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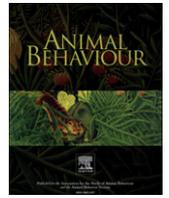
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Repeatability of mate preference functions in *Enchenopa* treehoppers (Hemiptera: Membracidae)

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Patterns of variation in mate preferences provide information about the processes influencing the evolution of mate choice behaviour. We measured variation in female mate preferences using preference functions, which are curves that describe female responses across variation in male signals. Over 3 weeks of testing, we repeatedly measured female *Enchenopa binotata* 'Ptelea' treehoppers (Hemiptera: Membracidae) for their preference for male signal frequency (Hz). We then calculated the consistency of differences in mate preference functions among females (i.e. repeatability) and variability across females for the preference functions. We then measured traits describing different aspects of variation in mate preferences, including peak preference and selectivity. We found repeatability in the overall preference functions, indicating a potential for preference functions to respond to selection. Furthermore, we found substantial repeatability and variability in selectivity traits and low repeatability and variability in peak preference. Finally, we found only weak covariation between peak preference and selectivity. We discuss the implications of our results within the framework of the evolution of mate preferences, both in terms of past selection shaping mate preferences, and the potential for preferences to respond to current selection.

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Mate choice is a strong and prevalent source of selection, a major cause of divergence in sexual traits and an important cause of the evolution of reproductive isolation and the formation of new species (West-Eberhard 1983; Andersson 1994; Hoekstra et al. 2001; Kingsolver et al. 2001; Panhuis et al. 2001; Kraaijeveld et al. 2011). Mate choice arises from patterns of variation in mate preferences and their relationship with variation in mating signals (Ritchie 1996; Jennions & Petrie 1997; Brooks et al. 2005; Rodríguez et al. 2006). Thus, there is much interest in how these traits vary (Jennions & Petrie 1997; Chenoweth & Blows 2006; Cotton et al. 2006; Bell et al. 2009; Chenoweth & McGuigan 2010).

Patterns of variation in mate preferences can offer insight into the processes that influence the evolution of mate choice behaviour (Wagner 1998). For example, the overall variability of a preference gives an indication of its evolutionary history: low variability suggests either recent strong selection that has depleted variation (Rodríguez et al. 2006; Reinhold 2011), or selection favouring environmental insensitivity (Arnold 1992; Flatt 2005). Also, the

consistency of behavioural differences among individuals (i.e. repeatability) gives an indication of the raw material available on which selection can act (Boake 1989). Repeatability in any behaviour may arise from genetic or environmental inputs (Boake 1989; West-Eberhard 1989; Dohm 2002). Both of these causes of repeatability are important for the response of behaviour to selection (Roff 1997; Lynch & Walsh 1998; West-Eberhard 2003, 2005). Considering variability and repeatability together can provide a comprehensive view of recent and likely future responses to selection. Furthermore, these patterns will inform us as to how mate choice will shape the course of sexual selection.

In describing variation in mate preferences, it is important to recognize that they are function-valued traits (Meyer & Kirkpatrick 2005; Stinchcombe et al. 2012). Mate preferences are expressed as a function of variation in mating signals, and therefore a full description of them involves a preference function. Preference functions are curves that describe female behavioural responses across a range of variation in signal traits (Wagner et al. 1995; Ritchie 1996). Most previous work has examined repeatability of mate preferences in terms of single features of these curves, such as the male signal trait having peak attractiveness (reviewed in Bell et al. 2009) or a descriptor of shape (Wagner et al. 1995; Brooks & Endler 2001; Verburg et al. 2008). The preference function

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approach allows assessing variation in all aspects of preference and may provide novel insights into the evolution of mate choice behaviour (cf. Stinchcombe et al. 2012).

Here, we use the function-valued approach of describing behaviour to assess variability and repeatability in mate preference functions. We then assess variation in various aspects of preferences in terms of peak preference (the preferred signal trait value) and three descriptors of the shape of the preference (Fig. 1). These descriptors (responsiveness, tolerance and strength defined in Fig. 1) describe how female response declines as signals deviate from peak preference. They can be summarized into a single composite trait, 'selectivity', that describes how female preference declines as a stimulus deviates from the peak preference (Fowler-Finn & Rodríguez 2012a, b). We also determine correlations between peak preference and preference selectivity traits, because the relationship between these features may influence how mate preferences evolve as well as how individual females contribute to the form of selection on male signals.

Our study species is a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). We are interested in the patterns of variation in mate preferences in *E. binotata* because mate choice plays a major role in the evolution of these insects: rapid speciation has occurred in concert with colonization of novel environments and diversification in sexual communication (Rodríguez et al. 2006, 2012; Cocroft et al. 2008, 2010). Like many phytophagous insects, members of the *E. binotata* complex communicate with plant-borne vibrational signals (Cocroft & Rodríguez 2005; Cocroft et al. 2008). Male advertisement signals consist of a pure tone that sweeps down in frequency from the beginning of the signal; signals begin with a whine portion and end with a series of pulses (Cocroft et al. 2008, 2010). Of the different features of the male signal, female mate choice exerts the strongest selection on signal frequency (i.e. pitch) (Rodríguez et al. 2006; Sullivan-Beckers & Cocroft 2010). *Enchenopa* female mate preferences for male signal frequency are curvilinear in shape: female behavioural response peaks at intermediate values of signal frequency and declines as signal frequency deviates above and below the peak (Rodríguez et al. 2006). Species in the *E. binotata* complex are highly divergent in preference for signal frequency. Furthermore, peak preference for each species corresponds to the average conspecific male signal frequency (Rodríguez et al. 2004, 2006).

We found significant overall repeatability of medium effect size in mate preference functions, low variability and significant but low repeatability in peak preference, and medium variability and significant medium repeatability in responsiveness, tolerance, strength and their combined trait 'selectivity'. Peak preference was weakly correlated with preference selectivity: females that preferred lower-frequency (Hz) signals were more selective. We discuss these results in terms of their implications for past selection on mate preferences, the potential for preferences to respond to current selection and the form of selection on male signals.

METHODS

We used the member of the *E. binotata* complex that grows on *Ptelea trifoliata* (Rutaceae) host plants in Missouri, U.S.A. As with the majority of the members of the complex, this species has not yet been formally described (Hamilton & Cocroft 2009), and we therefore refer to it by the name of its host plant, as follows: *E. binotata* 'Ptelea'. We kept voucher specimens in the collection of the R. L. Rodríguez Laboratory. The insects were collected as late-instar nymphs in the field (Boone County, MO, U.S.A.) in late May 2009 from several aggregations to ensure genetic diversity. We reared the insects on two large potted host plant exemplars in the

University of Wisconsin–Milwaukee greenhouse. The two plants were standardized for size, health and phenology so that each half of the insects experienced nearly identical rearing conditions. When the insects moulted to adults (31 May–4 June 2009), we immediately separated the sexes to ensure that females were sexually naïve. Males do not become sexually mature until 2–3 weeks post-adult moult, and females do not become sexually mature until 2 weeks later (Rodríguez et al. 2004; Cocroft et al. 2008). Thus, we could be sure that no female had mated at the time of experimentation. We then transferred groups of 20 haphazardly selected females each onto two potted host plant exemplars that were similar to the rearing plants.

We started testing individual female preferences 4–5 weeks after the adult moult, when females first become receptive (Rodríguez et al. 2004; Cocroft et al. 2008). We tested every female on three occasions, separated by 1-week intervals. Nineteen females responded in at least two of the three trials and are included in the analysis. We note that being unresponsive for any given trial represents a lack of receptivity on that day, rather than any aspect of the preference function itself. Repeatability has been shown to not vary with the number of tests of behaviour per individual (Bell et al. 2009). We similarly found that repeatability did not vary in any consistent manner across traits between females tested on 2 ($N = 7$) versus 3 ($N = 12$) weeks. Furthermore, variability did not differ in any trait between females tested on 2 versus 3 weeks. Therefore, we pooled data from all females in the analyses.

Assay and Description of Mate Preference Functions

We take advantage of the duetting behaviour that facilitates pair formation in *Enchenopa*: during mate searching, males produce advertisement signals and a receptive female produces a signal in response to the most preferred male signals (Rodríguez et al. 2004, 2006). This allows for a good behavioural assay of female preference across variation in male signals.

Using vibrational playbacks, we presented random sequences of synthetic signals in signal bouts varying in frequency in steps of 20, 50 and 100 Hz in both directions from the mean (385 Hz). This range allowed us to capture the shape of the entire preference function and cover the biologically relevant range of stimuli (Rodríguez & Cocroft 2006). Stimulus bouts were separated by 15 s. Each bout consisted of six signals, which is the mean number of signals/signal bout for *E. binotata* 'Ptelea' (Rodríguez & Cocroft 2006). We used custom script in Matlab v.7.5.0 (Mathworks, Inc., Natick, MA, U.S.A.) to create and deliver stimuli, and we used a piezo-electric controller and actuator (Thorlabs, Newton, NJ, U.S.A.) to impart stimuli to the stem of the plant on which a female was placed for testing. The stimuli were played at an amplitude of 0.30 mm/s.

We recorded female responses by focusing the beam of a laser vibrometer (Polytec CLV 2534; Polytec Inc., Auburn, MA) on a small (ca 2 mm²) piece of reflective tape secured to the plant stem within a few centimetres of the female. Signals from the laser were sent to an iMac computer and were recorded with the sound analysis program Audacity (v.1.2.5; <http://audacity.sourceforge.net/>). We analysed recordings using Audacity (v.1.2.5) by scoring the number of responses per stimulus for each female (i.e. 0–6 responses for each of the seven stimuli tested). In the *E. binotata* complex, a female's likelihood of responding tightly correlates with the number and length of responses (Rodríguez et al. 2004); we therefore used the number of responses as a proxy for an underlying continuous variable describing female preference, which allows us to describe preferences in greater detail than if we looked at the presence/absence of a response. Because each female was presented with the full range of variation in stimuli each week, we

used these responses to generate a full preference function for each individual for each trial.

We described variation in the full preference functions and then measured four aspects of the functions: peak preference, responsiveness, tolerance and strength (Fig. 1). We also calculated a fifth trait, 'selectivity', which is a composite of the three traits responsiveness, tolerance and strength. We measured peak preference from the frequency value eliciting the highest number of responses for a female (Fig. 1). We calculated responsiveness, tolerance and strength of preference separately (see Fig. 1) because they have the potential to evolve independently and therefore represent independent traits with different consequences for the evolution of mate choice. We then used a principal component analysis to summarize responsiveness, tolerance and strength into a single trait corresponding to selectivity that described 91.7% of the variation in the three constituent traits (eigenvalue = 2.8; eigenvectors: responsiveness = 0.58; tolerance = 0.59; strength = -0.56).

Repeatability

To approximate repeatability for the overall preference functions, we used a random-regression linear mixed model that included the linear and quadratic terms for stimulus frequency, testing week, replicate plant (random term) and individual female identity nested within replicate plant (random term). The linear term refers to female response as a linear function of signal frequency, whereas the quadratic term refers to female response as a curvilinear function of signal frequency. The female term indicates variation in the responsiveness of females (Fig. 1). We also included interaction terms for female \times linear stimulus frequency and female \times quadratic stimulus frequency. Because female preference for stimulus frequency is a curvilinear function in the *E. binotata* complex (Rodríguez et al. 2004, 2006), we estimated the

significance of repeatability for the overall preference function with the female \times quadratic stimulus frequency interaction term. We calculated the effect size for this term by converting the *P* value to the standard normal deviate equivalent using the table of *z* values (Cohen 1988), and then converted this value to the effect size ($r = z/\sqrt{N}$) with $N = 19$ females (Rosenthal 1991). We note that this does not correspond to the formal definition of repeatability (Lessels & Boag 1987), but it does provide a sense for the magnitude of consistent individual differences in preference functions.

The above analysis indicated significant repeatability in female preference functions (see Results). We therefore examined patterns of repeatability for five aspects of the preference functions. We tested the significance of the repeatability for each of these preference function traits with linear mixed models (EMS method). In each model, we included week, replicate plant (random term) and female identity nested within replicate (random term). We then used the same models, but implemented with the REML method for improved accuracy of the individual term percentage variance component, which provides the repeatability estimate. This variance component corresponds to repeatability as defined by Lessels & Boag (1987).

Overall Variability

We calculated variability with the among-individual coefficient of variation ($CV = (SD/mean) \times 100$), calculated from individual means for peak preference, responsiveness, tolerance and strength across all weeks. We did not calculate the CV for selectivity because the mean for a principal component is zero and thus the CV calculation amounts to division by zero.

Correlation between Preference Function Shape and Peak Preference

We calculated the correlation between peak preference and the three preference function shape traits as well as the composite trait selectivity. We assessed the significance of the correlations with linear mixed models with peak as the response variable, and week, replicate (random term), each of the other preference traits and female ID (random term) as the independent variables. We then assessed the effect size of the correlation in two ways. First, we calculated the Pearson product-moment correlation of the individual means. We then independently derived the effect size from the *P* value (see above) of the term in the model. We performed all statistical analyses in JMP (v.6.0; SAS Institute, Inc., Cary, NC, U.S.A.).

Ethical note

All experiments complied with the University of Wisconsin–Milwaukee's animal care regulations and with current laws of the United States.

RESULTS

Overall Repeatability of Mate Preference Functions

We found significant repeatability in female mate preference functions (effect size: $r = 0.41$; Table 1, Fig. 2). The significant individual \times quadratic stimulus frequency interaction term indicates consistent differences between females in the overall function shape, and the significant individual term specifically indicates consistent differences in responsiveness (Table 1). We found a significant effect of week, suggesting that responsiveness changed over time (Table 1).

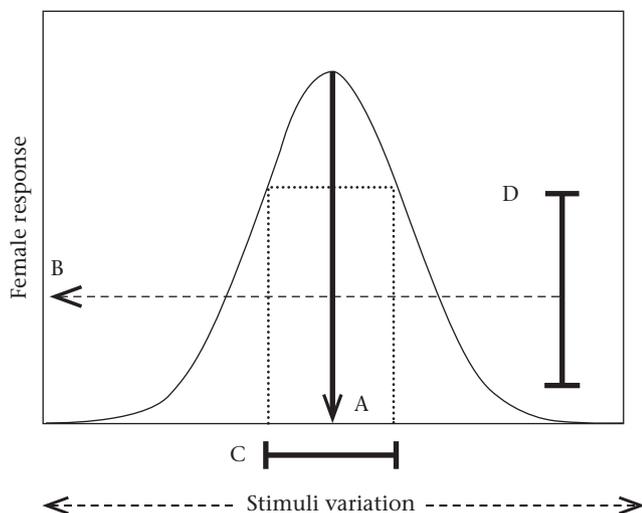


Figure 1. Variation in two main features that describe variation in mate preference functions. **A:** peak preference is the signal value eliciting the highest female response, which is indicated by the high value on each curve. **B–D:** traits that describe how female preference declines as the stimulus deviates from the peak preference; **B–D** can be summarized into a single composite trait, 'selectivity'. **B:** responsiveness is the mean elevation of response across all stimuli in the preference function (Bailey 2008). We measured it as the mean response across all stimuli for which a female was tested (Brooks & Endler 2001; Bailey 2008; Fowler-Finn & Rodríguez 2012a, b). **C:** tolerance describes the amount a male's signal can deviate from a female's peak preference and still be acceptable (McPeck & Gavrilts 2006). We measured it as the width of the preference function at the 33% drop from the highest response. **D:** strength of preference indicates how strongly females disfavour deviations from the peak. We estimated it with the square of the coefficient of variation in responses across the range of stimuli (Schluter 1988). Modified from Fowler-Finn & Rodríguez (2012a, b).

Table 1
Variation in mate preference functions for male signal frequency among *E. binotata* 'Ptelea' females

	df	F	P	Repeatability (r)
Whole model	56, 293	9.80	<0.0001	
Stimulus linear	1	22.12	<0.0001	
Stimulus quadratic	1	116.26	<0.0001	
Week	2	8.59	0.0002	
Replicate	1	0.11	0.7413	
Female (nested in replicate)	17	12.68	<0.0001	
Female×stim linear	17	1.81	0.0263	
Female×stim quadratic	17	1.68	0.0460	0.41

The overall test for significance in the repeatability of preference functions was obtained from the female × quadratic stimulus term (see section on Repeatability in Methods). Statistically significant terms indicated in boldface.

Repeatability and Variability of Preference Function Traits

For peak preference, we found low variability and low and nonsignificant repeatability (Table 2, Fig. 3). Peak preference showed a nonsignificant decrease over testing weeks (Table 2, Fig. 3). We found medium to high variability and significant repeatability of medium to high effect size for responsiveness, tolerance, strength and the composite trait selectivity (Table 2). Over the testing weeks, responsiveness and tolerance increased and strength decreased; this pattern was weak and nonsignificant and corresponded to an overall decrease in selectivity over testing weeks (Table 2, Fig. 3). The effect of week was nonsignificant, suggesting that preference functions traits did not change over time (Table 2).

Correlations between Peak and Selectivity Traits

We found significant relationships between peak and two of the three individual selectivity traits (responsiveness and tolerance; Table 3, Fig. 4): these correlations were positive and weak. Peak showed a nonsignificant negative association with strength (Table 3, Fig. 4). Finally, peak preference was significantly correlated with the composite selectivity trait: the correlation was negative and weak (Table 3), such that more selective females had somewhat lower peak preference (Fig. 4). Selectivity increased as responsiveness and tolerance decreased and strength increased; therefore, across all three components of selectivity, the most selective females preferred the lowest-frequency signals (Fig. 4).

DISCUSSION

We assessed variation in female mate preference functions for male signal frequency in a member of the *E. binotata* complex of treehoppers. When we considered the entire preference function as

Table 2
Variability and repeatability in descriptors of mate preference functions of *E. binotata* 'Ptelea' females

	df	F	P	Repeatability (r)	Variability (CV)
Peak				0.12	4.9
Whole model	20, 29	1.3	0.2459		
Week	2	2.2	0.1305		
Replicate	1	0.5	0.4977		
Female (within replicate)	17	1.2	0.3146		
Responsiveness				0.54	57.5
Whole model	29, 49	3.6	0.0009		
Week	2	2.4	0.1069		
Replicate	1	0.2	0.6514		
Female (within replicate)	17	3.8	0.0008		
Tolerance				0.46	47.8
Whole model	29, 49	2.9	0.0044		
Week	2	2.1	0.1456		
Replicate	1	0.2	0.6490		
Female (within replicate)	17	3.0	0.0043		
Strength				0.46	71.0
Whole model	29, 49	2.7	0.0076		
Week	2	1.1	0.3405		
Replicate	1	0.05	0.8225		
Female (within replicate)	17	2.9	0.0052		
Selectivity				0.51	
Whole model	20, 29	3.3	0.0019		
Week	2	2.0	0.1472		
Replicate	1	0.16	0.6945		
Female (within replicate)	17	3.4	0.0017		

We examined peak preference, three traits describing shape (responsiveness, tolerance and strength), and a single composite trait describing shape (selectivity; see Fig. 1). Statistically significant terms indicated in boldface.

the trait of interest, we found significant repeatability of medium effect size, indicating substantial consistent among-individual variation in preference functions. We then assessed repeatability and population-level variability of five preference function traits: peak preference, responsiveness, tolerance, strength and the composite trait 'selectivity'. We found low repeatability and variability in peak preference, and medium to high repeatability and variability in selectivity and its components.

Low repeatability in peak preference suggests that if selection were to favour a different peak preference, the response to selection would be weak. By contrast, high repeatability in responsiveness, tolerance, strength and selectivity suggests a high potential for selectivity traits to respond to selection. Thus, the relatively high effect size that we found for the repeatability of the overall preference functions likely reflects variation among females largely in the shape of the preferences rather than in peak preference (Fig. 2). The presence of repeatable variation in shape but not peak

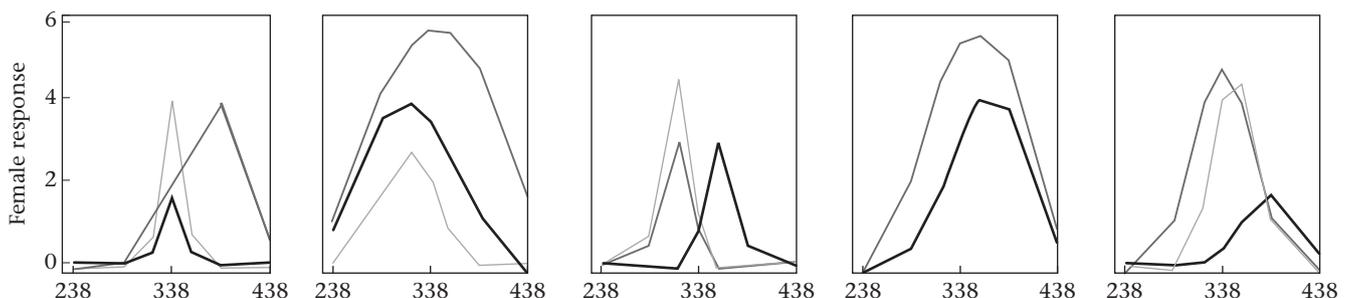


Figure 2. Example of between- and within-individual variation in mate preference functions in *E. binotata* 'Ptelea' females. Each panel shows the preference functions obtained for a single female over 3 successive weeks: Week 1 (—); Week 2 (---); Week 3 (---). The individual with two functions responded on only 2 weeks of testing.

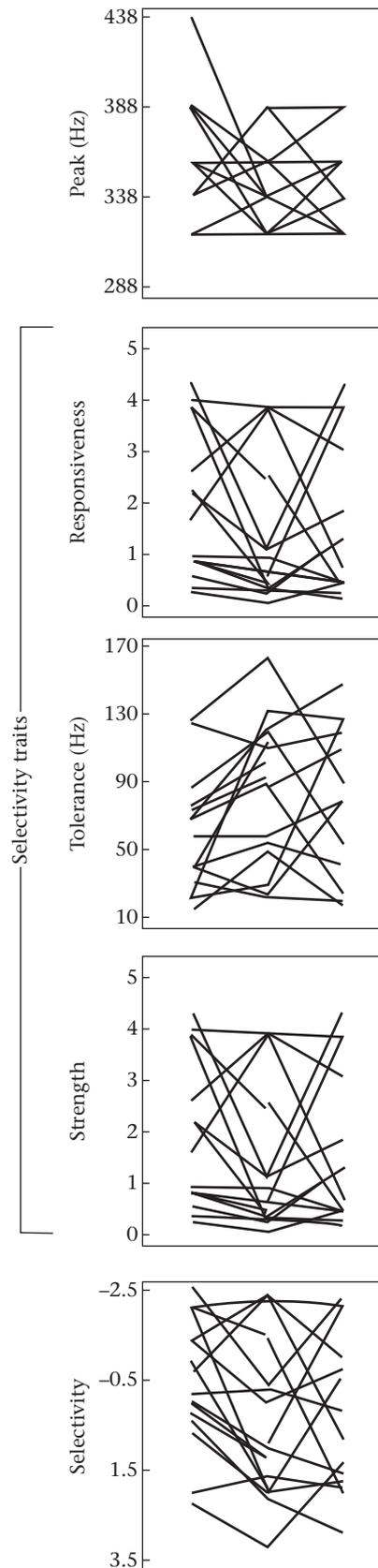


Figure 3. Variation in aspects of mate preference functions in *E. binotata* 'Ptelea' females over 3 weeks of testing ($N = 19$ females). Specifically shown are peak preference and selectivity, as well as the three constituent components of selectivity. Grey lines represent the values for each individual female; black lines represent the mean value across all females tested within a given week. Lines that appear to be discontinuous are females that were responsive in 2 out of 3 testing weeks.

Table 3

Estimating the correlation of peak preference with responsiveness, tolerance, strength and the composite trait 'selectivity' for female *E. binotata* 'Ptelea'

	<i>df</i>	<i>F</i>	<i>P</i>	Correlation (<i>r</i>)	Effect size (<i>r</i>) of relationship (from <i>P</i> value of term)
Responsiveness				0.27	0.45
Whole model	28, 49	1.7	0.0919		
Week	2	4.3	0.0229		
Replicate	1	0.13	0.7238		
Female	17	1.4	0.2059		
Responsiveness	1	5.5	0.0260		
Tolerance				0.29	0.52
Whole model	28, 49	1.9	0.0617		
Week	2	4.8	0.0163		
Replicate	1	0.13	0.7251		
Female	17	1.5	0.1695		
Tolerance	1	7.2	0.0119		
Strength				-0.18	-0.33
Whole model	28, 49	1.5	0.1491		
Week	2	3.3	0.0521		
Replicate	1	0.31	0.5829		
Female	17	1.4	0.2208		
Strength	1	3.4	0.0746		
Selectivity				0.26	0.47
Whole model	21, 48	1.8	0.0799		
Week	2	4.4	0.0216		
Replicate	1	0.16	0.6954		
Female ID	17	1.5	0.1743		
Selectivity	1	6.1	0.0196		

Statistically significant terms indicated in boldface.

preference is consistent with the observation of recent and rapid divergence in peak preference, with apparent lack of change in selectivity traits, across the *E. binotata* complex (Rodríguez et al. 2006). We interpret this in terms of the action of recent selection because the observed rapid divergence in peak preference argues against strong canalization or constraints. Accordingly, high variability in the selectivity traits and lower variability in peak preference suggest that recent selection has been stronger on peak preference and weaker on selectivity.

Our results suggest that the recent evolution of female mate preferences for male signal frequency involved divergent selection arising from changes among species in the signal frequency that females are selected to favour. Such changes may involve various mechanisms, such as Fisherian selection (West-Eberhard 1983; Mead & Arnold 2004; Prum 2010) or benefits of mate choice related to male condition or ecological performance (Lorch et al. 2003; Rundle & Nosil 2005; Ribak et al. 2009). Because speciation in the *E. binotata* complex, as in many plant-feeding insects, is closely tied to shifts in host plant species (Cocroft & Rodríguez 2005; Cocroft et al. 2008, 2010), an interaction between ecological and sexual selection is likely to be involved in divergence in mate preferences (Maan & Seehausen 2011). By contrast, divergent selection on selectivity, which appears to have been a weaker factor in the history of the complex, should arise from changes in the patterns of variation in the potential mate types available (Real 1990; Getty 1995; Jennions & Petrie 1997; McPeck & Gavrillets 2006). Interestingly, we have additional evidence for *E. binotata* 'Ptelea' and another member of the complex that plasticity in mate preference selectivity has evolved under selection related to the presence/absence of preferred mates and mate variability (Fowler-Finn & Rodríguez 2012a, b). Thus, some sources of selection appear to have favoured changes in peak preference during speciation in

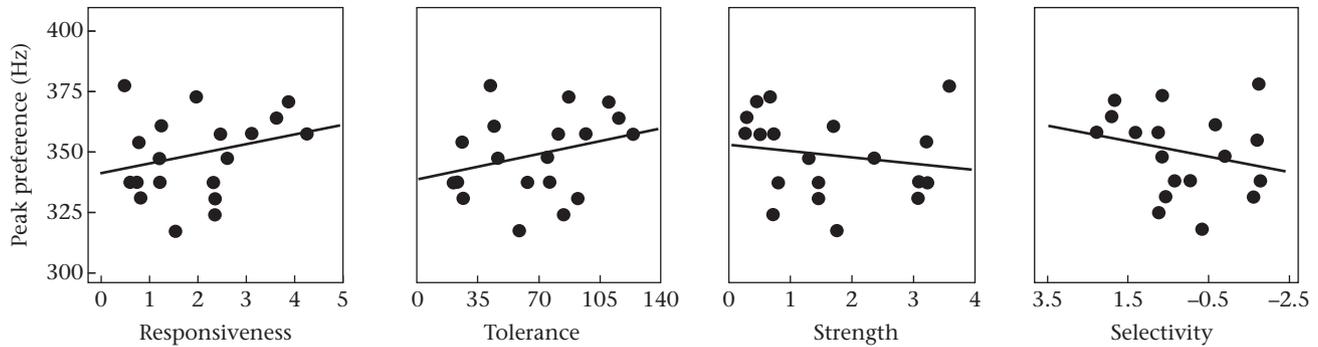


Figure 4. Relationship between peak preference and three traits describing the preference function shape (responsiveness, tolerance and strength) as well as their composite trait, selectivity, in *E. binotata* 'Ptelea' females. Data points correspond to individual means for each female across the testing weeks.

the complex, while other sources of selection may have favoured plasticity in preference selectivity.

Implications for Selection on Male Signals

Selection on male signals will arise from the relationship between patterns of variation in mate preferences and in mating signals (Ritchie 1996; Brooks et al. 2005; Bentsen et al. 2006; Rodríguez et al. 2006; Sullivan-Beckers & Coccoft 2010). Because peak is repeatable with low variability, selection on signal frequency should predominantly follow the mean peak preference of the population and be relatively consistent across the mating season. Male signal frequency in this species shows significant repeatability ($r = 0.33$) over a time span comparable to our trials for preferences (Sattman & Coccoft 2003), so selection stemming from mate choice should favour the same males throughout the season. However, large and repeatable differences among females in the selectivity traits suggests that some females might contribute to population-level selection on male sexual displays more than others. Given that females with lower peak preferences are (1) less likely to respond, (2) less tolerant of male signals deviating from their peak preference and (3) more likely to have strong preferences, we might expect stronger selection from females preferring lower-frequency signals. Note, however, that these correlations are weak. Overall, the predicted form of selection on male signal frequency should be stabilizing (because there is a match between signal preference and mean signal frequency; Rodríguez et al. 2006) with the potential for the strength of selection to vary across signal frequencies.

Conclusion

We used a function-valued approach to assess patterns of variation in mate preferences in terms of the overall preference function. Repeatability in mate preference functions indicates the ability of preferences to respond to selection. Our measures of variability and repeatability for peak preference and preference selectivity traits suggest that peak preference has a much lower potential to respond to changes in selection than does selectivity, and recent selection has likely been much stronger for peak preference than for selectivity. Against the background patterns of signal and preference diversity in *E. binotata* complex (Rodríguez et al. 2006; Fowler-Finn & Rodríguez 2012a, b), we infer that selection influencing peak preference has likely been divergent among species, but stabilizing within species. In contrast, selection influencing selectivity traits appears to have favoured the persistence of similar levels of variation across species with fine-tuning of

choice via adaptive plasticity (Rodríguez et al. 2006; Fowler-Finn & Rodríguez 2012a, b).

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