

GENETIC VARIANCE AND PHENOTYPIC PLASTICITY IN A COMPONENT OF FEMALE MATE CHOICE IN AN ULTRASONIC MOTH

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Abstract.—Female response to male advertisement signals in lesser waxmoths showed substantial genetic variation, phenotypic plasticity across rearing environments, and genotype-by-environment interactions resulting in crossing reaction norms. These results represent two previously underemphasized means by which genetic variation may be maintained in sexually selected traits: genetic variation in female response to male traits, and variation in the selection acting on both males and females. Genotype-by-environment interactions and reaction norms that cross indicate that divergent selection may act on male and female sexual traits if the level of environmental change is high. The processes that contribute to the maintenance of genetic variation may thus also contribute to population differentiation.

Key words.—*Achroia grisella*, cross-environmental genetic correlation, insect communication, Lepidoptera, Pyralidae.

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Female choice exerts strong and continuous selection on male sexual traits (Darwin 1871; West-Eberhard 1983). This process is expected to reduce genetic variation in male sexual traits and, consequently, in female preferences for them (Taylor and Williams 1982; Charlesworth 1987; Arnold 1994). Nevertheless, considerable genetic variation exists in sexual and life-history traits (Charlesworth 1987; Mousseau and Roff 1987; Houle 1992, 1998; Bakker and Pomiankowski 1995; Kruuk et al. 2000; Merilä and Sheldon 2000). This conundrum has linked the study of sexual selection to the area of evolutionary biology that analyzes the forces that contribute to the maintenance of genetic variation (e.g. Lewontin 1974; Roff 1997). Genetic variation may be maintained under selection by the influences of mutation/selection balance, environmental heterogeneity in conjunction with strong genotype-by-environment interaction (GEI), antagonistic pleiotropy, epistasis, condition-dependent trait expression, disruptive selection, cyclical selection, and exponential fitness increase with trait expression (e.g., Eshel and Hamilton 1984; Hamilton 1986; Charlesworth 1987; Pomiankowski and Møller 1995; Rowe and Houle 1996; Roff 1997; Brodie 2000; Kelly 2000; Kassen 2002). Several of these factors have been shown to operate on male sexual traits in various animals, including mutation/selection balance, GEI, antagonistic pleiotropy, condition-dependent trait expression, and disruptive selection (e.g., Eberhard 1980; Clutton-Brock et al. 1982; Charlesworth 1987; Andersson 1994; Emlen 1997; Jia and Greenfield 1997; Gray and Cade 1999a; Doty and Welch 2001; Jennions et al. 2001; Kotiaho et al. 2001; Brandt 2002).

When phenotypic plasticity is present, the interaction between genotype and environment determines the various phenotypes produced by a genotype in different environments, or the reaction norm (Falconer 1952; Via and Lande 1985; West-Eberhard 1989; Schlichting and Pigliucci 1998). The reaction norms of different genotypes may cross such that

the genotype with higher values in one environment has values lower than other genotypes in other environments (Roff 1997), with the consequence that the relative fitness ranking of genotypes may change across environments (Gillespie and Turelli 1989; Fry et al. 1996). Selection may thus favor different genotypes in different environments, and overall genetic variation can be maintained if there is migration between populations in different environments or if there is generation overlap in the case of temporal variation; at the very least the response to selection will be slowed down (Via and Lande 1987; Gillespie and Turelli 1989; Fry et al. 1996).

Genotype-by-environment and crossover interactions have been demonstrated in male sexual and life-history traits in lesser waxmoths, *Achroia grisella* F. (Lepidoptera: Pyralidae; Jia et al. 2000). Lesser waxmoths are widespread honeybee symbionts that employ ultrasound for sexual communication (Greenfield and Coffelt 1983; Jang and Greenfield 1996, 1998). Males produce sexual advertisement calls by fanning their wings and striking tymbals on their thorax to generate pairs of short (100 μ sec) pulses of broadband ultrasound (70–130 kHz). Females orient toward these calls and prefer high values for pulse rate (pulses-sec⁻¹) and amplitude, thus exerting directional selection on these traits (Jang and Greenfield 1996, 1998). There is nevertheless substantial additive genetic variance in these call traits, which have genetic correlations with life-history traits (Collins et al. 1999). Contributions to the maintenance of genetic variation in these traits may be made by the differential expression of male call and life-history traits in different environments experienced by larvae (Jia and Greenfield 1997), and by GEIs in which attractive genetic variants produce unattractive males in some environments, such that there are crossover interactions in reaction norms (Jia et al. 2000).

These findings raise the question whether similar influences act on female sexual traits. Variation in female choice has been verified in a number of species, and some of it has been shown to be caused by genetic variance (e.g., Andersson 1994; Hollocher et al. 1997; Bakker et al. 1999; Gray and Cade 1999b; Lesna and Sabelis 1999; Jang and Greenfield 2000; Qvarnström et al. 2000; Jiggins et al. 2001). Previous

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studies in lesser waxmoths have documented genetic variation in the relative weight that females place on different male signal characters when assessing mates (Jang and Greenfield 2000).

Here we show the presence of variation, genetic variance, phenotypic plasticity, and GEIs resulting in crossover interactions in a component of female choice in lesser waxmoths. Female lesser waxmoths have absolute values of male pulse rate below which calls are not attractive (Brandt 2002), which we term pulse rate thresholds. Thresholds of response are usually thought to optimize signal recognition by minimizing false acceptances and rejections (Dusenbery 1992; Sherman et al. 1997; Bradbury and Vehrencamp 1998), and pulse rate thresholds in lesser waxmoths influence recognition of sound stimuli as conspecifics or predatory bats. Stimuli with short pulses and high pulse rates are recognized as males, whereas stimuli with long pulses and low rates are recognized as bats (Greenfield and Weber 2000; Brandt 2002; Rodríguez 2002). Another conceivable function of these thresholds, avoidance of mismating with heterospecifics, has probably not been important in lesser waxmoths. Their natural history and geographic distribution does not expose them to many encounters with other ultrasonic insects (Greenfield and Coffelt 1983; for information on distributions of ultrasonic moths, see Conner 1999). Furthermore, receiver traits that function mainly in mate recognition tend to have “closed” preference functions (i.e., exert stabilizing selection on the signal trait), whereas receiver traits that function mainly in mate choice tend to have “open-ended” preference functions (i.e., exert directional selection; Gerhardt 1991; Gerhardt and Huber 2002). Pulse rate thresholds show repeatable variation among females ($r = 0.83$) and exert directional selection on male pulse rate: females with higher thresholds recognize a smaller proportion of the male population as potential mates, and only 30% of the males are found acceptable to all the females within the population from which our individuals were drawn (Brandt 2002; Rodríguez 2002). Variation in pulse rate thresholds thus reflects variation in the selection exerted on males.

We conducted a breeding experiment to evaluate genetic variation and phenotypic plasticity in the pulse rate thresholds of female lesser waxmoths, and to test for GEIs and crossover interactions. We used a full-sib, split-family design (Roff 1997) with two rearing temperatures as environmental treatments. This design cannot tease apart nonadditive genetic effects (Roff 1997, 1998), so we estimated broad-sense heritability (H^2), the proportion of phenotypic variance attributable to additive and nonadditive genetic variance (Roff 1997). A negative correlation between male size and pulse rate (Collins et al. 1999; Brandt 2002) led us to ask whether a correlation exists between female size and pulse rate thresholds. If so, variation in body size might provide a connection between variation in male signaling and female response. A correlation between body size and receiver response may be expected because of the potential influence of size on the sensitivity of ears. For example, individuals with larger ears have higher amplitude sensitivity in some insects (Bailey 1998). We therefore measured body and ear size, and larval development time, and tested for genetic and phenotypic correlations between these variables and pulse rate thresholds.

MATERIALS AND METHODS

Moths came from a captive colony established with over 200 individuals collected on abandoned beeswax in Lawrence, Kansas, in 1999. At the time of the experiments the population had been in the lab for approximately 12 generations, and the experiments were conducted with generations 12–13. The colony was maintained with a protocol that minimizes inbreeding. Larvae were fed a diet of flour, glycerol, brewer’s yeast, beeswax, honey and water (modified from Dutky et al. 1962), and kept on a 12:12 h light:dark cycle.

Experimental Design

We created 20 full-sib families by pairing 20 randomly chosen males and females. Each individual mated only once. We split their brood into two larval rearing temperatures (25°C or 22°C), chosen on the basis of a preliminary experiment (Rodríguez 2002). This experiment revealed no effect of food availability on pulse rate thresholds, but suggested the above rearing temperatures as the most likely to influence pulse rate thresholds. These temperatures were among the environments that resulted in GEIs and crossover interactions in male pulse rate (Jia et al. 2000). They also are within the range likely to be experienced on daily to annual scales by lesser waxmoth populations on honeybee colonies, abandoned hives, and the surrounding debris (Winston 1987). These changes in environmental conditions will thus be experienced at least every few generations (generation time = 5–10 weeks) and probably more often.

For each family, we placed 100 eggs in each temperature treatment. Food availability was 60 g of diet for 100 eggs, corresponding to established laboratory rearing procedure (Jang and Greenfield 1996). Of 20 families, there were four in which females laid too few eggs (from zero to <200 eggs) to be divided into the two rearing conditions. Our sample was thus reduced to 16 families. None of the pupae of one of these families eclosed at 22°C, so our sample was reduced to 15 families for that environment.

We isolated pupae in individual plastic cups to ensure that adults were sexually naive and responsive to playbacks of male calls. Experiments were performed on one-day-old virgin females. We measured 4–15 females per family per rearing environment (median = 12, mode = 14 females). Variation in sample size was due to the low percentage of adult eclosion in some families at 22°C. To sample females over the full range of eclosion times in each family, we staggered sampling by randomly choosing no more than two of the females that eclosed in a single day for each family, except during peak eclosion when many females eclosed at the same time and we randomly chose up to four females per family.

We conducted playback experiments during the first five hours of the scotophase in a 3.5 × 3.5 × 2.5-m semianechoic room. We kept test animals in the conditions in which they had been reared until shortly before the experiments, acclimatizing them to the conditions of the sound chamber (25°C) for at least 15 min. This time interval is sufficient for acclimatization because even moths cooled to 5°C for one hour recovered full motility and flight capability in <5 min. We thus ensured that differences in observed response among treatments reflected the effect of family and rearing environ-

ment, not the effect of bringing the moths into the chamber. We tested at 25°C to relate our results to previous work, in which that temperature has been the standard recording and testing temperature, and to eliminate the effect of temperature coupling (Walker 1957; Greenfield 2002) on female responses.

Signal Generation and Playback

We measured pulse rate thresholds with single-speaker broadcasts of a prerecorded male call modified digitally to vary in pulse rate. We recorded a calling male with a condenser microphone (ACO Pacifica Inc. 7016, Belmont, CA; frequency response ± 6 dB from 10 Hz to 160 kHz) fitted to a preamplifier (ACO model 4012). We transferred the signal to a 486 computer (66 MHz cpu) and used custom-designed software to generate playbacks and vary their pulse rate.

We placed females at the center of a screen arena (80-cm radius), 60 cm from a speaker outside. We broadcast playbacks with an Ultrasound Advice (London) S56 loudspeaker (frequency response ± 6 dB from 10 Hz to 200 kHz), amplified by an Ultra Sound Advice S55 amplifier (frequency response ± 3 dB from 18 Hz to 300 kHz). Recordings of our playbacks showed they had 0.1-msec pulses and a concentration of energy between 70–87 kHz (frequency cutoffs 6 dB below maximum energy), thus matching the mean values of these traits (Jang and Greenfield 1996). The peak amplitude of our playbacks was 86 dB SPL (0 dB = 20 μ Pa), calibrated with the peak equivalent method at the female's position in the arena. This amplitude is the mean of the population from which our moths were drawn, measured 10 cm away from the male (86 dB SPL, range = 73–92 dB SPL, $N = 25$).

Our playbacks had pulse rates spanning the range of male calls in the population (30–130 sec^{-1} in increments of 10 sec^{-1}), and were presented in random order until we had identified the lowest pulse rate to which the females responded with positive phonotaxis: the pulse rate threshold. If in the sequence of playbacks we had not done so, we checked that a randomly chosen higher rate also elicited phonotaxis to ensure that our measure was a threshold, and that the females had not habituated to the test stimuli. For each playback (each pulse rate), females were given 1 min to begin movement and an additional 1 min to complete phonotaxis. All females that responded started within 50 sec, some as soon as 1 sec. Time between exposure to different playbacks was at least 30 min. We put each female through a random sequence of playbacks only once. We checked for a possible effect of playback order in a preliminary study by testing for a correlation between the pulse rate of the first playback we presented to a female and her threshold. With habituation there should be a positive correlation; with sensitization, a negative one. We found no correlation (Pearson's product-moment correlation coefficient, $r = 0.02$, $P = 0.77$, $N = 148$; power ≥ 0.95 for $r \geq 0.29$), and concluded that our measures were not influenced by playback order. We noted the development time (interval in days between egg to eclosion from the pupa) of each female for which we obtained pulse rate thresholds.

To take the morphological measurements, we preserved females in 70% ethanol and later measured in the preserved moths the length of the right mid femur as an estimate of body size and the width of the right ear at the broadest point as an estimate of ear size. We used an optical grid attached to a dissecting microscope. These measurements involved sclerotized structures (femur) or structures enclosed by sclerotized tissue (ear), so they are not likely to have been affected by hardening of membranes during storage in ethanol. Femur length is a reliable predictor of body size (regression of \log_{10} -transformed femur length on \log_{10} of the cubic root of mass for one-day-old females: $R^2 = 0.68$, slope = 0.90, $F_{1,12} = 28.0$, $P < 0.001$).

Data Analysis

Our analysis had two aims: to estimate genetic variance and heritability, and to test for GEIs and crossover interactions. Different methods are best suited for these goals (Fry 1992). We tested the effects of family and rearing environment with mixed-model ANOVAs (Sokal and Rohlf 1995) performed with the general linear model, which allowed for an unbalanced design. Family was a random factor and environment was a fixed effect. This method corresponds to the Scheffé model in Fry (1992), in which the effect of family is tested by $F = MS_{\text{fam}}/MS_{\text{error}}$. When we found a significant effect of family, we calculated heritability and its standard error for a full-sib design with unequal family sizes (Roff 1997). We present two heritability estimates for pulse rate thresholds: one calculated from the mixed-model ANOVA, and one calculated from one-way ANOVAs performed separately for each rearing environment. We obtained these separate estimates because of the potentially confounding influence of a GEI that we detected.

Analyses of variance were performed on untransformed data when homogeneity of variances was present. Ear width and development time showed heteroscedasticity among rearing environments (and homoscedasticity among families). Various transformations did not achieve homoscedasticity in ear width, and we performed the ANOVA on the untransformed data because the bias introduced by heteroscedasticity is likely to be small: The group with the higher mean had the smaller variance, so that the detected effect of environment is unlikely to be spurious. A \log_{10} transformation reduced (but did not eliminate) heteroscedasticity in development time (see Fig. 2), and we performed an ANOVA on the transformed data. We calculated the power ($1 - \beta$) of tests that yielded nonsignificant results according to Sokal and Rohlf (1995) and Zar (1999). When calculating the power of a test for the difference found was impractical because $MS_{\text{within groups}} > MS_{\text{among groups}}$, we state the minimum effect that our test had the power to detect, calculated according to Rohlf and Sokal (1995).

To estimate GEIs, we first tested for family-by-rearing-environment interactions with the mixed-model ANOVA. If a significant interaction was detected, we evaluated GEI formally by calculating the cross-environment genetic correlation (r_g), which describes the GEI as the correlation between two genetically correlated traits expressed in two environ-

TABLE 1. Descriptive statistics and H^2 estimates for the traits measured in female lesser waxmoths, *Achroia grisella*. All traits show significant effects of family (see Tables 2–4), and substantial H^2 estimates. We present two estimates of H^2 for pulse rate thresholds: one estimated from the mixed-model ANOVA in Table 4 and one estimated from the one-way ANOVA for females reared at 25°C in Table 5.

	Femur length (mm)	Ear width (mm)	Pulse rate thresholds (pulse-sec ⁻¹)	Development time (days)
Mean	2.25	0.57	69.3	51.4
Variance	0.0101	0.00191	829.6	69.5
$H^2 \pm SE$	0.48 \pm 0.16	0.29 \pm 0.12	0.22 \pm 0.13 ¹ 0.40 \pm 0.17 ²	0.13 \pm 0.07

¹ Estimated from mixed-model ANOVA.

² Estimated from one-way ANOVA with females reared at 25°C.

ments (Falconer 1952). We used the SAS model of Fry (1992), thus:

$$r_g = \frac{MS_{fam}}{MS_{fam} + MS_{interaction}}$$

Crossover interactions in reaction norms are more likely when r_g values are $\ll 1$ (Fry 1992; Fry et al. 1996), but the only definitive assessment of crossover is by actual inspection of reaction norms. We employed Student’s t -tests to ask whether our calculated r_g was different from zero and < 1 (Sokal and Rohlf 1995). We then compared the number of reaction norms that crossed with the total number of possible comparisons between reaction norms.

We estimated genetic correlations between traits for a full-sib design according to Roff (1997) by calculating Pearson’s product-moment correlation coefficient for mean trait values for each family. We estimated phenotypic correlations between traits with Pearson’s product-moment correlation coefficient for individual trait values. We used untransformed data.

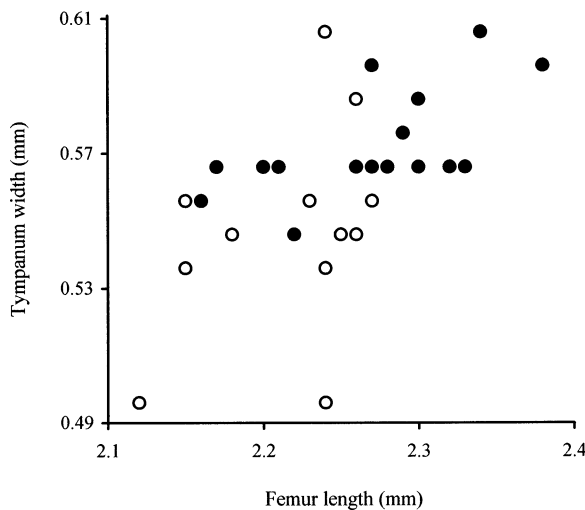


FIG. 1. Body and ear size in 15 full-sib families split into two rearing temperatures in female lesser waxmoths, *Achroia grisella*. Mean family values for femur length and ear width are shown. Solid circles indicate families reared at 22°C; open circles indicate families reared at 25°C.

RESULTS

A summary of overall descriptive statistics and H^2 estimates is presented in Table 1. Our analysis revealed strong effects of family and rearing environment on body and ear size and development time. Moths reared in the warmer environment were smaller, had smaller ears, and developed more quickly (Fig. 1; Table 2). From these ANOVAs (Tables 2 and 3), we estimated the heritability of femur length, ear width, and development time (Table 1). There was no interaction between the effects of family and rearing environment in any of these traits (Fig. 2A–C; Tables 2, 3).

The results for pulse rate thresholds were different. Family had a significant effect, rearing environment did not, and there was a significant interaction between the effects of family and rearing environment (Fig. 2D; Table 4). Thus, the effects of family and rearing environment on pulse rate thresholds depended on each other. We report an H^2 estimate based on this mixed-model ANOVA (Table 4) in Table 1. To eliminate the confounding influence of the family-by-rearing-environment interaction on our H^2 estimates, we performed one-way ANOVAs separately for each rearing environment. Family had a significant effect for females reared at 25°C (Fig. 3; Table 5), but not for females reared at 22°C (Table 5). Using the data of females reared at 25°C (Table 5), we estimated H^2 for pulse rate threshold in that environment (Table 1).

The family-by-rearing interaction detected for pulse rate thresholds suggested GEIs. We thus calculated r_g from the variance components of the mixed-model ANOVA (Table 4): $r_g = 0.53$. This estimate was significantly different from zero (two-tailed Student’s t -test, $t_\infty = 2.04$, $P < 0.05$) and significantly lower than one (one-tailed Student’s t -test, $N = 15$, $t = -1.96$, $P < 0.05$). This value of r_g is consistent with crossover interactions in reaction norms, but does not demonstrate them. Nevertheless, visual inspection of Fig. 2D reveals 88 reaction norms that crossed of a total of 105 possible combinations of the 15 reaction norms.

Inspection of Fig. 2D suggests that the GEI we detected may be due in large part to two families that showed markedly higher pulse rate thresholds at 25°C. Deleting these two families from the mixed-model ANOVA removed the effect of family and the effect of the interaction between family and rearing temperature ($F_{12,280} = 1.25$, $P > 0.10$; $F_{12,280} = 0.64$, $P > 0.75$, respectively), and an effect of temperature appeared

TABLE 2. Mixed-model ANOVA testing for the effect of family and rearing environment on body size (femur length) and ear size (ear width) in female lesser waxmoths. Both factors had significant effects, and there was no interaction between them.

	Femur length					Ear width				
	df	SS	MS	<i>F</i>	<i>P</i>	df	SS	MS	<i>F</i>	<i>P</i>
Family	15	0.73	0.049	6.86	<0.001	15	0.097	0.0064	4.25	<0.001
Environment	1	0.078	0.078	7.73	0.013	1	0.023	0.023	10.84	0.004
Family × environment	15	0.16	0.01	1.46	0.12	15	0.032	0.0021	1.4	0.15
Error	270	1.92	0.007			274	0.42	0.0015		
Total	301					305				

($F_{1,12} = 6.10$, $P = 0.03$). The detection of a GEI is thus dependent on those two families.

Analysis of the morphological data met the expectation of a phenotypic correlation between the size measures and development time. Overall, there was a positive correlation between femur length and ear width ($r = 0.50$, $P < 0.001$, $N = 302$, significant after Bonferroni correction for multiple tests performed on the same dataset), and both size measures were positively correlated with development time (femur length: $r = 0.29$, $P < 0.001$, $N = 302$; ear width: $r = 0.27$, $P < 0.001$, $N = 306$; both tests significant after the Bonferroni correction). But pulse rate thresholds were not correlated with femur length, ear width, or development time ($r = 0.02$, $P = 0.71$, $N = 302$; $r = -0.06$, $P = 0.33$, $N = 306$; $r = 0.07$, $P = 0.19$, $N = 352$, respectively; power was ≥ 0.95 for $r \geq 0.19$ – 0.21). We then examined phenotypic and genetic correlations separately for each temperature. For the phenotypic correlations, the pattern remained as above: the size measures and development time were positively correlated, but some of the correlations were nonsignificant after the Bonferroni correction (Table 6). We only detected a genetic correlation for femur length and ear width at 22°C, but power was low for these tests (Table 6).

DISCUSSION

Variation in four traits in female lesser waxmoths was to a large extent explained by the influence of family, suggesting substantial genetic variation. It is generally expected that heritability will be higher for morphological than behavioral traits, and that these will have higher heritabilities than life-history traits (Roff 1997). But our estimates of H^2 were comparable for body size, ear size, and pulse rate thresholds (Table 1). The life-history trait (development time) did have lower heritability. These findings are consistent with numerous studies that have verified the existence of genetic variation in sexual and life-history traits (Charlesworth 1987; Mousseau and Roff 1987; Houle 1992, 1998; Bakker and Pomiankowski 1995; Kruuk et al. 2000; Merilä and Sheldon

2000). The level of genetic variation was also comparable between male pulse rate, with h^2 estimates of 0.40–0.46 (Collins et al. 1999), and female pulse rate thresholds, with H^2 estimates of 0.22–0.40 (this study).

There are of course limitations of employing H^2 , because it cannot tease additive and nonadditive effects apart (Roff 1997, 1998). However, we expect that these limitations are not severe in our data. Dominance variance is expected to be low for behavioral traits (Roff 1997). Although we cannot rule out maternal effects, at least one is unlikely. There probably was no differential egg provisioning due to differences in female feeding rates (Lynch and Walsh 1998), because adult lesser waxmoths do not feed or drink (Greenfield and Coffelt 1983), so they eclose from their pupae with all the resources they will have available, and we used 1-day-old dams for our experiments. Finally, pulse rate thresholds had no genetic or phenotypic correlations with body or ear size or with development time (a correlation might have provided an indirect avenue of influence on pulse rate thresholds). Thus, our estimates should approximate h^2 .

The component of female choice reflected by pulse rate thresholds is therefore as capable of responding to selection as the male trait. The question of why females choose between males when they do not provide direct benefits is thus answered on two fronts: genetic variation in male call features and correlated life-history traits (Collins et al. 1999) indicates important indirect genetic benefits of female mate choice. Furthermore, genetic variation in female responses to male calls (Jang and Greenfield 2000; this study) provides the material for selection related to indirect benefits of mate choice to operate on females.

Our results also address the existence of genetic variation in male sexual traits: genetic variation in female response implies variation in the strength of the selection exerted on males. Because pulse rate thresholds exert directional selection on the males (Brandt 2002), GEIs and crossovers in pulse rate thresholds indicate that selection on males will vary in space and time if the level of environmental change is high.

TABLE 3. Mixed-model ANOVA testing for the effect of family and rearing environment on larval development time in female lesser waxmoths. Both factors had significant effects, and there was no interaction between them.

	df	SS	MS	<i>F</i>	<i>P</i>
Family	15	0.045	0.003	2.51	0.001
Rearing environment	1	1.12	1.12	668.37	<0.001
Family × environment	15	0.026	0.0017	1.44	0.125
Error	318	0.38	0.0012		
Total	349				

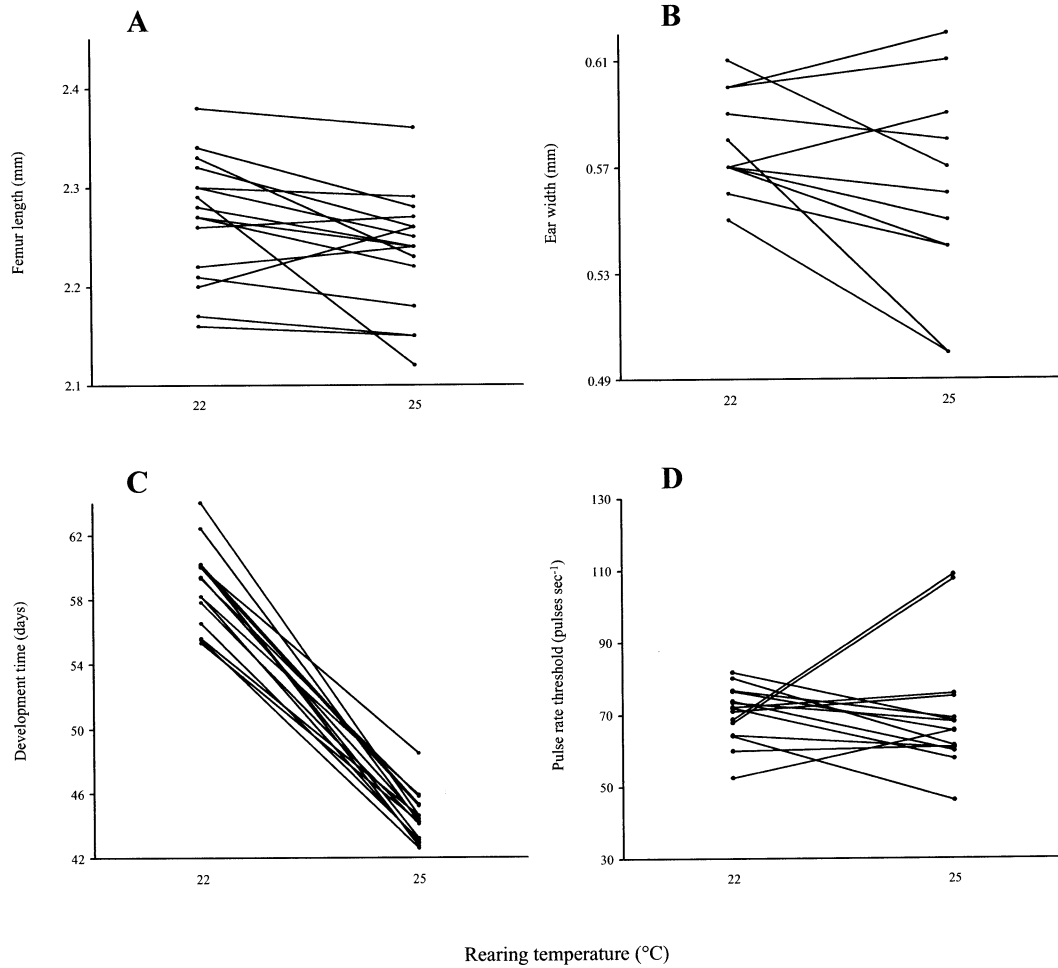


FIG. 2. Reaction norms of full-sib families split into two rearing temperatures in female lesser waxmoths. Mean family values in each rearing environment are shown. (A) Femur length. (B) Ear width. (C) Larval development time. The variance at 22°C was significantly larger ($F_{173,175} = 2.59, P < 0.001$). A \log_{10} transformation reduced heteroscedasticity but did not eliminate it ($F_{173,175} = 1.56, P < 0.001$). (D) Pulse rate thresholds. The variance was not significantly larger at 25°C than at 22°C ($F_{179,178} = 1.09, P > 0.10$).

Male mating success will thus be influenced by GEIs and crossover interactions in female thresholds mediated by the rearing environment they experienced as larvae, as well as by GEIs and crossover interactions in the male calls (Jia et al. 2000). The maximum difference between the mean threshold of families split across rearing environments was >40 pulses·sec⁻¹, with most changes being approximately 10 pulses·sec⁻¹ (Fig. 2D). These changes span at least one standard deviation and up to half the range of male pulse rates (Jang and Greenfield 1996). In terms of the influence of GEIs on

male pulse rate, the changes in female thresholds span at least the maximum change in male pulse rate, and up to one order of magnitude higher (Jia et al. 2000). Variation in selection on males and females caused by GEIs may thus be considerable, and it may further contribute to the maintenance of genetic variation. These results correspond to one of the situations outlined by Via and Lande (1987), in which $r_g < 1$ and fluctuations in selection contribute to the genetic variation in a population (see also Haldane and Jayakar 1963; Bertram 2002; Pfennig and Tinsley 2002).

TABLE 4. Mixed-model ANOVA testing for the effect of family and rearing environment on pulse rate thresholds in female lesser waxmoths. Family had a significant effect, rearing temperature did not, and there was an interaction between the effect of family and rearing environment. The test for the effect of environment had power = 0.90 for a difference of 10 pulses·sec⁻¹ and >0.95 for differences of ≥ 11 pulses·sec⁻¹.

	df	SS	MS	F	P
Family	14	24396.8	1742.6	2.34	<0.005
Rearing environment	1	4.9	4.9	0.003	0.96
Family × environment	14	21565.7	1540.4	2.07	0.013
Error	312	232100.1	743.9		
Total	341				

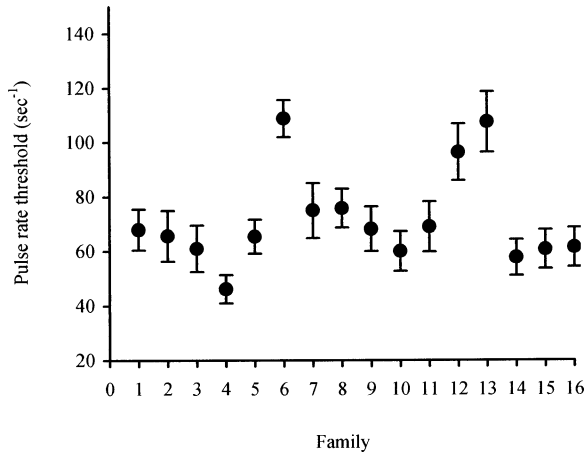


FIG. 3. Pulse rate thresholds in 16 full-sib families reared at 25°C in female lesser waxmoths. Mean \pm SE family values are shown.

The existence of genetic variation in female response to males also implies processes that maintain it. We found phenotypic plasticity in all traits measured, but GEIs were only present for pulse rate thresholds. Our estimate of the cross-environment genetic correlation ($r_g = 0.53$) is consistent with crossover interactions in reaction norms (Fry et al. 1996), and there was a high proportion (0.84) of crossing reaction norms for pulse rate thresholds (Fig. 2D). The detection of a significant GEI in pulse rate thresholds was dependent on two families (of 15) that had very different values among the two rearing temperatures. Removal of these two families from the dataset eliminated the significant GEI. Nevertheless, these families represent an interesting part of the population. They were tested in the course of normal sampling: one toward the middle and the other toward the end of our staggered sampling of families (see Materials and Methods). There is nothing to suggest that the traits of these families are the product of a bias, and further sampling should reveal further GEIs.

Crossover interactions in female response to male calls may thus contribute to the maintenance of their genetic variation. The connection between female response to male signals and female fitness is more indirect than in males, because a female's recognition of males as potential mates may influence her fitness to a lesser extent than a male's attractiveness will influence his fitness. Nevertheless, the expected influence of mate choice on offspring attractiveness and viability (Collins et al. 1999) will impact female fitness. Crossovers in female response to male signals are thus suggestive of changes in the fitness ranking of female genotypes, such

that different variants may be favored in different environments, and genetic variation may be maintained.

Genotype-by-environment interactions in female response to males also address a problem related to selection on females. Indirect (genetic) benefits of mate choice are often expected (Fisher 1958; West-Eberhard 1983; Andersson 1994; Höglund and Alatalo 1995), as is the case for female lesser waxmoths (Jang and Greenfield 1996, 1998; Jia and Greenfield 1997; Reinhold et al. 1998; Collins et al. 1999). But GEIs and crossover interactions in male sexual traits imply that male phenotypes may be unreliable predictors of offspring genotypes and phenotypes. For example, attractive males may sire attractive offspring that mature faster or have better feeding rates only in some environments (Jia and Greenfield 1997; Jia et al. 2000; Doty and Welch 2001). The link between male sexual traits and mate choice benefits may thus break down, and how female choice based on indirect (genetic) benefits can evolve becomes puzzling. Matching variation in the male and female traits may lessen this problem. For example, variation in female mate preferences matched seasonal or ecological variation in male parental care and growth rate in collared flycatchers and soil mites, respectively (Lesna and Sabelis 1999; Qvarnström et al. 2000). If crossover interactions in female preferences can result in predictable offspring traits, the benefits of mate choice may remain in spite of crossover interactions in male traits.

In our results, however, the reaction norms of female pulse rate thresholds did not match the male reaction norms. In an experiment involving artificial selection, high pulse rate genetic variants reared at lower temperatures exhibited lower rates than low pulse rate genetic variants in the same environments (Jia et al. 2000). Females reared at lower temperatures should thus have lower thresholds for the reaction norms to match. But there was no consistent pattern in this direction (Fig. 2D). Indeed, the presence of $r_g < 1$ and crossovers in female reaction norms indicates that some male and female genotypes must be mismatched across some environments, so females with high pulse rate thresholds will not consistently favor specific male genotypes across environments, and their offspring will not exhibit their sire's traits or their dam's thresholds consistently across environments. The scale at which lesser waxmoths will experience these environmental changes can be daily, weekly, and seasonal, because they inhabit the exterior portions of beehives, abandoned hives, and surrounding wax debris (Greenfield and Coffelt 1983), and do not therefore obtain the benefit of the colony's homeostasis (Winston 1987). The dynamics of mate choice may thus yield mate choice benefits and male-female

TABLE 5. One-way ANOVAs testing for the effect of family on pulse rate thresholds separately for females reared at 25°C and 22°C in lesser waxmoths. Family had an effect only at 25°C, which was significant after the Bonferroni correction for multiple tests performed on the same dataset. Power for the test performed on females reared at 22°C was 0.90 for a difference of 10 pulses·sec⁻¹ and ≥ 0.96 for differences ≥ 11 pulses·sec⁻¹.

	Females reared at 25°C					Females reared at 22°C				
	df	SS	MS	F	P	df	SS	MS	F	P
Family	15	39994	2666	3.79	<0.001	14	7931	567	0.70	0.78
Error	164	115286	703			158	128669	814		
Total	179	155280				172	136600			

TABLE 6. Genetic and phenotypic correlations between morphological, behavioral, and life-history traits in female lesser waxmoths. Pearson's product-moment correlation coefficients calculated with untransformed data. *P*-values are indicated between parentheses. Asterisks indicate significant correlations after the Bonferroni correction. For genetic correlations: *N* = 15–16 families; power ≥ 0.95 for $r \geq 0.76$ – 0.78 . For phenotypic correlations: *N* = 148–176 females; power ≥ 0.95 for $r \geq 0.18$ – 0.29 . NS, nonsignificant.

	At 25°C			At 22°C		
	Pulse rate threshold	Femur length	Ear width	Pulse rate threshold	Femur length	Ear width
Genetic correlations						
Families						
Pulse rate threshold	—			—		
Femur length	−0.02 (0.93) NS	—		−0.10 (0.72) NS	—	
Ear width	0.12 (0.65) NS	0.40 (0.12) NS	—	−0.04 (0.90) NS	0.65 (0.00)*	—
Development time	0.25 (0.34) NS	0.15 (0.57) NS	−0.11 (0.69) NS	0.25 (0.37) NS	0.12 (0.67) NS	0.18 (0.50) NS
Phenotypic correlations						
Females						
Pulse rate threshold	—			—		
Femur length	−0.08 (0.31) NS	—		−0.12 (0.14) NS	—	
Ear width	−0.15 (0.06) NS	0.48 (<0.001)*	—	0.10 (0.24) NS	0.50 (<0.001)*	—
Development time	−0.02 (0.76) NS	0.334 (<0.001)*	0.14 (0.08) NS	0.18 (0.02) NS	0.18 (0.03) NS	0.12 (0.13) NS

coevolution only on short-term and local scales. At broader scales of temporal, environmental, and geographical variation, the overall selection on males and females may be weaker, and the response to selection slower, than usually expected for sexually selected traits.

In conclusion, we have documented three previously underemphasized factors that can contribute to the maintenance of genetic variation in sexual traits: genetic variation in female response to male signals can maintain genetic variation in the male trait, genetic variation in the female trait can be sustained by GEIs and crossing reaction norms, and there may be differences in the selection that acts on males and females, mediated by environmental heterogeneity and GEIs and crossing reaction norms in males and females. We finally note that, although these results can contribute to the maintenance of genetic variation, they may also contribute to rapid population divergence. Phenotypic plasticity resulting from GEIs can facilitate divergence when conditions appear that favor the expression of a portion of the total phenotype variability (West-Eberhard 1989). Thus, GEIs and crossover interactions in sexual traits may result in divergent selection and accentuate the process of population differentiation that may result from sexual selection by female choice.

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