JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12061

Curves as traits: genetic and environmental variation in mate preference functions

R. L. RODRÍGUEZ, A. C. HALLETT, J. T. KILMER & K. D. FOWLER-FINN

Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, WI, USA

Keywords:

Auchenorrhyncha;
function-valued trait;
genotype-environment interaction.

Abstract

Study of the genetic and developmental architecture of mate preferences lags behind the study of sexual ornaments. This is in part because of the challenges involved in describing mate preferences, which are expressed as a function of variation in ornaments. We used the function-valued approach to test for genetic and environmental components of variation in female mate preferences in *Enchenopa* treehoppers (Hemiptera: Membracidae). These insects communicate with plant-borne vibrational signals, and offer a case study of speciation involving sexual selection and environmental change. We focused on female preferences for male signal frequency, the most divergent signal trait in *Enchenopa*. Obtaining complete, individual-level descriptions of mate preferences in a full-sib, split-family rearing experiment, we document substantial genetic variation in mate preference functions. Focusing on traits describing variation in the shape of the preference functions, we further document considerable broad-sense heritability and evidence of weak genotype × environment interaction in most traits. Against the background of recent and rapid divergence in *Enchenopa*, these results indicate potent mechanisms that maintain variation and sustain the involvement of mate preferences in sexual selection.

Introduction

The genetic and developmental architecture of sexual traits have particular relevance for understanding evolutionary processes such as sexual selection and speciation (Andersson, 1994; Ritchie & Phillips, 1998; Coyne & Orr, 2004; Ritchie, 2007). For example, the presence and magnitude of genetic variation in a trait determine whether the trait can be given a role in divergence and speciation by the process of Fisherian selection; and the trait's relationships with individual viability and condition influence how other components of natural and sexual selection will act on it (Fisher, 1958; West-Eberhard, 1983; Mead & Arnold, 2004; Andersson & Simmons, 2006; Kokko *et al.*, 2006; Prum, 2010; Hill, 2011; Clark, 2012). Additional evolutionary consequences may arise from phenotypic plasticity in sexual traits, either facilitating divergence or weakening sexual selection (Qvarnström, 2001; West-Eberhard, 2003, 2005; Bailey & Zuk, 2008; Chaine & Lyon, 2008; Verzijden *et al.*, 2012). And genetic variation in plasticity – that is, genetic variation in reaction norms, also termed genotype × environment interaction (G × E) (Via & Lande, 1985; Lynch & Walsh, 1998) – can have varied consequences for evolution via sexual selection, which range from sustaining sexual selection, altering its course, or halting it altogether (Greenfield & Rodríguez, 2004; Lehmann *et al.*, 2007; Bussière *et al.*, 2008; Kokko & Heubel, 2008; Rodríguez *et al.*, 2008; Higginson & Reader, 2009; Ingleby *et al.*, 2010). There is, consequently, considerable interest in the patterns of genetic and environmental variation in traits such as sexual ornaments and mate preferences (Chenoweth & McGuigan, 2010).

A challenging aspect of research on variation in sexual traits involves describing mate preferences. Mate preferences are function-valued traits (Meyer & Kirkpatrick, 2005; Stinchcombe *et al.*, 2012), which is to say that mate preferences are expressed as a function of the features of the sexual ornaments encountered during

Correspondence: Rafael L. Rodríguez, Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin–Milwaukee, Lapham Hall, 3209 N. Maryland Ave., Milwaukee, WI 53201, USA.
Tel.: +1 414 226 3445; fax: +1 414 229 3926; e-mail: rafa@uwm.edu

mate choice. Accurate and complete description of mate preferences thus requires using preference functions to depict variation in sexual response according to variation in ornaments (Ritchie, 1996). This approach emphasizes that any aspect of mate preferences can generate and respond to sexual selection. For example, preferences may evolve to favour different traits or trait values among closely related species, creating divergent sexual selection (Ritchie, 1996; Rodríguez *et al.*, 2006). Alternatively, preferences may diverge in the strength with which they disfavour deviation from preferred values for different ornament traits, creating variation in the strength of sexual selection and in the concomitant match between preferred values and ornament values (Rodríguez *et al.*, 2006). Describing preference functions is challenging, however, and most work characterizing variation in mate preferences deals with component features such as response thresholds; receptivity; preference strength; and preferred traits and values (Bakker & Pomiankowski, 1995; Jennions & Petrie, 1997; Bakker, 1999; Qvarnström, 2001; Chenoweth & Blows, 2006; Chenoweth & McGuigan, 2010; Narraway *et al.*, 2010; Schielzeth *et al.*, 2010; Wiley & Shaw, 2010). The function-valued approach has been used to estimate genetic variation from comparison of the preferences of closely related species and their hybrids (Ritchie, 1996, 2000); and to estimate genetic variation from partial descriptions of individual functions (Brooks & Endler, 2001; McGuigan *et al.*, 2008; Delcourt *et al.*, 2010). The function-valued approach with complete individual-level preference functions has only recently been used to assess plasticity, repeatability (Fowler-Finn & Rodríguez, 2012a,b, unpublished data), and genetic variation (Ritchie *et al.*, 2005) in mate preferences.

Here, we assess genetic and environmental variation in mate preference functions, using complete, individual-level functions for each female. Evaluating the evolutionary implications of these measures requires a study species wherein the role of mate preferences in divergence is well understood. We used a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae), a clade of herbivorous insects wherein changes in advertisement signals and mate preferences are central to a process of speciation mediated by the colonization of novel environments, that is, host plant species (Wood, 1993; Cocroft *et al.*, 2008). Pair formation in the *E. binotata* complex involves male–female duets of plant-borne vibrational signals (Hunt, 1994; Cocroft *et al.*, 2008). Males fly from plant to plant producing advertisement signals, and females attracted by a male signal back to engage him in a duet and prompt him to search locally. Male and female signals are species-specific and function in mate choice (Rodríguez *et al.*, 2004, 2006, 2012; Rodríguez & Cocroft, 2006; Cocroft *et al.*, 2010). Female mate preferences appear to be the main cause of between-species divergence in male signals (Rodríguez *et al.*, 2006), as well as the main cause of within-species variation in male mating success (Sullivan-Beckers & Cocroft, 2010). We focused on female mate preferences for male signal frequency – which is, with host use, the most divergent adult trait in the complex, and the signal trait for which females have the strongest mate preferences (Rodríguez *et al.*, 2006; Cocroft *et al.*, 2008, 2010).

We discuss our results in terms of the role of mate preferences in sexual selection and divergence. The basic mechanisms of sexual selection (e.g. Fisherian selection) require genetic variation in sexual traits (Fisher, 1958; Mead & Arnold, 2004; Kokko *et al.*, 2006; Prum, 2010). We therefore focus on genetic variation in mate preference functions. In addition, plasticity and G × E can have varied consequences for sexual selection. For example, expressed across environments that populations encounter regularly, G × E may halt sexual selection by disrupting patterns of assortative mating and genetic covariance (Greenfield & Rodríguez, 2004; Lehmann *et al.*, 2007; Kokko & Heubel, 2008; Higginson & Reader, 2009) – but expressed across environments encountered less frequently, G × E may promote divergence by changing the dynamics of sexual selection, that is, by changing the patterns of assortative mating and sexual genetic covariance that create Fisherian selection (Rodríguez *et al.*, 2008). There is evidence suggesting that G × E may be important in the early stages of divergence in the *E. binotata* complex: G × E is expressed in male signals across host plant species (Rodríguez *et al.*, 2008). Here, we report results for plasticity and G × E in mate preferences across environments that the treehoppers experience regularly, that is, exemplars of their native host plant. At this low scale of environmental heterogeneity, G × E should be weak (Rodríguez, 2012).

Materials and methods

Study species

In our field sites (Saukville, WI, USA), there are two members of the *E. binotata* complex that live on *Viburnum lentago* plants (Caprifoliaceae): a species whose males produce low-frequency signals (mean dominant frequency = 180 Hz), and another whose males produce higher frequency signals (315 Hz). These species await taxonomic description, but male signal frequency is useful to distinguish species in the complex (Rodríguez *et al.*, 2004; Hamilton & Cocroft, 2009; Cocroft *et al.*, 2010). We used the low signal frequency species, and kept voucher specimens in 95% EtOH.

Rearing experiment

We partitioned variation among families (as a proxy for genotype) and environments with a full-sib, split-family rearing design (Roff, 1997). This design provides an

estimate of broad-sense heritability (H^2), which includes additive and nonadditive genetic variation and maternal effects (Roff, 1997). H^2 does not predict the short-term response to selection with the breeder's equation, but our goal was to evaluate overall genetic variability that may influence evolution, where nonadditive variation and maternal effects are relevant (Day & Bondurianksy, 2011).

We assessed variation due to developmental environments with a sample of the treehoppers' host plants (*V. lentago*) acquired from a local native-plants source that grows the plants from seed (Johnson's Nursery, Inc., Menomonee Falls, WI, USA). Rearing plants were all similar in size (0.5–0.9 m in height), condition and phenology, and thus represent a fraction of the range of conditions the treehoppers experience in nature.

To establish the full-sib families, we collected mated females at the end of the summer at the UWM Field Station, Saukville, WI, USA. Because *E. binotata* females mate only once (Wood, 1993; Sullivan-Beckers & Cocroft, 2010), each female's brood is a full-sib family. We allowed females to lay eggs on potted exemplars of their host plant, one female per plant. On nymph eclosion, we divided the broods in half, placing each half on its own rearing plant. We only used broods large enough to allow 20 nymphs/plant (40 nymphs/family). We thus started the experiment with 25 families. Of these, we obtained full, individual-level preference functions for females from both rearing plants for $n = 20$ families, with a median sample of $n = 10$ females/family (mean = 11, range = 2–26). For each replicate plant, median sample size was $n = 5$ females (mean = 6, range = 1–14). The total sample of females contributing full preference functions was $n = 222$. Restricting the experiment to larger broods would allow more replication within families, but at the cost of undersampling the diversity of families in the population. We reared the insects in the greenhouse at temperatures corresponding to outside conditions, except that on very warm days we used vents and shading to prevent extremes, and on very cloudy days we used supplemental lighting. Males were removed from rearing plants within a week of the adult moult (before they began to signal), to prevent mating and ensure female receptivity in the playback experiments. We tested females at the peak of their sexual receptivity at 6–8 weeks post-adult moult.

Describing mate preference functions

We used laser vibrometry and vibrational playbacks. Laser vibrometry allows monitoring substrate-borne vibrational signals without contacting the substrate (preventing any alteration of its signal propagation features), and is well suited for the low-amplitude signals used by *Enchenopa*. We used the number of female response signals produced when duetting with playback stimuli as the preference assay. The number of female responses correlates well with the likelihood to respond to stimuli, and provides a finer-detail description of variation in female response according to variation in stimulus traits (Rodríguez *et al.*, 2004, 2012; Fowler-Finn & Rodríguez, 2012a,b). We imparted stimuli onto the playback plant with a piezo-controller and actuator, and recorded the stimuli and the resulting female responses with the laser vibrometer. To capture the full shape of the preference functions, we used 19 playback stimuli that spanned and slightly exceeded the species range for male signal frequency. Each female received a different random stimulus sequence. Each stimulus consisted of a bout of three signals, corresponding to the structure of male signals for this species, with all traits but frequency set to the species mean. We generated the stimuli and controlled the playbacks with custom MATLAB scripts (available upon request). More detailed methods are provided in Fowler-Finn & Rodríguez (2012a,b).

We generated preference functions with cubic spline regressions, which do not make assumptions about shape other than smoothness (Schluter, 1988). We generated the splines in R v. 2.14.1 (R Development Core Team, 2011) with the mgcv package and the *gam* function, following Schluter (http://www.zoology.ubc.ca/%7Eschluter/zoo502stats/Rtips.models.html#gam), and optimizing the smoothing parameter for each individual female (Fig. 1).

In addition to the above analysis, we deconstructed variation in the shape of preference functions with four traits extracted from the spline curves (Bailey, 2008; Fowler-Finn & Rodríguez, 2012a). We provide graphical definitions of the traits in Fig. 3. The traits were as follows: (i) Peak preference: the signal frequency eliciting the highest response for each female. (ii) Tolerance, or preference width: how broadly a female continues to show high response as stimuli deviate from peak preference; measured at 67% of the peak in each female's function. (iii) Responsiveness: the mean level of response for each female across stimuli. (iv) Preference strength: how strongly a female disfavours deviation from peak preference. We used two different measures of preference strength. First, we used the square of the coefficient of variation of each female's responses across stimuli (CV^2; henceforth, preference strength_{CV^2}; Schluter, 1988; Fowler-Finn & Rodríguez, 2012a,b). This measure results in greater strengths for less responsive females (because CV = SD/mean). We also used a measure that is independent of responsiveness: the range of variation in female response divided by the standard deviation (range/SD) across the preference function (henceforth, preference $\text{strength}_{\text{range/SD}}$), modified from Gray & Cade (1999). Of the above traits, tolerance, responsiveness and strength are correlated with each other (Fowler-Finn & Rodríguez, 2012a,b). We study them separately because they may have

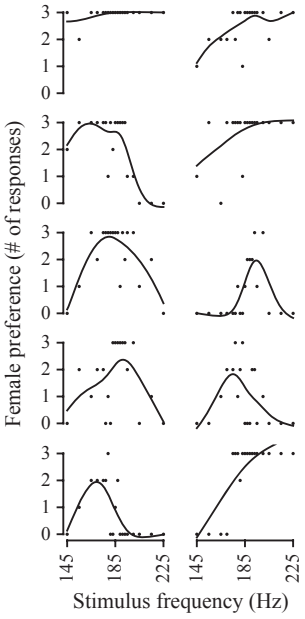

Fig. 1 Example of the individual-level mate preference functions that are the basis for our analysis of variation in *Enchenopa* preferences for male signal frequency. The data in this Figure correspond to one of 20 full-sib families. Each panel shows the data points and cubic spline preference function generated for a female. Columns correspond to the two replicate rearing plants used for this family.

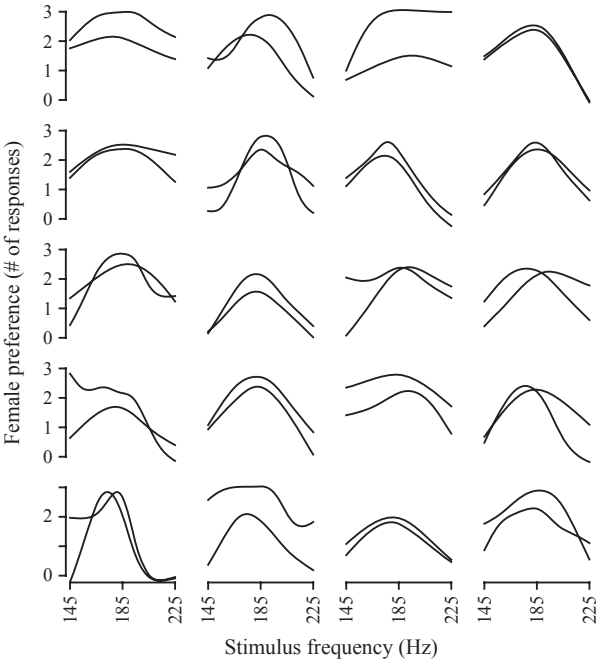

Fig. 2 Variation among and within full-sib families in *Enchenopa* mate preferences for male signal frequency. Each panel corresponds to one family, and the two preference functions in each panel correspond to the replicate-level cubic splines generated with the mean response values of the females in each split family.

different consequences for sexual selection. Although this increases the risk of spurious significance (Rice, 1989), corrections for multiple testing compromise statistical power (Nakagawa, 2004). We thus base interpretation on effect sizes (Nakagawa & Cuthill, 2007) and focus on the magnitude of the effects: H^2; variance components; and r_g (see below).

Estimating genetic and environmental variation in preference functions

We used a linear mixed model in JMP 7.0.1 (SAS Institute, Cary, NC, USA). The dependent variable was the number of responses females produced whilst duetting with the playbacks (see above). The independent variables were as follows: family; replicate nested within family; individual female nested within replicate and family; and linear and quadratic terms for stimulus frequency and their interaction with family. All terms including family, replicate or female are random effects. In the *E. binotata* complex, mate preferences for signal frequency are 'closed' or 'unimodal': females prefer signals of intermediate frequency, and species differ in which frequency is favoured (Rodríguez *et al.*, 2006). Consequently, the term that tests for genetic variation in these closed preference functions is the family × quadratic stimulus frequency interaction (Rodríguez *et al.*, 2006; Fowler-Finn & Rodríguez, 2012a,b). Note that this test assumes a quadratic preference shape, but we use it only for significance testing, and depict preferences with the cubic splines (Figs 1 and 2). The term for replicate rearing plant tests for plasticity in the overall elevation of the curves.

Estimating genetic and environmental variation in preference function traits

We estimated H^2, plasticity and G × E for each trait. We calculated H^2 and its standard error (SE) for a full-sib design with unequal family sizes (Roff, 1997), and obtained 95% confidence intervals from the SE. We used a linear mixed model (JMP, no intercept) with each trait as the dependent variable and family and replicate nested within family as independent random effects. Significance for the hypothesis that $H^2 > 0$ is provided by the *F*-ratio for the family term, calculated with the family Mean Square as the numerator and a combination of the replicate and residual Mean Squares as the denominator. As this test takes into account variation between the two rearing plants of each family, the H^2 estimate is not confounded by common environments within rearing plants.

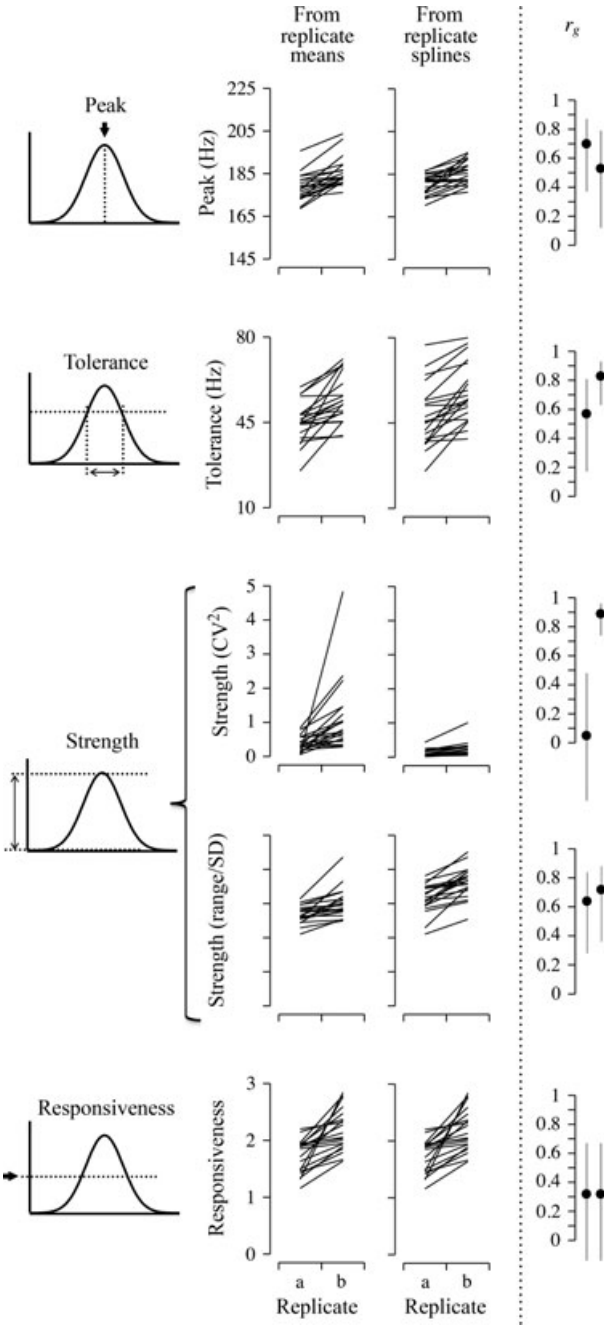

Fig. 3 Reaction norms of the traits that decompose variation in the shape of *Enchenopa* mate preference functions for signal frequency. Graphical trait definitions in the left column. The two columns with reaction norms correspond to the two methods to estimate split-family values; left: means; right: splines as in Fig. 2. The right-most column shows the two corresponding estimates for r_g. Black symbols indicate the r_g estimate; grey bars indicate the 95% CIs. In each reaction norm plot, the *y*-axis indicates the phenotypic range of variation for each trait, except for preference strength, where we show the range excluding three outlier females that were beyond the 98% percentile (but note that those females still influenced the replicate means).

The term for replicate rearing plant tests for nondirectional plasticity in each trait, arising from variation among developmental environments and from the social groupings comprised by each split-family. Thus, this term provides a random sample of variation in developmental and social environments. This means that each family was reared across slightly different environmental gradients. Consequently, family reaction norms may differ (Fig. 3) either due to G × E or to differences in the gradients experienced by each family. We consider that non G × E effects are likely to be minimal, for the following reasons: gradient differences across pairs of rearing plants were small (see above); removal of males from the rearing plants (see above) eliminated one known cause of preference plasticity (experience of signalling; Fowler-Finn & Rodríguez, 2012a,b); and although social induction of preference plasticity may occur throughout the treehoppers' lives, this effect varies very little within families (Rebar & Rodríguez, unpublished data). Thus, our test for G × E (see below) is likely to be only minimally confounded by differences in the gradients experienced across split-families. Nevertheless, we interpret our results with caution as a general indication of the presence of G × E, rather than as an indication of its true magnitude. We also report the percentage variance components for family and rearing plant to give a sense of their relative effect sizes.

We tested for nonparallel reaction norms (G × E) with the cross-environmental genetic correlation (r_g): reaction norms are nonparallel when $r_g < 1$ (Via & Lande, 1985; Roff, 1997). We estimated r_g with the Pearson correlation between split-family values across replicate plants (Roff, 1997; Astles *et al.*, 2006; Zhou *et al.*,

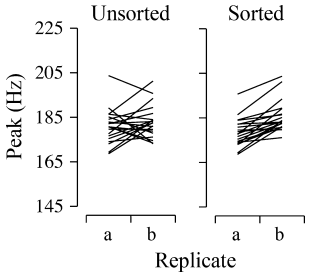

Fig. 4 Example of two potential arrangements for the family reaction norms (lines) for peak preference that may be used to calculate r_g. Each family was split among two replicate plants. With replicate plant nested within family, there is no across-family correspondence in the rearing conditions presented by replicates 'a' and 'b' (but those differences are small). An example of one such arrangement is shown in the 'unsorted' panel on the left. To estimate r_g in a way that would be conservative for the test for nonparallel reaction norms, we sorted replicates such that 'a' always had the lower split-family value. This arrangement is the 'sorted' panel on the right (as shown in Fig. 3). This makes it as hard as possible for reaction norms to cross and for r_g to be low. It also creates the artefact of a positive slope in the reaction norms.

Table 1 Function-valued assessment of genetic variation in *Enchenopa* mate preference functions for male signal frequency. The following terms are random effects: family; replicate rearing plant (nested within family); female individual (nested within replicate and family); and their interactions (see below). The term of interest is the family × quadratic stimulus frequency interaction, which tests for differences among families in the overall shape of the preference functions. We include the main linear and quadratic stimulus frequency terms for completeness. The family, replicate and individual terms test for variation among families in overall responsiveness – that is, in the 'intercept' of the functions. Bold indicates significant terms.

	Model testing whether $H^2 > 0$			
Term	MS	*F*	d.f.	*P*
Fam	9.24	1.07	19,22.794	0.44
Replicate	11.85	1.31	20,182	0.18
Fem indv	9.02	10.19	182,3956	**<0.0001**
Freq	25.68	29.01	1,3956	**<0.0001**
$Freq^2$	670.59	305.05	1,23.644	**<0.0001**
Fam × freq	4.36	4.91	19,3974.7	**<0.0001**
Fam × $freq^2$	2.65	3.00	19,3956	**<0.0001**

2008), and considered reaction norms nonparallel when the upper 95% confidence limit of r_g was < 0.9. We estimated split-family values in two ways: with the mean for each split-family, and from split-family preference functions (Fig. 2). As replicate plant is nested within family, any one arrangement for plotting reaction norms or calculating r_g is arbitrary, that is, there is no correspondence across families in the conditions presented by replicates 'a' or 'b' (although the differences are small). To calculate r_g, we therefore first arranged the replicates such that 'a' had the lower value for each split-family (Fig. 4). This restricts variation between family reaction norms and makes the test for $r_g < 1$ conservative.

Results

Genetic variation in preference functions

Preference functions for signal frequency varied considerably among families and individual females (Figs 1 and 2; Tables 1 and 2). The significant family × quadratic frequency interaction (Table 1) points to genetic variation in mate preferences as a whole. Visual inspection of the preference functions shows considerable variation in various aspects of their shape (Fig. 2), which we proceeded to deconstruct with traits describing different preference aspects. The term for replicate rearing plants was not significant (Table 1), indicating little environmental variation in the overall elevation of the curves.

H^2, plasticity and tentative G × E in preference function traits

The family term was significant or marginally significant for four of the five traits (peak, tolerance, $strength_{range/SD}$ and responsiveness); the corresponding H^2 estimates were large and the 95% CIs did not overlap zero (Table 2). Only preference $strength_{CV^2}$ showed no heritability (Table 2). We interpret these results as evidence of substantial genetic variation in most preference traits.

The term for replicate plant was significant for all traits but preference $strength_{CV^2}$, and percentage variance components were comparable with those for family (Table 2).

Reaction norms were often nonflat and nonparallel, and there were reaction norm crossover for all traits, especially tolerance and responsiveness (Figs 3 and 4). The 95% CI for the r_g estimates were below 0.9 for most traits, but often overlapped 0.8; the lowest r_g estimates were for responsiveness (Fig. 3). The two

Table 2 Estimates of broad-sense heritability (H^2) and overall plasticity in traits describing variation in the shape of *Enchenopa* mate preference functions for male signal frequency (see also Fig. 3). Family and replicate rearing plant were random effects; replicate was nested within family. Bold indicates significant or marginally significant terms.

Trait	$Term_{(d.f.num,d.f.den)}$	MS	*F*	*P*	$H^2 \pm 95\%$ CL	% var comp
Peak	$Fam_{(19,20.105)}$	1 13 146	1.84	**0.091**	0.53 ± 0.33	14.8
	$Repl_{(20,183)}$	61 613	2.67	**0.0003**		21.9
	$Res_{(183)}$	23089.4				63.3
Tolerance	$Fam_{(19,20.137)}$	8563.97	2.13	**0.051**	0.47 ± 0.31	16.4
	$Repl_{(20,183)}$	4040.86	2.04	**0.0075**		14.8
	$Res_{(183)}$	1980.47				68.7
$Strength_{CV^2}$	$Fam_{(19,20.282)}$	5.5233	0.97	0.53	−0.01 ± 0.12	−0.3
	$Repl_{(20,183)}$	5.70133	0.99	0.47		−0.1
	$Res_{(183)}$	5.74104				100.5
$Strength_{range/SD}$	$Fam_{(19,\ 20.124)}$	27.2678	2.06	**0.058**	0.50 ± 0.31	16.5
	$Repl_{(20,183)}$	13.2828	2.25	**0.0026**		17.2
	$Res_{(183)}$	5.8992				66.3
Responsiveness	$Fam_{(19,20.159)}$	12.5726	2.30	**0.036**	0.43 ± 0.29	17.0
	$Repl_{(20,183)}$	5.48892	1.76	**0.028**		11.3
	$Res_{(183)}$	3.11541				71.6

methods for obtaining split-family values to calculate r_g (mean vs. splines) were discordant for preference strength$_{CV^2}$, but here the split-family means may be affected by an outlier (Fig. 3); excluding the outlier yields $r_g = 0.58$, 95% CI = 0.17–0.82. We interpret these results as suggestive of weak G × E, perhaps stronger for responsiveness. Note that the impression of a positive slope in the reaction norms is an artefact of the sorting to calculate r_g (Fig. 4).

Discussion

We tested for genetic variation in female mate preferences using full, individual-level preference functions as the trait of interest. We focused on female mate preferences for male signal frequency in a member of the *E. binotata* complex. We found evidence of genetic variation in mate preference functions. We also found substantial heritability and some plasticity in most of the traits describing variation in the shape of the preference functions, and tentative evidence of weak G × E.

Substantial genetic variation in mate preference functions suggests that they can readily respond to selection, and this included most of aspects of variation in shape. In the *E. binotata* complex, mate preferences for signal frequency have a recent history of rapid divergence, with marked species differences in peak preference (Rodríguez *et al.*, 2006). Against this background, high levels of genetic variation in mate preferences are remarkable – especially for peak preference, the trait that showed the highest heritability but that has the lowest repeatability, plasticity and overall variability (Fowler-Finn & Rodríguez, 2012a,b, unpublished data; Rebar & Rodríguez, unpublished data). We interpret these results as indicating that peak preference does not vary a great deal between individuals, but that a considerable amount of the variation that is present has genetic underpinnings. This points to the action of mechanisms that maintain variation in mate preferences under strong selection and rapid evolution, and underscores the potential for mate preferences to have an important and ongoing role in potential Fisherian selection.

Social and developmental causes of plasticity are confounded in our experiment. The variation in reaction norms that we observed thus represents G × E plus differences in the gradient of conditions experienced by different families. However, because the conditions offered by rearing plants and split-family groupings likely varied only slightly across families (see above), confounding factors likely account for a small fraction of the among-family differences in reaction norms. However, we use caution in interpreting the data, and we take nonparallel reaction norms only as a suggestion of G × E. Based on our r_g estimates, any such G × E is weak, except perhaps for responsiveness. In turn, weak G × E expressed across environments that the treehoppers regularly experience may only have minimally disruptive effects on sexual selection (cf. Greenfield & Rodríguez, 2004). This finding is also in agreement with the prediction that G × E will be weak across environments encountered regularly (Rodríguez, 2012). This follows from the hypothesis that selection can best act on genetic variation in plasticity (G × E) at fine scales of environmental heterogeneity – in contrast, selection loses this ability at broader scales of heterogeneity, wherein G × E may be sustained (Rodríguez, 2012). Our estimates do not test this hypothesis (because we biased them towards weak G × E), but are consistent with it, and we take them as a guideline for future work on the effect of the scale of environmental heterogeneity on the expression of G × E.

In conclusion, preference functions and most aspects of variation in their shape, including rapidly evolving traits, showed a combination of high heritability and low G × E that is consistent with sustained Fisherian selection as a mechanism of sexual selection, and the on-going evolution of mate choice.

Acknowledgments

We thank Flavia Barbosa, Gerlinde Höbel, Christine Miller, Darren Rebar and two anonymous reviewers for helpful comments to the manuscript. We also thank Tom Schuck for logistic support and Mishal Al-Wathiqui for help with playback experiments. Funding was provided by NSF grant IOS–1120790 to RLR and KDFF; by NSF grant IOS–0919962 to RLR; and by UWM SURF fellowships to ACH and RLR.

References

Andersson, M. 1994 *Sexual Selection*. Princeton, Princeton, NJ.

Andersson, M. & Simmons, L.S. 2006. Sexual selection and mate choice. *Trends Ecol. Evol.* **21**: 296–302.

Astles, P.A., Moore, A.J. & Preziosi, R.F. 2006. A comparison of methods to estimate cross–environmental genetic correlations. *J. Evol. Biol.* **19**: 114–122.

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. & Zuk, M. 2008. Acoustic experience shapes female mate choice in field crickets. *Proc. Biol. Sci.* **275**: 2645–2650.

Bakker, T.C.M. 1999. The study of intersexual selection using quantitative genetics. *Behaviour* **136**: 1237–1266.

Bakker, T.C.M. & Pomiankowski, A. 1995. The genetic basis of female mate preferences. *J. Evol. Biol.* **8**: 129–171.

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

Bussière, L.F., Hunt, J., Stölting, K.N., Jennions, M. & Brooks, R. 2008. Mate choice for genetic quality when environments vary: suggestions for empirical progess. *Genetica* **134**: 69–78.

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

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

Chenoweth, S.F. & McGuigan, K. 2010. The genetic basis of sexually selected variation. *Annu. Rev. Ecol. Evol. Syst.* **41**: 81–101.

Clark, C.J. 2012. The role of power versus energy in courtship: what is the 'energetic cost' of a courtship display? *Anim. Behav.* **84**: 269–277.

Cocroft, R.B., Rodríguez, R.L. & Hunt, R.E. 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. In: *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (K. Tilmon, ed.), pp. 88–100. University of California Press, Berkeley, CA.

Cocroft, R.B., Rodríguez, R.L. & Hunt, R.E. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biol. J. Linn. Soc.* **99**: 60–72.

Coyne, J.A. & Orr, H.A. 2004 *Speciation*. Sinauer, Sunderland, MA.

Day, T. & Bonduriansky, R. 2011. A unified approahc to the evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.* **178**: E18–E36.

Delcourt, M., Blows, M.W. & Rundle, H.D. 2010. Quantitative genetics of female mate preferences in an ancestral and a novel environment. *Evolution* **64**: 2758–2766.

Fisher, R.A. 1958 *The Genetical Theory of Natural Selection. A Complete Variorum Edition*. Oxford University Press, New York, NY.

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

Fowler-Finn, K.D. & Rodríguez, R.L. 2012b. The evolution of experience-mediated plasticity in mate preferences. *J. Evol. Biol.* **25**: 1855–1863.

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

Greenfield, M.D. & Rodríguez, R.L. 2004. Genotype-environment interaction and the reliability of mating signals. *Anim. Behav.* **68**: 1461–1468.

Hamilton, K.G.A. & Cocroft, R.B. 2009. Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Entomol. News* **120**: 554–565.

Higginson, A.D. & Reader, T. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. *Proc. Biol. Sci.* **276**: 1153–1159.

Hill, G.E. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol. Lett.* **14**: 625–634.

Hunt, R.E. 1994. Vibrational signals associated with mating behavior in the treehopper *Enchenopa binotata* Say (Hemiptera: Homoptera: Membracidae). *J. NY Entomol. Soc.* **102**: 266–270.

Ingleby, F.C., Hunt, J. & Hosken, D.J. 2010. The role of genotype-by-environment interactions in sexual selection. *J. Evol. Biol.* **23**: 2031–2045.

Jennions, M.D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev.* **72**: 283–327.

Kokko, H. & Heubel, K. 2008. Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica* **132**: 209–216.

Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.

Lehmann, L., Keller, L.F. & Kokko, H. 2007. Mate choice evolution, dominance effects, and the maintenance of genetic variation. *J. Theor. Biol.* **244**: 282–295.

Lynch, M. & Walsh, B. 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

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

Mead, L.S. & Arnold, S.J. 2004. Quantitative genetics models of sexual selection. *Trends Ecol. Evol.* **19**: 264–271.

Meyer, K. & Kirkpatrick, M. 2005. Up hill, down dale: quantitative genetics of curvaceous traits. *Philos. Trans. R. Soc. B Biol. Sci.* **360**: 1443–1455.

Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav. Ecol.* **15**: 1044–1045.

Nakagawa, S. & Cuthill, I.C. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* **82**: 591–605.

Narraway, C., Hunt, J., Wedell, N. & Hosken, D.J. 2010. Genotype-by-environment interactions for female preference. *J. Evol. Biol.* **23**: 2550–2557.

Prum, R.O. 2010. The Lande–Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. *Evolution* **64**: 3085–3100.

Qvarnström, A. 2001. Context-dependent genetic benefits from mate choice. *Trends Ecol. Evol.* **16**: 5–7.

R Development Core Team. 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

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

Ritchie, M.G. 2000. The inheritance of female preference functions in a mate recognition system. *Proc. R. Soc. Lond. B* **267**: 327–332.

Ritchie, M.G. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**: 79–102.

Ritchie, M.G. & Phillips, S.D.F. 1998. The genetics of sexual isolation. In: *Endless Forms. Species and Speciation* (D.J. Howard & S.H. Berlocher, eds), pp. 291–308. Oxford University Press, New York, NY.

Ritchie, M.G., Saarikettu, M. & Hoikkala, A. 2005. Variation, but no covariance, in female preference functions and male song in a natural population of *Drosophila montana*. *Anim. Behav.* **70**: 849–854.

Rodríguez, R.L. 2012. Grain of environment explains variation in strength of genotype × environment interaction. *J. Evol. Biol.* **25**: 1897–1901.

Rodríguez, R.L. & Cocroft, R.B. 2006. Divergence in female duetting signals in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Ethology* **112**: 1231–1238.

Rodríguez, R.L., Sullivan, L.E. & Cocroft, R.B. 2004. Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution* **58**: 571–578.

Rodríguez, R.L., Ramaswamy, K. & Cocroft, R.B. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feedings insects. *Proc. Biol. Sci.* **273**: 2585–2593.

Rodríguez, R.L., Sullivan, L.M., Snyder, R.L. & Cocroft, R.B. 2008. Host shifts and the beginning of signal divergence. *Evolution* **62**: 12–20.

Rodríguez, R.L., Haen, C., Cocroft, R.B. & Fowler-Finn, K.D. 2012. Males adjust signalling effort based on female mate preference cues. *Behav. Ecol.* **23**: 1218–1225.

Roff, D.A. 1997 *Evolutionary Quantitative Genetics*. Chapman & Hall, New York, NY.

Schielzeth, H., Bolund, E. & Forstmeier, W. 2010. Heritability of and early environment effects on variation in mating preferences. *Evolution* **64**: 998–1006.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* **42**: 849–861.

Stinchcombe, J.R., Function-valued Traits Working Group & Kirkpatrick, M. 2012. Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol. Evol.* **27**: 637–647.

Sullivan-Beckers, L. & Cocroft, R.B. 2010. The importance of female choice, male–male competition, and signal transmission as causes of selection on male mating signals. *Evolution* **64**: 3158–3171.

Verzijden, M.N., ten Cate, C., Servedio, M.R., Kozak, G.M., Boughman, J.W. & Svensson, E.I. 2012. The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* **27**: 511–519.

Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 505–522.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**: 155–183.

West-Eberhard, M.J. 2003 *Developmental Plasticity and Evolution*. Oxford University Press, New York, NY.

West-Eberhard, M.J. 2005. Developmental plasticity and the origin of species differences. *Proc. Natl Acad. Sci. USA* **102**: 6543–6549.

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

Wood, T.K. 1993 Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In: *Evolutionary Patterns and Processes* (D.R. Lees, D. Edwards, eds), pp. 299–317. Academic Press, New York, NY.

Zhou, Y., Kuster, H.K., Pettis, J.S., Danka, R.G., Gleason, J.M. & Greenfield, M.D. 2008. Reaction norm variants for male calling song in populations of *Achroia grisella* (Lepidoptera: Pyralidae): toward a resolution of the lek paradox. *Evolution* **62**: 1317–1334.

Data deposited at Dryad: doi: 10.5061/dryad.td18m

Received 1 August 2012; revised 10 October 2012; accepted 25 October 2012