkpatrick 2005; Kirkpatrick 2009). This approach uses random-coefficient mixed models to quantify the genetic basis of female preference within the context of a classic quantitative genetic breeding design, even when individual females are used in only a single mate choice trial. The basis of the approach is to calculate preference functions not for individual females, but rather for groups of related females within the breeding design for which multiple observations (i.e., mate-choice trials) are available. Variance in preference among appropriate groups of females, such as offspring of separate sires, provides an estimate of the additive genetic basis of preference. When preferences are modeled as a function of multiple male traits, genetic variation in them is represented by a covariance function ($\mathcal{G}$) in place of the standard covariance matrix (**G**) for scalar-valued traits (Kirkpatrick and Meyer 2004).

The nature of individual variation in mate preferences, and in particular, the question of whether female mate preferences are condition dependent, has received increased attention of late (Owens et al. 1994; Hingle et al. 2001; Bro-Jørgensen 2002; Mazzi 2004; Hunt et al. 2005; Cotton et al. 2006b; Baugh and Ryan 2009; Holveck and Riebel 2010; reviewed in Cotton et al. 2006a). Mate preferences are thought to be costly (Pomiankowski 1987; Reynolds and Gross 1990; Byers et al. 2005; Rundle et al. 2009) and if these costs, or the fitness benefits the preferences provide, vary with female quality then the strength of the preferences should evolve to depend on condition for reasons analogous to the evolution of condition dependence for male sexual displays (Cotton et al. 2006a). Preferences are expected to be weaker in low-condition females and stronger in high-condition females. Condition-dependent expression of mate preferences is of interest because it may alter the strength and direction of sexual selection within an environment due to variation in female condition, and it may affect the degree to which sexual selection may promote adaptation to a novel environment (Lorch et al. 2003). Condition-dependent mate preferences may also contribute to the maintenance of genetic variation in male display traits. The preferred approach for demonstrating condition dependence has been to estimate changes in preference when female condition is experimentally manipulated environmentally, often through alterations in diet quality (Cotton et al. 2004, 2006a). Although studies of the phenotypic consequences of such manipulations are accumulating, the genetic basis of condition-dependent mate preferences is generally unknown (but see Bakker et al. 1999; Rodriguez and Greenfield 2003).

Here, we conduct a standard diet manipulation of female condition within the context of a quantitative genetic half-sibling breeding design, allowing us to estimate the genetic basis of female preference and providing insight into its condition dependence. Our experiment uses *Drosophila serrata*, an Australian species in which population-level mate preferences and the sexual selection they generate have been extensively studied via a series of quantitative genetic and evolution experiments. Females use a suite of contact pheromones, composed on long-chain cuticular hydrocarbons (CHCs), in mate choice within populations (Chenoweth and Blows 2003, 2005; Petfield et al. 2005) and species recognition (Blows and Allan 1998; Higgie et al. 2000; Higgie and Blows 2007, 2008). As in past studies (Hine et al. 2002; Chenoweth and Blows 2003; Hine et al. 2004; Chenoweth and Blows 2005; Petfield et al. 2005; Skroblin and Blows 2006; Rundle et al. 2009), we quantify sexual selection by female choice on male CHCs using replicate laboratory mating trials in which individual females are allowed to choose between two or more males which subsequently have their CHCs extracted and quantified using gas chromatography. Selection gradients are estimated using a standard first-order multiple regression (Lande and Arnold 1983) that models the mating success of males as a function of their CHCs. Selection gradients estimated in this way are equivalent to the population-level female mate preference for these traits (Wagner 1998; Chenoweth and Blows 2006).

A number of lines of evidence in *D. serrata* demonstrate that male CHCs are the direct target of sexual selection arising from female mate preferences, and that the quantification of such preferences via laboratory mate choice trials is biologically relevant to our understanding of preferences in nature. Male CHCs exhibit a pattern of reproductive character displacement along the Australian east coast that corresponds with the presence versus absence of the related species, *D. birchii* (Higgie et al. 2000; Higgie and Blows 2007). Laboratory mate choice trials reveal genetically based differences in female mate preferences for male

CHCs between sympatry and allopatry, and the resulting divergent sexual selection estimated in the laboratory corresponds with the pattern of character displacement in nature (Higgie and Blows 2007). In addition, in a laboratory evolution experiment that manipulated the opportunity for sexual selection, male CHCs and female preferences for them evolved to match the observed character displacement (Higgie and Blows 2008). This effect was attributable to female choice alone (the additional opportunity for both male–male competition and sperm competition had no significant effect), providing strong manipulative evidence that the measure of preference, and the experimental design for the mate choice trials, are relevant to the field. Finally, female preferences for male CHCs have also been shown to evolve in other contexts, for example in response to altered natural selection (Rundle et al. 2005, 2009), indicating that females exercise choice during laboratory trials and revealing the existence of genetic variation in this choice.

We estimated the linear genetic covariance function describing female mate preferences for eight CHCs in males using a paternal half-siblings breeding design involving a laboratory-adapted *D. serrata* population. This population was created almost four years earlier by pooling collections from multiple geographic population (potential effects on the genetic basis of preference are addressed in the Discussion). We performed the breeding design across two environments, one involving a larval diet to which the population was well adapted (its "ancestral" yeast food) and the other involving a novel larval diet (corn food). The corn-food diet reduces female productivity by 35% on average (Delcourt et al. 2009), creating an experimental manipulation of condition. If preference expression is condition dependent, it will vary between the diet treatments; such phenotypic changes would be manifested as a genotype × environment interaction in a quantitative genetic analysis of preference.

Material and Methods

HALF-SIBLING BREEDING DESIGN

A paternal half-sibling breeding design was conducted using a previously described laboratory stock population of *D. serrata* (Rundle et al. 2006; Chenoweth et al. 2008). Ninety-two sires were each mated to four virgin females (dams) and these females were subsequently allowed to oviposit for 20 h in one environment (yeast or corn) and then 48 h in the other. The order of oviposition environments was alternated among females and difference in oviposition time ensured similar larval rearing densities given a decline in female egg laying rate with time. The breeding design was conducted in three blocks consisting of 30, 31, and 31 sires that spanned five generations of the laboratory population. The results of fitness assays conducted on male and female offspring from this breeding designed are presented in Delcourt et al. (2009). The number of sires in the current dataset differs slightly from Delcourt et al. (2009) due to incomplete data records. Unique offspring (i.e., different individuals from the fitness assays) were used in the mate preference assays described below.

FEMALE MATE PREFERENCE ASSAYS

Preference assays were performed using 3-day-old virgin daughters from each half-sibling family, raised in each environment, that were collected at emergence and separated by sex using light CO_2 anesthesia. These females were held in vials in groups of 10 on their respective larval food media with abundant live yeast sprinkled on top. Female preference was estimated using replicate mate choice trials in which a single daughter was placed in a vial along with five virgin males of similar age randomly chosen from the stock population and raised in the yeast environment. Five males were used to give females a greater opportunity to express their preference by increasing the range of male phenotypes among which she could choose. Vials were observed until the female successfully mated with one of the five males, at which point the chosen male, along with one of the four rejected males (randomly selected), were removed using CO_2 anesthesia for CHC extraction. The remaining three rejected males were discarded, along with the female. Mating vials contained food that matched the environment in which the daughter was raised (i.e., yeast or corn). Five replicate mating trials using five separate daughters were conducted using the offspring of each dam raised in each environment, generating more than 7000 males for CHC extraction.

CHC EXTRACTION AND ANALYSIS

CHCs were extracted from single flies by washing individuals in 100 μL of hexane for approximately 3 min and then vortexing for 1 min. Individuals were then removed and samples were analyzed using an Agilent Technologies (Wilmington, DE) 6890N gas chromatograph fitted with a HP5 column of 50 m × 0.32 mm internal diameter, pulsed splitless inlet (at 200°C), and a flame ionization detector (at 250°C), using the temperature program given in Rundle et al. (2005). Individual CHC profiles were determined by integration of the area under nine peaks, corresponding to those used in past studies, and identified in order of their retention times as: (Z,Z)-5,9-$C_{24:2}$; (Z,Z)-5,9-$C_{25:2}$; (Z)-9-$C_{25:1}$; (Z)-9-$C_{26:1}$; 2-Me-C_{26}; (Z,Z)-5,9-$C_{27:2}$; 2-Me-C_{28}; (Z,Z)-5,9-$C_{29:2}$; and 2-Me-C_{30} (Howard et al. 2003). After integration, relative abundances were calculated separately for each individual by dividing the area integrated for each of their CHCs by the total area for all nine CHCs. Expressing the abundances of each CHC as a proportion of the total corrects for technical error associated with quantifying the absolute CHC abundance. Proportions were then transformed to log-contrasts, using (Z,Z)-5,9-$C_{24:2}$ as the divisor,

to break the unit-sum constraint and thereby permit multivariate analyses to be performed (Atchison 1986). Log-contrast CHC values were standardized (mean = 0, standard deviation = 1) prior to statistical analyses.

STATISTICAL ANALYSES

Standardized linear sexual selection gradients on the eight log-contrast CHCs, equivalent to the population-level female mate preference for these traits, were estimated separately for each environment using a standard first-order multiple regression model (Lande and Arnold 1983)

$$w = \alpha + B + O + BO + \sum_{i=1}^{8} \beta_i z_1 + \varepsilon, \qquad (1)$$

in which w is the mating success score (0 = rejected, 1 = chosen) of individual males, z_i are the eight log-contrast CHC values for a given male, and ε is unexplained error. Fixed effects include the intercept (α), experimental block (B), the order of oviposition environments (O), and their interaction (BO). This regression yielded a column vector of linear selection gradients (β_i) characterizing directional selection on each of the eight log-contrast CHCs. Because these gradients arise from female mate choice, they provide an estimate of population-level linear preference functions of females for these male traits (Wagner 1998; Chenoweth and Blows 2006). Although the selection gradients were estimated using standard least squares, significance was determined using logistic multiple regression because mating success is binomially distributed (Fairbairn and Preziosi 1996; Rundle et al. 2009). This was done using a generalized linear model with a logistic link function, fit via maximum likelihood, as implemented in the GENMOD procedure in SAS version 9.2 (SAS Institute, Cary, NC). To determine whether sexual selection, and hence population-level mate preferences, differed between the two environments, data from both environments were combined in a single analysis using the multiple regression described in equation (1) with the inclusion of a fixed effect term representing the main effect of environment and eight additional terms representing the interaction of each log-contrast CHC with environment. A likelihood ratio test was then used to compare the fit of this full model with a reduced one lacking the eight interaction terms.

Genetic variation in female preferences for the eight log-contrast CHCs in males was estimated using the following multivariate random-coefficient model (Meyer and Kirkpatrick 2005; Littell et al. 2006; McGuigan et al. 2008):

$$\mathbf{y}_{ijkl} = \alpha + E + B + O + BO + \mathbf{X}_{kl}\mathbf{b} + \mathbf{Z}^{(d)}_{kl}\boldsymbol{\delta}^{(d)}_{kl} + \mathbf{Z}^{(s)}_{kl}\boldsymbol{\delta}^{(s)}_{kl} + \varepsilon_{kl}, \qquad (2)$$

in which $\mathbf{y}$ is a response vector containing the binomial mating success scores of the ith male from a mating trial employing the jth daughter of the kth dam within the lth sire. Fixed effects are as described in equation (1) with the addition of environment (E). The vector of population-wide linear partial-regression slopes ($\mathbf{b}$) for the eight log-contrast CHCs, represented in the design matrix $\mathbf{X}$, is equivalent to the vector of directional selection gradients ($\boldsymbol{\beta}$) in equation (1) that quantifies linear sexual selection on the eight log-contrast CHCs. Although more complex (e.g., second-order) functions can be fit using random regression, consistent with previous investigations (Chenoweth and Blows 2005), population-level mate preferences in this population of *D. serrata* appear to be predominately linear (H. Rundle, unpubl. data).

The genetic basis of female preference functions was estimated through the random-effect terms in equation (2). These terms treat the partial regression coefficients (i.e., the slopes, β, of the population-level linear preference functions for each logcontrast CHC) as random samples from some population of possible coefficients—here those at the dam and sire levels of our breeding design. In particular, $\mathbf{Z}^{(d)}_{kl}\boldsymbol{\delta}^{(d)}_{kl}$ represents the departure of the regression slope for the kth dam nested with the lth sire, and $\mathbf{Z}^{(s)}_{kl}\boldsymbol{\delta}^{(s)}_{kl}$ represents the departure of the regression slope for the lth sire from the population-wide regression, $\mathbf{X}_{kl}\mathbf{b}$. $\mathbf{Z}^{(d)}_{kl}$ and $\mathbf{Z}^{(s)}_{kl}$ are the design matrices at the dam and sire levels, respectively, and the variances $\boldsymbol{\delta}^{(d)}_{kl}$ and $\boldsymbol{\delta}^{(s)}_{kl}$ are assumed to be normally distributed with means of zero and variances Σ_d and Σ_s, respectively. The vector of residual (error) parameters was reduced because we lacked replication to estimate the covariance among female preference functions at this level (Meyer 1991; McGuigan et al. 2008). Separate errors were estimated for groups of sires that had different numbers of daughters to account for heterogeneity as a consequence of family size. Hypothesis tests assumed a normal distribution because samples sizes were large and the probability of either outcome (chosen or rejected males) was equal by design (Zar 1999; Chenoweth and Blows 2003).

Equation (2) was fit by restricted maximum likelihood, as implemented in the MIXED procedure of SAS version 9.2 (SAS Institute, Cary, NC), using the factor analytic approach to estimate the covariance matrices of the random effects at the dam and sire level. Of particular interest here is the sire-level covariance matrix because, when multiplied by four, it provides an estimate of the additive genetic covariance function ($\mathcal{G}$) of female preference for male CHCs. Factor analytic modeling directly estimates the eigenfunctions of a covariance function, representing mutually orthogonal aspects of trait covariation within $\mathcal{G}$, and is analogous to the direct estimation of the genetic principal components (i.e., eigenvectors) of an additive genetic covariance ($\mathbf{G}$) matrix (Kirkpatrick and Meyer 2004; Hine and Blows 2006). The factor-analytic approach provides a powerful method for directly testing the number of statistically supported eigenfunctions underlying $\mathcal{G}$, or in other words, the number of independent dimensions of genetic variance for female preference (Hine and Blows 2006; Meyer and Kirkpatrick 2008). For this analysis, $\mathcal{G}$ was constrained to be

from eight through zero dimensions and a series of nested likelihood ratio tests were used to determine whether excluding each dimension significantly worsened the fit of the model. The covariance function at the dam level was fixed at three dimensions for these tests, corresponding to the number of eigenfunctions with nonzero (i.e., positive) eigenvalues at this level.

To test whether the genetic basis of female preference functions differed overall between the two environments, sire × environment and dam × environment random effect terms were added to equation (2). A nested likelihood ratio test was then used to determine the significance of the first eigenfunction at the sire × environment level, thereby providing a test of whether the additive genetic basis of female preference differed between environments in an approach analogous to the standard technique for detecting genotype × environment interactions (Lynch and Walsh 1998). For this analysis, the sire, dam, and dam × environment covariance functions were fixed at the number of dimensions (i.e., eigenfunctions) with nonzero eigenvalues, corresponding to four, three, and three dimensions respectively.

Results

At the population level, female mate preferences generated significant linear sexual selection on the eight log-contrast CHCs in males in both environments (Table 1), explaining 21.2% (likelihood ratio test: $\chi^2 = 811.3$, df = 8, $P < 0.0001$) and 20.4% (likelihood ratio test: $\chi^2 = 789.7$, df = 8, $P < 0.0001$) of the variance in male mating success in the "ancestral" yeast and novel corn environments, respectively. Although the population-level preferences differed significantly between these environments (likelihood ratio test: $\chi^2 = 16.67$, df = 8, $P = 0.033$), the differences were minor: the vector correlation of the selection gradients in yeast versus corn was 98.2%. The significance of this difference likely reflects the high statistical power (6598 males were phenotyped). Consistent with numerous past studies using various populations of *D. serrata* (Blows et al. 2004; Hine et al. 2004; Rundle et al. 2006; Skroblin and Blows 2006), sexual selection was significant on the three methyl-branched alkanes, acting in opposite directions on 2-Me-C_{28} versus 2-Me-C_{26} and 2-Me-C_{30}. Selection was also strong on the diene (Z,Z)-5,9-$C_{29:2}$, exceeding the median absolute value of 0.18 for directional sexual selection gradients across taxa in nature (Kingsolver et al. 2001). Strong sexual selection on this diene has been previously observed in other *D. serrata* populations (Blows et al. 2004; Chenoweth and Blows 2005).

The genetic basis of female mate preference for the eight log-contrast CHCs in males did not differ between the ancestral (yeast) and the novel (corn) environments, as indicated by a nonsignificant sire × environment interaction in the random regression model (eq. 2; $\chi^2 = 0.678$, df = 8, $P = 0.99$). Consequently, within this model the vector of population-wide regression slopes across these two environments (**b**), representing the fixed effects of each log-contrast CHC on male mating success, was very similar to the environment-specific vectors of sexual selection ($\boldsymbol{\beta}$; Table 1). A single additive genetic covariance function ($\mathcal{G}$) of female preference was therefore estimated across the two environments.

Genetic variance in female preference is present for all eight log-contrast CHCs, although both positive and negative covariances give rise to strong genetic correlations that suggest a shared genetic basis of preference for these traits (Table 2). The absolute genetic correlation among the three methyl-branched alkanes (2-Me-C_{28}, 2-Me-C_{26} and 2-Me-C_{30}), for example, averages 0.83. This nonindependence is reflected in the eigenfunctions of $\mathcal{G}$, with their associated eigenvalues declining rapidly in magnitude

Table 1. Vectors of standardized directional sexual selection gradients on eight log-contrast CHCs in males, equivalent to population-level female mate preferences, as estimated from a standard multiple regression (eq. 1) conducted separately in each environment (β), and from a single random regression (b) across environments (eq. 2). The first ($\mathcal{G}_{max}$) and second ($\mathcal{G}_2$) eigenfunctions of the additive genetic covariance function ($\mathcal{G}$; Table 2) of female preference estimated from equation (2), accounting for 64.6% and 22%, respectively of the total genetic variance in preference.

Log-contrast CHC	$\boldsymbol{\beta}$ (yeast)	$\boldsymbol{\beta}$ (corn)	**b**	$\mathcal{G}_{max}$	$\mathcal{G}_2$
(Z,Z)-5,9-$C_{25:2}$	0.021*	−0.019*	−0.002	0.154	−0.166
(Z)-9-$C_{25:1}$	−0.059**	−0.037*	−0.044**	−0.337	0.347
(Z)-9-$C_{26:1}$	−0.037**	−0.029*	−0.036**	−0.023	−0.020
2-Me-C_{26}	−0.066**	−0.100**	−0.086**	−0.212	0.031
(Z,Z)-5,9-$C_{27:2}$	−0.048**	−0.053**	−0.058**	0.137	0.102
2-Me-C_{28}	0.150**	0.179**	0.163**	0.645	−0.037
(Z,Z)-5,9-$C_{29:2}$	0.251**	0.243**	0.268**	−0.122	0.813
2-Me-C_{30}	−0.131**	−0.123**	−0.114**	−0.607	−0.423

*$P<0.05$; **$P<0.001$.

Table 2. Genetic covariance function ($\mathcal{G}$) of linear female preferences for the eight log-contrast CHCs in males, as estimated from the sire covariance matrix in a four-dimensional factor analytic model. Genetic variances are displayed along the diagonal (bold) with covariances below. Genetic correlations are given above the diagonal in italics.

Log-contrast CHC	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**0.00524**	*−0.160*	*−0.889*	*−0.553*	*−0.460*	*0.441*	*−0.314*	*−0.303*
(Z)-9-$C_{25:1}$	−0.00098	**0.00717**	*−0.095*	*0.747*	*−0.431*	*−0.788*	*0.610*	*0.519*
(Z)-9-$C_{26:1}$	−0.00096	−0.00012	**0.00022**	*0.526*	*0.539*	*−0.315*	*−0.144*	*0.344*
2-Me-C_{26}	−0.00195	0.00309	0.00038	**0.00238**	*−0.077*	*−0.853*	*0.183*	*0.727*
(Z,Z)-5,9-$C_{27:2}$	−0.00173	−0.0019	0.00042	−0.00020	**0.00270**	*0.500*	*−0.018*	*−0.580*
2-Me-C_{28}	0.00404	−0.00845	−0.00059	−0.00527	0.00329	**0.01603**	*−0.276*	*−0.911*
(Z,Z)-5,9-$C_{29:2}$	−0.00223	0.00506	−0.00021	0.00088	−0.00009	−0.00342	**0.00961**	*−0.121*
2-Me-C_{30}	−0.00283	0.00568	0.00066	0.00458	−0.00389	−0.01489	−0.00154	**0.01667**

(Table 3). This indicates that, overall, the genetic covariances severely constrain the multivariate patterns of genetic variation in female preferences for this suite of male CHCs.

The first eigenfunction of $\mathcal{G}$ ($\mathcal{G}_{max}$) accounts for well over half (64.6%) of the total genetic variance in female preference and is the only trait combination for which genetic variance is statistically supported by factor analytic modeling ($P = 0.0001$; Table 3). The three methyl-branched alkanes contributed strongly to this eigenfunction (Table 1), with 2-Me-C_{28} loading in opposite direction to 2-Me-C_{26} and 2-Me-C_{30}. This suggests that genes segregating in this population that strengthen female preference for some methyl-alkanes tend to weaken it for others. The second eigenfunction ($\mathcal{G}_2$) accounts for an additional 22.0% of the genetic variance in preference and approaches significance ($P = 0.142$ is an upper bound from a known-conservative test; Self and Liang 1987; McLachlan and Basford 1988), suggesting the existence of a second, independent genetic dimension underlying female preferences. This trait combination represents, to a large degree, female preference for the diene (Z,Z)-5,9-$C_{29:2}$, although moderate loadings also contrast (Z)-9-$C_{25:1}$ with 2-Me-C_{30} (Table 1).

Discussion

Quantifying the genetic basis of female mate preference presents significant empirical challenges (Chenoweth and Blows 2006), in particular because they often target sexual displays that are composed of multiple components (e.g., the various properties of a visual or acoustic display, or the constituent components of a pheromone) and/or that involve traits of different types or modes (Candolin 2003). Insight may be gained, however, by treating preferences not as scalar values but rather as continuous functions of the display traits they target (McGuigan et al. 2008). Here, we have used this approach in *D. serrata* to estimate the quantitative genetic basis of female preference for a multicomponent sexual display in males composed of a suite of contact pheromones (CHCs), and to test for condition dependence in the expression of these preferences. Mate preferences, estimated as the population-level sexual selection gradients on male CHCs arising from female choice, were relatively strong in this population, explaining 20–21% of the variance in male mating success. In comparison, a survey of nine natural *D. serrata* populations found that directional selection on CHCs explained on average approximately 6.1% of the variance in male mating success (Rundle et al. 2008), although values as high as 59% have been observed in other laboratory populations (Rundle et al. 2005).

Our results reveal genetic variance in female preference for male CHCs, one effect of which may be to aid in the maintenance of genetic variance in the male display traits (CHCs) they target. The presence of genetic variance in preference is confirmed by the evolution of these traits when selection was manipulated in a

Table 3. Model fit statistics of the number of effective dimensions of the genetic covariance function ($\mathcal{G}$) of female preference, as determined from a factor-analytic model of the sire-level covariance matrix. The percent of the total genetic variance in female preference (% variance) was calculated from the full (i.e., eight-dimensional) factor analytic model.

No. of dimensions	% variance	No. of parameters	−2LL	AIC[1]	*P*-value[2]
8	0	81	–	–	–
7	0	80	–	–	–
6	0	78	–	–	–
5	0	75	8076.3	8222.3	1
4	2.8	71	8076.3	8212.3	0.989
3	10.7	66	8076.9	8204.9	0.722
2	21.8	60	8080.5	8200.5	0.142
1	64.6	53	8091.4	8197.4	0.0001
0	——	45	8122.9	8212.9	——

[1]Akaike's information criterion.

[2]Results of a likelihood ratio test of whether the fit of a model with one fewer genetic dimensions is significantly worse than the fit of the current model.

recent laboratory experiment using this same population (Rundle et al. 2009). Strong genetic covariances among preferences for different CHCs were also detected such that the majority of the genetic variance in preference (64.6%) was accounted for by a single underlying trait ($\mathcal{G}_{max}$, the first eigenfunction of $\mathcal{G}$, equivalent to $\mathbf{g}_{max}$ for scalar traits). This indicates that much of the genetic basis for female preference for the different CHCs is shared, similar to that observed in the related species *D. bunnanda* (McGuigan et al. 2008).

This first eigenfunction of $\mathcal{G}$ ($\mathcal{G}_{max}$) represents the combination of male traits for which there is the greatest genetic variance in female preference within this population. This eigenfunction was dominated by the three methyl-alkanes (Table 1), with 2-Me-C_{28} loading in opposite direction to 2-Me-C_{26} and 2-Me-C_{30}, reflecting the very strong genetic correlations estimated between preferences for these three traits (Table 2). Female mate preferences for CHCs in males vary substantially among natural population of *D. serrata*, with much of this variation being associated with the presence versus absence of a related species, *D. birchii* (Higgie and Blows 2007, 2008; Rundle et al. 2008). Among nine natural populations, the major axis of variation in population-level mate preferences (termed $\boldsymbol{b}_{max}$ because it also represents the major axis of variation in sexual selection on male CHCs generated by these population-level preferences) also strongly contrasts 2-Me-C_{28} with the other two methyl-alkanes (Chenoweth et al. 2010). This similarity between $\boldsymbol{b}_{max}$ and $\mathcal{G}_{max}$ indicates that the combination of male traits for which genetic variance in female preference is greatest is also the combination of traits for which mate preferences have diverged most among these populations in nature.

The second eigenfunction of $\mathcal{G}$ ($\mathcal{G}_2$) accounted for a substantial proportion of the remaining genetic variance in preference (22%). Although this dimension was not statistically significant ($P = 0.142$), likelihood ratio tests comparing nested mixed models that differ in more than one random-effect parameter are known to be conservative (Self and Liang 1987; McLachlan and Basford 1988). This suggests the possible existence of an independent genetic dimension of preference variation. The potential existence of a second dimension of genetic variance in female preference implies genetic variance for changes in preference direction, or in other words, the combination of male CHCs that females find attractive. Consistent with this, such variation has been shown to exist in nature (Chenoweth et al. 2010) and has evolved in the laboratory (Rundle et al. 2009). Our laboratory population was created (almost four years prior to the experiment) by pooling collections from multiple populations sympatric and allopatric with *D. birchii* (Rundle et al. 2006). The presence of two underlying genetic dimensions may therefore reflect a distinct genetic basis to preference variation within versus among populations in nature, with the latter arising from the reinforcement of mate recognition in sympatry (Higgie et al. 2000; Higgie and Blows 2008).

Notwithstanding the potential existence of a second independent dimension to the genetic variance in female preference, our results strongly suggest the absence of independent genetic variance in preference for all eight individual log-contrast CHCs in males. Although very similar to preference variation for CHCs in *D. bunnanda* (McGuigan et al. 2008), this contrasts with findings in guppies in which female preferences for orange and black coloration respond individually to selection, implying an independent genetic basis (Brooks and Couldridge 1999). Additional studies are much needed from a diversity of species and a variety of traits, but if results such as ours are common, it suggests that explanations for the evolution of multicomponent sexual displays may not lie in their conveying multiple messages (e.g., signaling different aspects of condition) or acting as redundant signals (Candolin 2003), but rather in part in the tendency of researchers

to overparse such displays relative to how they are perceived by female sensory systems. Whether the genetic basis of preference for multimodal signals shows similar nonindependence may appear less likely, although conclusions await direct empirical study.

Finally, we found no evidence that the genetic basis of female preference differed between the yeast and corn environments, despite the fact the novel corn-food reduced female productivity by 35% on average compared to the yeast-food to which the population is adapted (Delcourt et al. 2009). A lack of statistical power is one possible explanation given that the random-coefficient mixed model relies on groups of individuals (in our case, offspring of different sires) to estimate preference. However, at the phenotypic level females chose mates with respect to CHCs in essentially the same way in yeast and corn, with the correlation in population-level mate preferences (i.e., the β vectors) between these two environments being extremely high (98.2%). Our results therefore suggest that preference expression in *D. serrata* females is independent of their condition, or in other words, that the combination of male CHCs that a female prefers does not depend on her health or resources. Our results also provide no evidence that genetic variance in preference is greater overall in the more harsh corn-food environment, as has been predicted for condition-dependent preferences and shown in male display traits (Bjorksten et al. 2000; Cotton et al. 2006a).

The absence of condition-dependence contrasts with both expectation and the results of a number of studies showing changes in preference strength or choosiness (the effort an individual is willing to invest in choice; Jennions and Petrie 1997) when female condition was manipulated (reviewed in Cotton et al. 2006a). For example, female zebra finch raised in larger broods develop into lower quality adults and prefer the mating songs of lower quality males (Holveck and Riebel 2010), and in túngara frogs, females of low body condition were more likely to reverse their initial choice as compared to females of high body condition when male call attractiveness parameters were altered (Baugh and Ryan 2009). The expectation for condition-dependence arises if preferences are costly and high-condition individuals are more efficient at converting preference into fitness (Cotton et al. 2006a; Getty 2006). In a recent evolution experiment in this same population, female preferences for male CHCs weakened overall when their expression was prevented by randomly creating monogamous pairs every generation for mating (Rundle et al. 2009). The selective removal of preferences when their benefits were prevented suggests that they are costly. Our current results suggest that this cost does not depend on the condition of the female. Condition dependence of other aspects of female preference (e.g., choosiness; Jennions and Petrie 1997; Hunt et al. 2005) is nevertheless possible and remains to be explored.

In summary, elucidating the quantitative genetic basis of mate preferences is a challenging endeavor. We have taken advantage of the random regression approach to the analysis of function-valued traits to explore female preferences for a sexual display trait in males that is composed of multiple contact pheromones (CHCs). Our results demonstrate genetic variance and in female preference, with strong covariances among preferences for different CHCs that are likely to constrain preference evolution. We find little evidence to suggest that preferences expression is condition dependent at the phenotypic or genetic level.

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