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REACTION NORM VARIANTS FOR MALE CALLING SONG IN POPULATIONS OF *ACHROIA GRISELLA* (LEPIDOPTERA: PYRALIDAE): TOWARD A RESOLUTION OF THE LEK PARADOX

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Significant additive genetic variance often occurs for male advertisement traits in spite of the directional selection imposed by female choice, a problem generally known in evolutionary biology as the lek paradox. One hypothesis, which has limited support from recent studies, for the resolution of this paradox is the role of genotype × environment interaction in which no one genotype exhibits the superior performance in all environments—a crossover of reaction norms. However, these studies have not characterized the actual variation of reaction norms present in natural populations, and the extent to which crossover maintains genetic variance remains unknown. Here, we present a study of genotype × environment interaction for the male calling song in populations of *Achroia grisella* (Lepidoptera: Pyralidae; lesser waxmoth). We report significant variance among reaction norms for male calling song in two North American populations of *A. grisella* as measured along temperature, food availability, and density gradients, and there is a relatively high incidence of crossover of the temperature reaction norms. This range of reaction norm variants and their crossover may reflect the co-occurrence of plastic and canalized genotypes, and we argue that the different responses of these variants along environmental gradients may contribute toward the maintenance of genetic variance for male song.

KEY WORDS: **Acoustic communication, mate choice, phenotypic plasticity, sexual selection, signal evolution.**

The presence of additive genetic variance for male sexual traits such as mating signals remains a major problem in evolutionary and behavioral biology. Directional selection normally imposed by female choice is expected to reduce additive genetic variance for male traits greatly, which, in turn, should relax selection pressure maintaining female choice for indirect (genetic) benefits. Nonetheless, behavioral studies of many vertebrate and invertebrate species have indicated that female choice for indirect benefits does occur and may represent a major component of sexual selection (Andersson 1994; Shuster and Wade 2003).

Moreover, quantitative genetic studies routinely demonstrate substantial additive genetic variation for both male signal traits (as reviewed in Bakker and Pomiankowski 1995) and female response traits (e.g., Brooks and Endler 2001; Meffert and Regan 2002), although the body of data for the latter remains small owing to difficulties in sampling. This apparent contradiction between theoretical expectation and observations of natural populations has become known as the "paradox of the lek" (Taylor and Williams 1982; Kirkpatrick and Ryan 1991), a reference to the exaggerated levels of sexual selection often witnessed at classical leks. However, the problem is a general one, and it may be encountered wherever female choice based on indirect benefits exists.

In broader terms, the lek paradox represents an aspect of the fundamental question: what maintains genetic variance in natural populations (Lewontin 1974)? Evolutionary geneticists propose no fewer than seven distinct mechanisms to maintain additive genetic variation (Roff 1997; Lynch and Walsh 1998), and empirical testing of these possibilities has been done in various species. However, for the sexually selected traits on which the lek paradox focuses, there is a striking imbalance between theory and data, and only a few studies have attempted to identify the specific mechanism(s) responsible for observed genetic variance. To date, these studies have concentrated on four possibilities, frequency-dependent selection (e.g., Fitzpatrick et al. 2007), mutation–selection balance (e.g., Rowe and Houle 1996; Kotiaho et al. 2001; Tomkins et al. 2004; see also Hine et al. 2004 for negative evidence; see Zhang and Hill 2005 for a general overview of mutation–selection balance and genetic variance), genetic trade-offs (e.g., Brooks 2000; see also Jennions et al. 2001 for negative evidence), and genotype × environment interaction (GEI) (e.g., Qvarnström 2001; Welch 2003; see Turelli and Barton 2004 for an overview of these several mechanisms). Although limited evidence in support of each of the several mechanisms has been found in different species, the research has neglected full genetic characterization of natural populations and assessment of field conditions believed to sustain variance. For example, theory predicts that strong GEI accompanied by environmental change over time or space is conducive to maintaining additive genetic variance (V_A): If GEI is sufficiently strong that ecological crossover (intersection) occurs between the reaction norms of the various genotypes, no single genotype will be the superior one in all environments (Felsenstein 1976; Slatkin 1978; Charlesworth and Hughes 2000). Given this framework, random dispersal among environments or unpredictable change in environmental conditions accompanied by overlapping generations would maintain V_A (Charlesworth 1988; Gillespie and Turelli 1989; Ellner and Hairston 1994). Studies of both vertebrates (e.g., Qvarnström 1999; Mills et al. 2007) and insects (e.g., Jia et al. 2000) support this hypothesis, but no investigation has examined both the genetics underlying the diversity of reaction norm variants within a population and the requisite temporal or spatial heterogeneity in the field. In the absence of this ecological information, the role of GEI in maintaining V_A remains uncertain.

Our previous studies of the lesser wax moth (*Achroia grisella*; Lepidoptera: Pyralidae) illustrate the difficulties outlined above. Male *A. grisella* broadcast an ultrasonic calling song attractive to females (Spangler et al. 1984), and recordings and playback experiments indicate that songs vary considerably among males in features that influence attractiveness (Jang and Greenfield 1996, 1998). Importantly, breeding studies show that these features are both repeatable within individual males and heritable (Jang et al. 1997; Collins et al. 1999). Thus, the continued presence of unattractive male songs represents the lek paradox in *A. grisella* populations. We have found no evidence of genetic trade-offs between song attractiveness and developmental features of any kind (Brandt and Greenfield 2004; Danielson-François et al. 2006), but ample evidence supports the occurrence of GEI affecting song. Both artificially selected (Jia et al. 2000) and inbred lines randomly extracted from various populations of *A. grisella* (Danielson-François et al. 2006) exhibit markedly different reaction norms across diet (cf. Olvido and Mousseau 1995), population density, and temperature gradients, and the incidence of ecological crossover is approximately 30% for the lines and environmental conditions tested. Much of the observed crossover appears to result from the co-occurrence of relatively canalized lines, which maintain a consistent song performance across an environmental gradient, and relatively plastic lines, which exhibit superior performance when developing under favorable conditions but suffer markedly when under stress (Danielson-François et al. 2006). However, at this point we do not know (1) whether the variation in reaction norms and the incidence of crossover interactions observed in selected and inbred laboratory lines reflect the composition of populations, (2) whether the degree of environmental heterogeneity over space or time is sufficient to maintain the various genotypes exhibiting different reaction norms in the population, and (3) whether genotypes that exhibit a superior phenotype over entire environmental gradients exist and are selected in populations. Here, we address these questions in *A. grisella* and provide a more thorough analysis of whether and how GEI and environmental heterogeneity may resolve the lek paradox in this species.

We approached the stated objectives of our study by implementing three fundamental protocols. These three steps extended our understanding of GEI from the setting of heavily inbred and selected laboratory lines to the genetic and environmental milieu of populations taken directly from the field. First, we sampled a large number of *A. grisella* individuals from natural populations at two different geographical locations in North America and determined

laboratory reaction norms of moths from these sampled populations within one or two generations following collection. Thus, while acknowledging that some changes may have occurred during the interval between collection and measurements for reaction norms, we reduced the inevitable effects of laboratory selection and genetic drift to the extent possible. Moreover, in sampling the moths from which we determined reaction norms, we used a breeding design that included a maximum amount of genetic variance potentially found in the collected population. This measure ensured that the diversity of reaction norms we observed in the laboratory reflected the much of the range existing in the natural population in the field.

Second, we measured reaction norms along three environmental gradients that moths likely encounter in nature. Across space and time, larvae developing in the wild are likely to experience dramatic differences in (1) the quantity of available food, (2) population density, and (3) temperature. As symbionts of honey bee (*Apis mellifera*) colonies, *A. grisella* larvae feed on organic material that the honey bee workers store, honey bee brood, and detritus within and surrounding the hive (Künike 1930; Milum 1940). Typically infesting weakened colonies with declining worker populations, *A. grisella* experience a steadily diminishing and nonrenewable food supply. Consequently, the food availability to which *A. grisella* larvae are exposed will generally depend on the number of moth generations since the infestation began, as well as the initial abundance of organic material in a given honey bee colony. These factors imply that the range of food availability is potentially high, extending from a nearly ad libitum situation to scarcity in which only the most competitive individuals or those with special metabolic features may survive and develop normal adult traits. Similarly, moth population density will vary in accordance with the time since the beginning of infestation, as well as the number of *A. grisella* adults founding that infestation, and the amount of available food. Temperature changes seasonally, but it also depends on the number of honey bee workers present in the hive and the distance that developing *A. grisella* larvae are from the core area of the hive, which honey bee workers thermoregulate to some extent.

Third, we monitored environmental conditions in honey bee colonies at the specific geographical localities from which our *A. grisella* populations were collected. These conditions include the actual food availabilities, moth population densities, and temperatures that *A. grisella* may experience throughout a year, values that correspond to levels along the three environmental gradients for which reaction norms were determined. Consequently, we could estimate the expected performances of the various *A. grisella* genotypes—the reaction norm variants studied in the laboratory—in the field during different times of the year and at specific locations.

Methods

POPULATIONS STUDIED

We collected *A. grisella* larvae in September and October 2005 from honey bee colonies in Beltsville, Maryland (39°01′ N, 76°52′ W) and Baton Rouge, Louisiana (30°25′ N, 91°13′ W), localities chosen to represent the northern and central portions of the species' geographical range in North America. *Achroia grisella* population levels at Beltsville, Maryland are generally small and adult presence is restricted to several months during late summer and early autumn, whereas population levels at Baton Rouge, Louisiana are higher and more widespread, and adult presence lasts for six or more months of the year. At both localities we sampled several dozen *A. grisella* at honey bee colonies in bee yards that were relatively isolated from other honey bee populations: Hives were never moved into these bee yards from other locations, and the sites were at least 2 km distant from neighboring yards. Thus, we suggest that most of the gene flow to which our two *A. grisella* populations were subject was the occasional immigration of adult moths, which are relatively poor fliers. Consequently, we effectively consider these populations as distinct units that might be characterized genetically.

We monitored environmental conditions at honey bee colonies in both localities over a 12-month period following collection of *A. grisella* larvae. Temperature was recorded within six different hive boxes at each locality with digital thermochron buttons (ibuttons; Maxim Integrated Products, Sunnyvale, CA) placed at the peripheral sites where *A. grisella* larvae normally reside. These thermal devices registered temperature ($\pm$ 0.25°C) at 4-h intervals throughout the monitoring period. An observer sampled adult moth populations in and surrounding 10 hive boxes at monthly intervals.

EXPERIMENTAL DESIGN

For both *A. grisella* populations, we transferred the collected larvae to plastic boxes containing a standard synthetic diet (see Jang and Greenfield 1996) and reared them in growth chambers at 25°C under a 12:12 L:D photoperiod. Following one or two generations of laboratory rearing in which we ensured that a maximum number of males and females mated and contributed to the next generation, we sampled 15 and 10 newly eclosed, unmated adult males from the Maryland and Louisiana populations, respectively, and used these males as sires for our investigations of GEI. We paired each sire with 4–6 different newly eclosed, unmated females (dams) sampled randomly from the same population, making the successive pairings of a given sire on consecutive days. These pairings led to four successful matings and dam families for all but two sires from the Louisiana population, in which only three dam families were generated. Unmated insects were used for standardization because female *A. grisella* generally mate but once and become

unreceptive thereafter (Greenfield and Coffelt 1983). Following mating, we placed the female in a 30-mL plastic cup supplied with 12-g diet, where she typically oviposited and on which newly hatched larvae fed. We transferred the female to a new cup every day until death, generally 5–7 d later, while retaining all previous cups. Thus, we had a record of the precise oviposition date of each of a female's offspring, and we used these dates to determine their developmental period from oviposition to adult eclosion.

Approximately 12 d after the mean oviposition date of each dam (pairing), we split her offspring among six different environmental conditions that represented high and low values along the diet availability, population density, and temperature gradients. Offspring were late first- or early second-instar larvae at this juncture. The various environmental conditions used for this split-family design are presented in Table 1 and were chosen in accordance with variation in food, density, and temperature observed in natural populations of *A. grisella*. Moreover, previous studies had indicated phenotypic plasticity in the responses of laboratory populations of *A. grisella* to these values (Jia and Greenfield 1997; Jia et al. 2000; Rodriguez and Greenfield 2003; Danielson-François et al. 2006). To test GEI under environmental conditions as close as possible to those experienced by natural populations, throughout the split-family experiment we reared *A. grisella* larvae on food obtained from honey bee colonies at their population of origin: honeycomb, stored pollen, honeybee brood, and organic detritus. We homogenized this material to provide each larva from a given population with diet of comparable quality. The 12:12 L:D photoperiod was retained for all six environmental conditions.

In each of the six environmental conditions (Table 1), we reared the larvae in small groups within 30-mL plastic cups, either three (standard density), two (low), or six (high) larvae per cup. We then adjusted diet availability to 2 g (standard quantity), 1 g (low), or 3 g (high) per cup. Thus, we examined the separate influences of population density and diet availability on developmental and signal traits of the various sire and dam families. This design allowed us to consider the possibility that specific social effects of different population density levels might exert influences independent of the diet available per larva: We could compare performance under 3 larvae per 3 g diet (1.0 g diet per larva; environmental rearing condition 6 from Table 1) with that under 2 larvae per 2 g diet (1.0 g diet per larva, the same amount of available diet but a rather different larval density per cup; environmental rearing condition 3), or 3 larvae per 1 g diet (0.33 g diet per larva; environmental rearing condition 5) with that under 6 larvae per 2 g diet (0.33 g diet per larva; environmental rearing condition 4). Our test individuals completed their larval development, pupated, and eclosed to the adult stage within the 30-mL cups.

The set of six environmental conditions used in our split-family design (Table 1) was incomplete in that it did not include all possible combinations; for example, high temperature (29°C) and low diet availability (1 g per cup). This incomplete set was used because measuring phenotypic parameters from the many sire and dam families would not have been possible with a fully factored design ($3 \times 3 \times 3 = 27$ environmental conditions). To offset the limitation of our design and to address the basic objective of the project, we specifically chose environmental conditions that allowed us to evaluate reaction norms relevant to expected field conditions; for example, the combination of high temperature and low diet availability noted above would not be expected naturally, as the former normally occurs in mid-summer whereas the latter would generally be an autumn phenomenon. Finally, one must note that our experimental design does allow the evaluation of reaction norms other than the basic comparisons along a single environmental gradient (i.e., rearing conditions 1 versus 2, 3 versus 4, and 5 versus 6 in Table 1), and we do make these additional

Table 1. Six environmental rearing conditions used in the split-family experimental design; these six rearing conditions involved three different environmental gradients, each with three levels—high, low, and standard. Values indicate level along a given gradient; values in boldface indicate gradient that was varied between high and low levels for a given pair of environmental rearing conditions (1 and 2, 3 and 4, 5 and 6).

Environmental rearing condition	Environmental gradient			Number of replicates*
	Temperature (°C)	Density (larvae/cup)	Food availability (g/cup)	
1 Low temperature	**22**	3	2	5
2 High temperature	**29**	3	2	5
3 Low density	25	**2**	2	8
4 High density	25	**6**	2	3
5 Low food	25	3	**1**	5
6 High food	25	3	**3**	5

*Number of rearing cups per dam family.

evaluations where warranted (e.g., supplementary evaluations of population density as described in the preceding paragraph).

DEVELOPMENTAL AND SIGNAL MEASUREMENTS

Eclosed adults were collected daily, and the developmental period of each individual was noted. We weighed (± 0.005 mg) each male on the day of adult eclosion. Because adult *A. grisella* neither eat nor drink and rely entirely on energy reserves acquired during larval development (Künike 1930), body weight at adult eclosion represents a reliable index of expected adult longevity (females: 5–7 d, in the laboratory; males: 10–14 d), a female's potential fecundity (200–400 eggs), and the energy that a male can devote to acoustic signaling. Previous studies had confirmed these expected relationships for adult longevity (Brandt and Greenfield 2004) and male signaling (Reinhold et al. 1998) in laboratory populations of *A. grisella*.

From each of the six groups (environmental rearing conditions) into which we had split the offspring of each dam, we sampled 5–8 male offspring and recorded their calling signals within 30 h following eclosion. All recordings were made within an acoustically insulated chamber (see Jang and Greenfield 1998) during the initial 6 h of the night, the normal activity period of *A. grisella*. Temperature in the chamber was maintained at 25 ± 1°C to standardize recordings (see Greenfield and Medlock 2007).

To facilitate signal recording, we placed males individually within small screen cages (1.5 cm diam., 2.0 cm height) in which they readily broadcast song with normal characteristics (Jang and Greenfield 1996). The cages were separated by at least 30 cm and isolated by acoustic insulation foam to reduce influences of neighbors on a male's singing and to permit clear recordings of a focal male without neighbors' songs in the background. We used a condenser microphone (model 7016, ACO Pacific; Belmont, CA; frequency response: ±2 dB from 10 to 100,000 Hz, ±6 dB from 10 to 160,000 Hz), pointed toward the cage at a standard distance of 10 cm, to record the male song. The microphone output was amplified (model 4012 preamplifier, model PS9200 amplifier, ACO Pacific), filtered (model 3202 variable filter, Krohn-Hite, Brockton, MA), and digitized (National Instruments DAQCard 6062E, National Instruments, Austin, TX; 500,000 samples per sec), and we saved a 1-sec sample of this digitized song to a file on a laptop computer using Batsound 4.0 software (Petterson Elektronik AB, Uppsala, Sweden).

Previous studies indicated that the attractiveness of an *A. grisella* male's song to females is influenced primarily by three signal characters: the rate at which pairs of sound pulses are delivered (PR), the peak amplitude of sound pulses (PA), and the so-called asynchrony interval that elapses between the onset of the first pulse in a pair and the onset of the second (AI). Playback experiments conducted with several *A. grisella* populations showed that females prefer a faster PR, more intense PA, and a longer AI (Jang and Greenfield 1998). We therefore measured these three signal characters from each recorded song using software modified from Spike2 (Cambridge Electronic Design, Cambridge, UK; see Brandt and Greenfield 2004 for complete procedure). These measured values represented a male's signal performance under a given rearing environment.

Because actual female evaluation of males is based on a composite feature of song, we calculated an overall attractiveness index (AT) that was determined as a function of the three separate signal characters (cf. Scheuber et al. 2004). This function was determined by testing the relative attractiveness of males to females in a laboratory arena, recording and measuring the song characters of the various males, and then employing a selection gradient analysis (see Lande and Arnold 1983) that identified the predicted contributions of the several characters to attractiveness. For both our Maryland and Louisiana populations of *A. grisella*, we followed the procedure outlined in Jang and Greenfield (1998) and monitored the relative attractiveness of four males to 20 females, each female tested individually. The females used for these tests came from stock populations representing the two geographic sites, and both stock populations were maintained in environmental chambers at 25°C and a 12:12 L:D photoperiod. We replicated this process 10 times in both populations, each replicate testing different sets of males and females. With these data on relative male attractiveness and song characters, we determined separate functions for the two populations and then each male's AT using the function determined for its population.

In applying selection gradient analysis, we considered both linear and nonlinear models as potential predictors of attractiveness. We found that none of the quadratic coefficients for any signal character was significant in both of our populations, implying either that stabilizing and/or disruptive selection were weak or that our samples were not sufficiently large. Because only linear selection gradients were significant, we retained a linear model as the predictor of relative attractiveness of *A. grisella* males.

QUANTITATIVE GENETIC ANALYSES

We used several assessments of the level of GEI in our two *A. grisella* populations to evaluate different aspects of the interactions and the likelihood that they are responsible for maintaining genetic variance. In each assessment we evaluated GEI for male body weight, male developmental period, the three separate male song characters, and overall male song attractiveness. These assessments were made along each of the three environmental gradients tested, population density, food availability, and rearing temperature.

We first employed a two-way mixed-model analysis of variance (ANOVA) (general linear model) in which sire and dam (nested within sire) were treated as random effects and the environmental gradient as a fixed effect (see Fry 1992). The *F*-test for

the interaction between sire and environmental value was then a measure of the significance of GEI for a specific population, index of performance, and environmental gradient. Here, we considered the sire effect to represent genotype, as it encompassed four different dams (full-sibling families), which reduced the influence of any specific maternal effect due to a given female parent. But, we also report the results of the F-test for the interaction between dam (nested within sire) and environmental value because of the increased number of samples (dams) available.

We examined all data for normality (Kolmogorov–Smirnov test) prior to implementing the two-way ANOVA, and we transformed those data that did not conform to the normal distribution. Thus, developmental period was inverse-transformed, PA was log-transformed, and AI was square root-transformed; these procedures removed approximately one half of the departures from normality among the various subsets of our data (departures defined by a Kolmogorov–Smirnov statistic with $P < 0.05$). Following these transformations, we employed Levene's test to examine the equality of variances among data within each population. We applied a sequential Bonferroni procedure (Holm 1979) to correct significance values for multiple tests in our ANOVA.

To provide a more specific measure of GEI, we calculated the Pearson product-moment correlation (r_m) among family means across the two values along an environmental gradient (Via 1984). This statistic essentially measured the extent to which the reaction norms for the various sires were parallel. An r_m value significantly less than 1.0 indicates nonparallel reaction norms and the likelihood that GEI is present. Although r_m may underestimate GEI when family sizes are small, we used this statistic rather than the cross-environment genetic correlation, r_g—an index specifically designed to measure GEI (see Fry et al. 1996)—because the latter becomes unreliable when family variance components are small (Windig 1997; Astles et al. 2006). We estimated confidence limits for r_m with the z-transformation as described in Sokal and Rohlf (1995). As above, we provide r_m values for both sire and dam families. For assessment along the population density gradient, we compared high density/standard food and temperature versus low density/standard food and temperature (rearing conditions 3 vs. 4 from Table 1) and also two additional comparisons in which the amount of food per larva was constant but the number of larvae per cup varied: high food quantity/standard density and temperature versus low density/standard food and temperature (rearing conditions 4 vs. 5) and low food quantity/standard density and temperature versus high density/standard food and temperature (rearing conditions 3 vs. 6).

Because the litmus test for GEI that can maintain additive genetic variance within a population is the incidence of ecological crossover (see Roff 1997; Lynch and Walsh 1998), we analyzed all pairs of reaction norms for intersections. For all performance indices and environmental gradients in both populations, we constructed reaction norms connecting sire family means and we report the percentage of pairs of sire families for which reaction norms intersect. We then employed a modification (Baker 1988; Cornelius et al 1992) of the Azzalini–Cox test (Azzalini and Cox 1984) to estimate the significance of changes in rank order for each intersection, determined by measuring whether the difference in performance between the two sire families is significantly greater than zero in one environment and significantly less than zero in the other. The first step in this special modification was calculating the appropriate critical value ($C = t_\alpha \sigma \sqrt{2}$) for each environment. Based on a randomized complete block analysis within each environment (general linear model in which sire was considered as a primary factor and dam as a blocking factor), we calculated σ, the standard error of the performance in each environment, as $\sqrt{(MS_{error}/n)}$, where n is the number of dams per sire. The critical multiplier t_α was computed as the one-tailed Student's t value, given a t distribution probability of 0.1581 and the degrees of freedom of the error term obtained from the randomized complete block analysis (see http://homepage.usask.ca/~rjb609/gxe5.html for more details and examples). Second, we calculated performance differences between all possible pairs of sires in each environment and then compared the difference of each pair with the critical value calculated for the specific environment. If the difference between two sires exceeds the critical value in one environment, and the difference between them is less than the negative critical value in another environment, we reject the null hypothesis that no crossover interaction occurred between the two reaction norms. For example, if Y_{i1}, Y_{j1} are the mean performance indices of sire i and j under environment 1, Y_{i2}, Y_{j2} are the mean performance indices of sire i and j under environment 2, and C_1, C_2 are, respectively, the critical values estimated for environment 1 and 2, we assume that crossover interaction occurs when $Y_{i1} - Y_{j1} > C_1$ and $Y_{i2} - Y_{j2} < -C_2$, or $Y_{i1} - Y_{j1} < -C_1$ and $Y_{i2} - Y_{j2} > C_2$. As above, we report the percentage of pairs of sire families for which reaction norm intersections are significant by this criterion.

Our sets of reaction norms allowed us to investigate patterns in canalization or plasticity among genotypes (sire families). Reaction norms were categorized as canalized when performance at one level along an environmental gradient was not significantly different ($P > 0.05$; two-way nested ANOVA, general linear model) from performance at the other level and plastic when a significant difference occurred. We then asked whether genotypes that were canalized (or plastic) for a given performance index and environmental gradient were also canalized (or plastic) for that performance index along another gradient. Similarly, we asked whether genotypes that were canalized (or plastic) along a given environmental gradient and for one performance index were also canalized (or plastic, with increased performance in the same direction along the gradient) for another performance index.

To distinguish the relative strengths of genetic and maternal influences on developmental and song characters in *A. grisella*, we estimated the variance components using a restricted maximum likelihood method (REML in JMP, version. 6.0). We first estimated the causal variance components of sires and dams (within sires) along each environmental gradient using a nested full-sibling/half-sibling breeding design (Statistical model: $y = \mu + s + d(s) + e$; μ, s, $d(s)$, e denote grand mean, sire, dam(sire) and residual error, respectively). Additive genetic variance (V_A), maternal effects (V_M), and environmental variance (V_E) were estimated as 4 × among-sire variance, the among-dam variance minus the among-sire variance, and the residual variance minus 2 × among-sire variance, respectively (Falconer and Mackay 1996; Rauter and Moore 2002). Because significant dam (sire) × environment interaction terms were found in the mixed-model ANOVA (Table 2; see Results), we partitioned the phenotypic variances of male developmental and signal traits into causal variance components following Via (1984) and Groeters (1988). This procedure allowed us to further examine the relative importance of environment × sire and environment × dam terms for each of the three environmental gradients tested.

Results

Observations at the Maryland population revealed small numbers of adults present at several of the sampled hive boxes between May and September. Ambient temperatures during this period ranged between 3.0 and 35.5°C, with mean monthly temperatures ranging from 16.0 in May to 25.4°C in July (averages of all 12 thermochron buttons for all temperature records; see Fig. 1 for one example of the full year's records). At the Louisiana population, adults were observed at several sampled hive boxes between April and November, ambient temperatures ranged between 1.5 and 41°C during this period, and mean monthly temperatures ranged from 16.1 in November to 28.9°C in June (averages of all six thermochron buttons for all temperature records; see Fig. 1 for one example of the full year's records). Daily temperature ranges at both the Maryland and Louisiana sites were approximately 10 °C.

Our tests of the preferences of female *A. grisella* for males revealed slightly different functions determining male attractiveness (AT) in the two populations. Selection gradient analysis of data from the Maryland population indicated that AT = −1.32 + 0.022 PR + 0.0057 PA + 0.00034 AI ($r^2 = 0.22$, $P = 0.012$), whereas analysis of data from the Louisiana population indicated that AT = −1.27 + 0.018 PR + 0.008 PA + 0.00031 AI ($r^2 = 0.27$, $P = 0.010$).

Two-way mixed-model ANOVA indicated significant interaction between temperature and sire family for the attractiveness index of male song (AT) in both our Maryland and Louisiana populations of *A. grisella* (Table 2; Levene's tests showed that in both populations variances among families were equivalent for AT as measured along all environmental gradients, $P > 0.05$). Our Maryland population also showed significant interaction between sire family and population density for the attractiveness index (AT), and between sire family and temperature for the pulse-pair rate in male song (PR). However, we note that these values, as well as some others in the ANOVA table, were no longer significant following the Holm correction for multiple tests. Overall, we interpret these results as potential GEI.

Further analyses indicated significant interaction between all three environmental factors and dam (nested within sire) for developmental period and body weight at eclosion (Table 2). However, we hesitate to interpret these results as GEI, as they may reflect maternal effects in addition to, or rather than, genotype (see below).

Results of r_m calculations further confirmed our above interpretation that GEI existed for indices of male performance along the various environmental gradients (Table 3). For both sire and dam families, we found r_m values that were significantly less than 1.0 for all 30 pairwise combinations of environmental gradients and male performance indices in both Maryland and Louisiana populations. Moreover, eight and nine r_m values for sire families in the Maryland and Louisiana populations, respectively, were negative. That is, we found no indication that sire families in which performance indices were relatively high (or low) when reared at one level along an environmental gradient also tended to have relatively high (or low) indices when reared at a different level.

Construction and analyses of reaction norms (Fig. 2) revealed high incidences of crossover interaction between sire and environmental gradient for the various performance indices in both populations (Table 4). For example, for the attractiveness index of male song (AT), crossover occurred in at least 25% of all sire pairs along all environmental gradients in both populations. Similarly, for the temperature gradient, crossover also occurred in at least 25% of all sire pairs for all performance indices in both populations. When we restricted crossover to those cases in which the interaction was significant ($P < 0.05$) by the modified Azzalini–Cox test, we found that its incidence remained relatively high (15% and 22% in the Maryland and Louisiana populations, respectively) for the attractiveness index of male song (AT) and for the temperature gradient. In general, we found few sire families that exhibited superior performance at both levels tested along an environmental gradient, and no such examples for the attractiveness index of male song (AT) when tested along the temperature gradient.

Examination of phenotypic plasticity in both populations revealed no patterns of relationships between different indices of performance or between different environmental gradients. That is, sire families that exhibited high levels of phenotypic plasticity for male attractiveness (AT) did not necessarily exhibit plasticity

Table 2. Results of mixed-model two-way ANOVA (general linear model) on developmental performance indices (DP, development period; BW, body weight at eclosion) and song performance indices (PR, pulse-pair rate; PA, peak amplitude; AI, asynchrony interval; AT, attractiveness index of male song) in Louisiana and Maryland populations of *Achroia grisella*. The two different levels along each environmental gradient represent the fixed factor, and the sire or dam families represent the random factor. *F* values were calculated as: $F_{environment}=MS_{environment}$ / $MS_{environment\times sire}$; $F_{sire}=MS_{sire}$ / ($MS_{dam(sire)}$ + $MS_{environment\times sire}$ − $MS_{environment\times dam(sire)}$); $F_{dam(sire)}=MS_{dam(sire)}$ / MS_{error}; $F_{environment\times sire}=MS_{environment\times sire}$ / $MS_{environment\times dam(sire)}$; $F_{environment\times dam(sire)}=MS_{environment\times dam(sire)}$ / MS_{error}.

Louisiana population

Environmental gradient	Source	df	DP^{-1}		BW		PR		log (PA)		$\sqrt{(AI)}$		AT	
			MS ($\times10^{-5}$)	*F*	MS	*F*	MS	*F*	MS ($\times10^{-2}$)	*F*	MS	*F*	MS	*F*
Temperature	Environment	1	413.38	**1503.68*****	394.93	**40.75*****	4111.73	**39.54*****	49.47	**24.30*****	79.55	0.69	0.0169	0.14
	Sire	9	0.68	1.47	11.88	1.13	43.36	0.32	1.95	0.82	99.64	0.68	0.0654	0.47
	Dam (sire)	29	0.68	1.42	5.99	1.12	119.16	1.34	1.36	1.25	86.18	1.48	0.0636	1.26
	Environment × Sire	9	0.27	0.59	9.93	1.89	106.44	1.22	2.11	1.94	119.45	2.04	0.1275	2.54*
	Environment × Dam (Sire)	29	0.48	**3.15*****	5.356	1.68**	88.64	1.45	1.09	1.04	58.29	0.95	0.0505	1.08
	Error	268[1]	0.15		3.19		61.16		1.05		61.1		0.0466	
Density	Environment	1	21.30	**43.14*****	239.80	**15.46****	73.44	0.78	15.02	**14.58*****	7.65	0.10	0.2680	4.08
	Sire	9	2.27	2.49	4.71	0.26	231.25	2.47	3.29	1.71	62.43	0.68	0.1126	1.13
	Dam (sire)	29	0.86	1.87	11.31	1.17	118.22	0.92	1.89	2.09*	83.75	1.21	0.0818	1.54
	Environment × Sire	9	0.53	1.18	16.61	1.77	103.01	0.84	1.03	1.12	78.99	1.16	0.0731	1.4
	Environment × Dam (Sire)	29	0.46	**5.75*****	9.63	**2.82*****	128.32	**2.41*****	0.91	0.84	69.16	1.16	0.0532	1.29
	Error	230[2]	0.08		3.41		53.15		1.07		59.45		0.0413	
Food availability	Environment	1	7.31	**25.30*****	280.65	**33.82*****	66.40	1.94	20.14	9.44**	22.13	0.42	0.5745	8.12*
	Sire	9	0.43	0.48	2.04	0.14	157.28	2.57	1.66	0.66	65.81	2.23	0.0692	0.96
	Dam (sire)	29	0.83	**3.89*****	12.38	2.06*	80.35	1.64	1.57	1.17	60.22	0.73	0.0557	0.95
	Environment × Sire	9	0.31	1.47	8.77	1.48	31.82	0.65	2.29	1.73	49.84	0.62	0.0745	1.3
	Environment × Dam (Sire)	29	0.21	1.70**	6.02	1.43	48.90	0.95	1.34	1.29	82.05	1.28	0.0585	1.37
	Error	303[3]	0.13		4.22		51.59		1.04		64.21		0.0426	

Continued.

Table 2. Continued.

Maryland population

Environmental Gradient	Source	df	DP^{-1}		BW		PR		PA		$\sqrt{(AI)}$		AT	
			MS ($\times 10^{-5}$)	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Temperature	Environment	1	804.03	**4273.64*****	4609.859	**1386.85*****	14187.12	**81.08*****	133886.10	**272.26*****	1233.85	**21.52*****	0.0000	0
	Sire	14	0.27	0.78	7.458	1.35	110.68	0.58	689.60	1.26	100.83	1.63	0.0594	0.51
	Dam (sire)	45	0.32	2.00**	4.924	1.82*	53.53	1.38	324.60	1.22	56.13	1.09	0.0625	1.88*
	Environment × Sire	14	0.19	1.19	3.337	1.24	175.89	**4.53*****	492.60	1.84	57.38	1.12	0.0873	2.61**
	Environment × Dam (Sire)	45	0.16	**3.2*****	2.698	**1.72****	38.68	0.81	267.10	0.72	51.49	1.07	0.0333	0.71
	Error	490[4]	0.05		1.573		47.62		373.10		48.31		0.0470	
Density	Environment	1	2.06	10.44**	104.12	**57.66*****	1171	9.44**	10470.90	**27.34*****	54.49	1.50	0.0064	0.07
	Sire	14	0.25	0.75	1.903	0.59	113.23	0.73	292.90	0.55	131.6	2.14	0.0895	0.61
	Dam (sire)	45	0.32	1.81*	4.156	1.52	103.79	1.44	517.20	1.43	61.72	1.71*	0.0855	**2.66*****
	Environment × Sire	14	0.2	1.16	1.806	0.66	124.51	1.72	383.80	1.06	36.27	1.00	0.0942	**2.88****
	Environment × Dam (Sire)	45	0.17	**2.13*****	2.73	1.5*	72.01	0.78	361.40	1.12	36.14	0.76	0.0321	0.44
	Error	436[5]	0.08		1.83		92.77		324.00		47.31		0.0732	
Food availability	Environment	1	2.44	**14.48****	17.839	7.86*	126.55	2.61	6731.40	**14.19****	341.8	7.27*	0.00084	0.02
	Sire	14	0.32	1.06	3.701	1.21	153.81	2.86	622.10	1.29	102.27	1.77	0.1338	2.15
	Dam (sire)	45	0.28	1.96*	3.706	1.27	78.63	1.08	399.00	1.02	75.47	1.17	0.0708	1.47
	Environment × Sire	14	0.17	1.20	2.274	0.78	48.18	0.66	475.90	1.22	46.93	0.73	0.0398	0.83
	Environment × Dam (Sire)	45	0.14	**2.33*****	2.922	**1.83*****	73.04	0.95	391.40	1.19	64.55	1.28	0.0480	0.84
	Error	433[6]	0.06		1.59		77.22		328.20		50.61		0.0569	

[1] df for BW=268; df for PR, PA, and AI=220.
[2] df for BW=236; df for PR, PA, AI, and AT=181.
[3] df for BW=226; df for PR, PA, AI, and AT=177.
[4] df for BW=434; df for PR, PA, AI, and AT=467.
[5] df for BW=473; df for PR, PA, AI, and AT=453.
[6] df for BW=461; df for PR, PA, AI, and AT=435.

$^{*}P < 0.05$; $^{**}P = 0.01$; $P = 0.001$. *F*-values in boldface indicate $P<0.05$ following correction for multiple tests by the Holm adjustment.

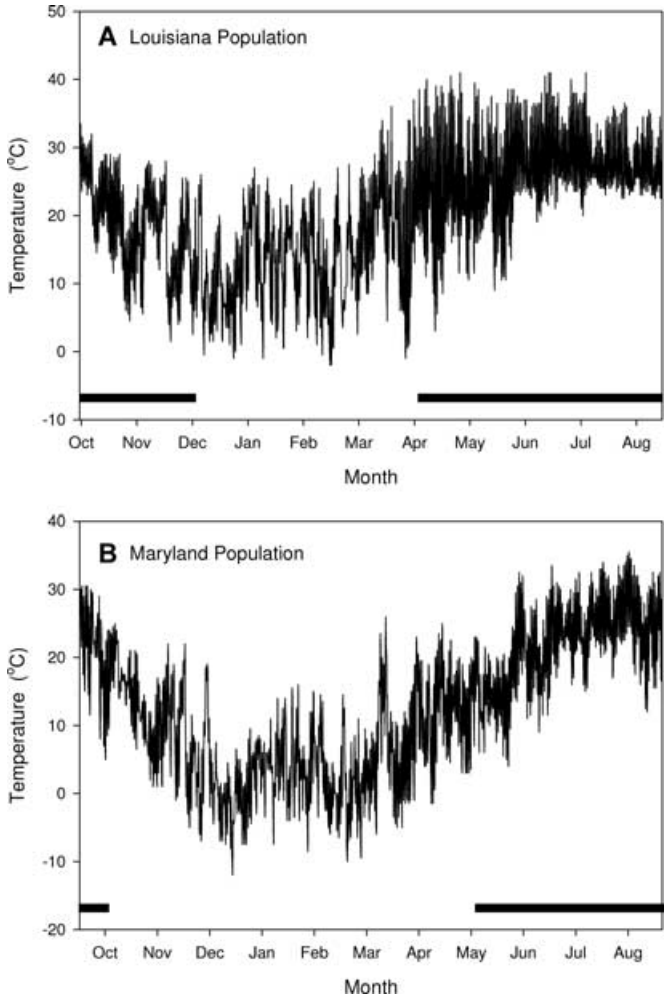

Figure 1. (A) Example of temperature recordings over a one-year period (September 2005–August 2006) in Baton Rouge, Louisiana. (B) Example of temperature recordings at Beltsville, Maryland. Records indicate values taken every 4 h with a Dallas Semiconductor ibutton placed at a location inside a honey bee colony typical of *A. grisella* infestations. Thick horizontal bars indicate months during which adult *A. grisella* were observed in the field. Tick marks along x-axis indicate first day of month.

for body weight or developmental period along a given environmental gradient (Fisher's exact test; $P > 0.05$ for all comparisons). From the other perspective, sire families that exhibited high levels of phenotypic plasticity along one environmental gradient did not necessarily exhibit plasticity along another gradient for a given performance index, either AT, body weight or developmental period ($P > 0.05$ for all comparisons).

Estimates of the variance components from our REML analysis showed that additive genetic variance and maternal effects varied among traits, environmental conditions, and populations (see online Supplementary Table S1). For developmental rate, additive genetic effects were weak in both populations, as judged by the low estimates of V_A under most of the environmental conditions. Maternal effects, on the other hand, explained 27–75% and 27–40% of the total phenotypic variance for developmental rate in the Louisiana and Maryland populations, respectively. These relatively strong maternal effects were observed across environmental conditions, save for low temperature in the Louisiana population and low density in the Maryland population, where additive genetic effects were stronger than maternal effects. For body weight, weak maternal effects, but strong sire effects (V_A), were found under both high and low temperatures in the Louisiana population, whereas maternal effects were relatively stronger than sire effects under other environmental conditions. The effects of both V_A and V_M on body weight were weak in the Maryland population across all environmental conditions. For male song traits, V_A explained 0–32%, 0–42%, 0–32%, and 0–58% of the total phenotypic variances for PR, PA, AI and the attractiveness index (AT), respectively, across all environmental conditions in the Louisiana population. Similarly, in the Maryland population, V_A explained 0–39%, 0–20%, 0–20%, and 0–26% of the total phenotypic variances for these respective traits. The effects of V_M were generally weak for individual signal traits as well as the AT in both populations.

The REML estimates of the variance components between environments are generally consistent with the results of the mixed-model ANOVA, where most of the dam × environment interaction terms, but not sire × environment terms, were significant for developmental rate and body mass under the three environmental gradients tested (see Table 2 and online Supplementary Table S2). Under the assumption that epistatic interactions among more than two loci are negligible (see Groeters 1988), the estimate of the nonadditive genetic component plus the maternal-effect component of the GEI ($V_{I(M+NA))}$ for developmental rate and for body mass comprised 25–70% and 10–76%, respectively, of the total phenotypic variance (Vp) in the Louisiana population. In the Maryland population, the respective values were 50–59% and 27–52% of V_P. For comparison, the additive genetic components of the GEI ($V_{I(A))}$ for the same two traits were 0–7% and 0–32%, respectively, in the Louisiana population, and 0–4 and 0–8%, respectively, in the Maryland population. Similarly, the contribution of $V_{I(M+NA)}$ to Vp was generally higher than the contribution of $V_{I(A)}$ for the several male song traits as well as the attractiveness index under all the three environmental gradients in both populations. However, within each population the contribution of $V_{I(M+NA)}$ varied among song traits and environmental treatments.

Discussion

Our studies of reaction norms in two natural populations of *A. grisella* in North America reveal considerable variation among these environmental responses, that this variation has a significant

Table 3. Pearson product-moment correlations for sire families (r_{m1}) and dam families (r_{m2}) in both Louisiana and Maryland populations of *Achroia grisella* for developmental performance indices (DP, development period; BW, body weight at eclosion) and song performance indices (PR, pulse-pair rate; PA, peak amplitude; AI, asynchrony interval; AT=attractiveness index of male song). Values are given for three environmental gradients; parenthetic values are 95% confidence limits (Sokal and Rohlf 1995).

Environmental gradient		DP	BW	PR	PA	AI	AT
Louisiana population							
Temperature	r_{m1}	0.486	0.256	0.094	−0.278	−0.486	−0.433
		(0.197, 0.697)	(−0.065, 0.529)	(−0.233, 0.402)	(−0.549, 0.046)	(−0.697, −0.197)	(−0.661, 0.132)
	r_{m2}	0.277	0.056	0.050	0.156	0.032	0.077
		(−0.047, 0.548)	(−0.264, 0.365)	(−0.274, 0.364)	(−0.173, 0.453)	(−0.291, 0.348)	(−0.249, −0.387)
Food availability	r_{m1}	0.572	−0.047	0.819	−0.101	−0.025	−0.528
		(0.304, 0.756)	(−0.365, 0.281)	(0.676, 0.902)	(−0.412, 0.231)	(−0.342, 0.297)	(−0.728, −0.246)
	r_{m2}	0.560	0.153	0.349	0.080	−0.085	−0.058
		(0.288, 0.748)	(−0.180, 0.454)	(0.97, 0.559)	(−0.251, 0.394)	(−0.398, 0.246)	(−0.375, 0.271)
Density[1]	r_{m1}	0.717	−0.552	0.347	0.520	0.100	0.072
		(0.512, 0.845)	(−0.741, −0.282)	(0.031, 0.600)	(0.240, 0.720)	(−0.227, 0.407)	(−0.254, 0.383)
	r_{m2}	0.248	−0.235	−0.096	0.313	−0.006	0.063
		(−0.083, 0.529)	(−0.516, 0.092)	(−0.403, 0.231)	(−0.007, 0.453)	(−0.325, 0.314)	(−0.262, 0.375)
Density[2]	r_{m1}	0.492	0.146	0.375	0.32	0.228	0.003
		(0.200, 0.704)	(−0.187, 0.449)	(0.063, 0.620)	(−0.004, 0.584)	(−0.099, 0.510)	(−0.321, 0.327)
	r_{m2}	0.415	0.225	0.158	0.281	0.022	0.064
		(0.105, 0.651)	(−0.107, 0.512)	(−0.107, 0.402)	(−0.047, 0.555)	(−0.304, 0.344)	(−0.266, 0.380)
Density[3]	r_{m1}	0.604	0.303	0.616	0.105	0.454	−0.013
		(0.348, 0.776)	(−0.023, 0.571)	(0.369, 0.782)	(−0.227, 0.415)	(0.157, 0.676)	(−0.336, 0.312)
	r_{m2}	0.372	0.333	0.201	0.142	0.216	0.071
		(0.055, 0.621)	(0.01, 0.593)	(−0.063, 0.439)	(−0.191, 0.446)	(−0.116, 0.505)	(−0.259, 0.386)

Continued.

Table 3. Continued.

Environmental gradient		DP	BW	PR	PA	AI	AT
Maryland population							
Temperature	r_{m1}	0.256	0.395	−0.245	0.198	0.386	−0.173
		(0.002, 0.479)	(0.157, 0.590)	(−0.470, 0.010)	(−0.059, 0.430)	(0.146, 0.583)	(−0.409, 0.085)
	r_{m2}	0.332	0.319	−0.047	0.077	0.209	0.123
		(−0.085, 0.540)	(0.071, 0.530)	(−0.297, 0.209)	(−0.180, 0.325)	(−0.047, 0.440)	(−0.135, 0.366)
Food availability	r_{m1}	0.239	0.247	0.511	0.108	0.527	0.59
		(−0.016, 0.465)	(−0.007, 0.471)	(0.295, 0.677)	(−0.150, 0.352)	(0.315, 0.689)	(0.395, 0.734)
	r_{m2}	0.251	0.154	0.197	0.024	0.216	0.301
		(−0.003, 0.475)	(−0.104, 0.393)	(−0.060, 0.429)	(−0.231, 0.276)	(−0.040, 0.445)	(0.051, 0.516)
Density[1]	r_{m1}	0.032	−0.029	−0.058	−0.198	0.55	−0.04
		(−0.224, 0.284)	(−0.281, 0.227)	(−0.307, 0.199)	(−0.430, 0.059)	(0.344, 0.705)	(−0.291, 0.216)
	r_{m2}	0.216	0.244	0.089	0.119	0.329	0.28
		(−0.040, 0.445)	(−0.011, 0.469)	(−0.169, 0.335)	(−0.139, 0.362)	(0.082, 0.538)	(0.028, 0.498)
Density[2]	r_{m1}	−0.076	−0.082	0.227	0.197	0.013	0.428
		(−0.324, 0181)	(−0.329, 0.176)	(−0.029, 0.455)	(−0.060, 0.429)	(−0.242, 0.266)	(0.195, 0.615)
	r_{m2}	0.191	0.265	0.131	0.100	0.137	0.195
		(−0.066, 0.424)	(0.012, 0.486)	(−0.127, 0.373)	(−0.158, 0.345)	(−0.121, 0.378)	(−0.062, 0.428)
Density[3]	r_{m1}	0.565	0.546	0.43	0.532	0.423	0.566
		(0.363, 0.716)	(0.339, 0.703)	(0.198, 0.617)	(0.322, 0.692)	(0.189, 0.611)	(0.364, 0.717)
	r_{m2}	0.441	0.227	0.104	0.078	0.193	0.214
		(0.211, 0.625)	(−0.029, 0.455)	(−0.154, 0.349)	(−0.179, 0.325)	(−0.064, 0.426)	(−0.042, 0.444)

[1]Density gradient assessed by comparison of rearing conditions 3 versus 4, Table 1.

[2]Density gradient assessed by comparison of rearing conditions 4 versus 5, Table 1.

[3]Density gradient assessed by comparison of rearing conditions 3 versus 6, Table 1.

genetic component, and that it may be sufficient to maintain the genetic variance observed among male advertisement signals. As previously found in laboratory populations of selected (Jia et al. 2000) and inbred lines (Danielson-François et al. 2006), we report that between 1/3 and 1/2 of all sire family pairs exhibit crossover interactions for the traits and environmental gradients that we tested. Moreover, for the attractiveness of male song, a rank-order statistical test designed to evaluate crossover interaction indicates that a substantial proportion of the crossovers observed for the attractiveness of male song along the temperature gradient is significant.

We speculate that male song attractiveness may show a higher incidence of crossover than the other traits we measured because attractiveness represents a composite of several traits that are

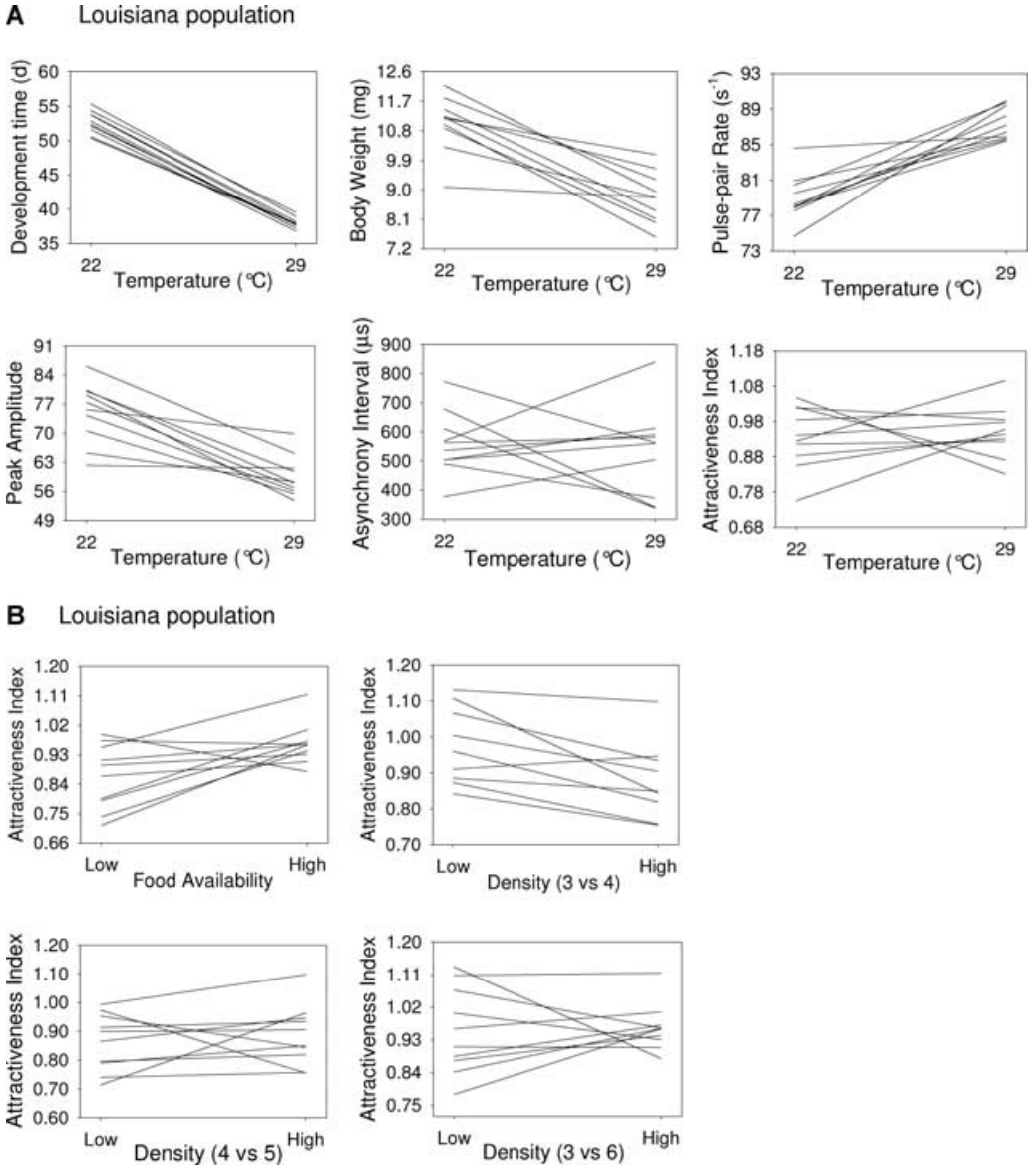

Figure 2. **Reaction norms for sire families of *A. grisella* populations as measured for developmental performance indices (development period, body weight) and male song performance indices (pulse-pair rate, peak amplitude, asynchrony interval, attractiveness index) and along five different environmental gradients (rearing temperature, food availability, density). Density is evaluated along three different gradients: environmental rearing conditions 3 versus 4, 4 versus 5, and 3 versus 6 (see Table 1 for definitions of environmental rearing conditions). (A) Reaction norms in the Louisiana population for all six performance indices along the temperature gradient. (B) Reaction norms in the Louisiana population for male song attractiveness along the food availability and all three density gradients. (C) Reaction norms in the Maryland population for all six performance indices along the temperature gradient. (D) Reaction norms in the Maryland population for male song attractiveness along the food availability and all three density gradients. Values for peak amplitude and the attractiveness index are given in arbitrary units along a linear scale.**

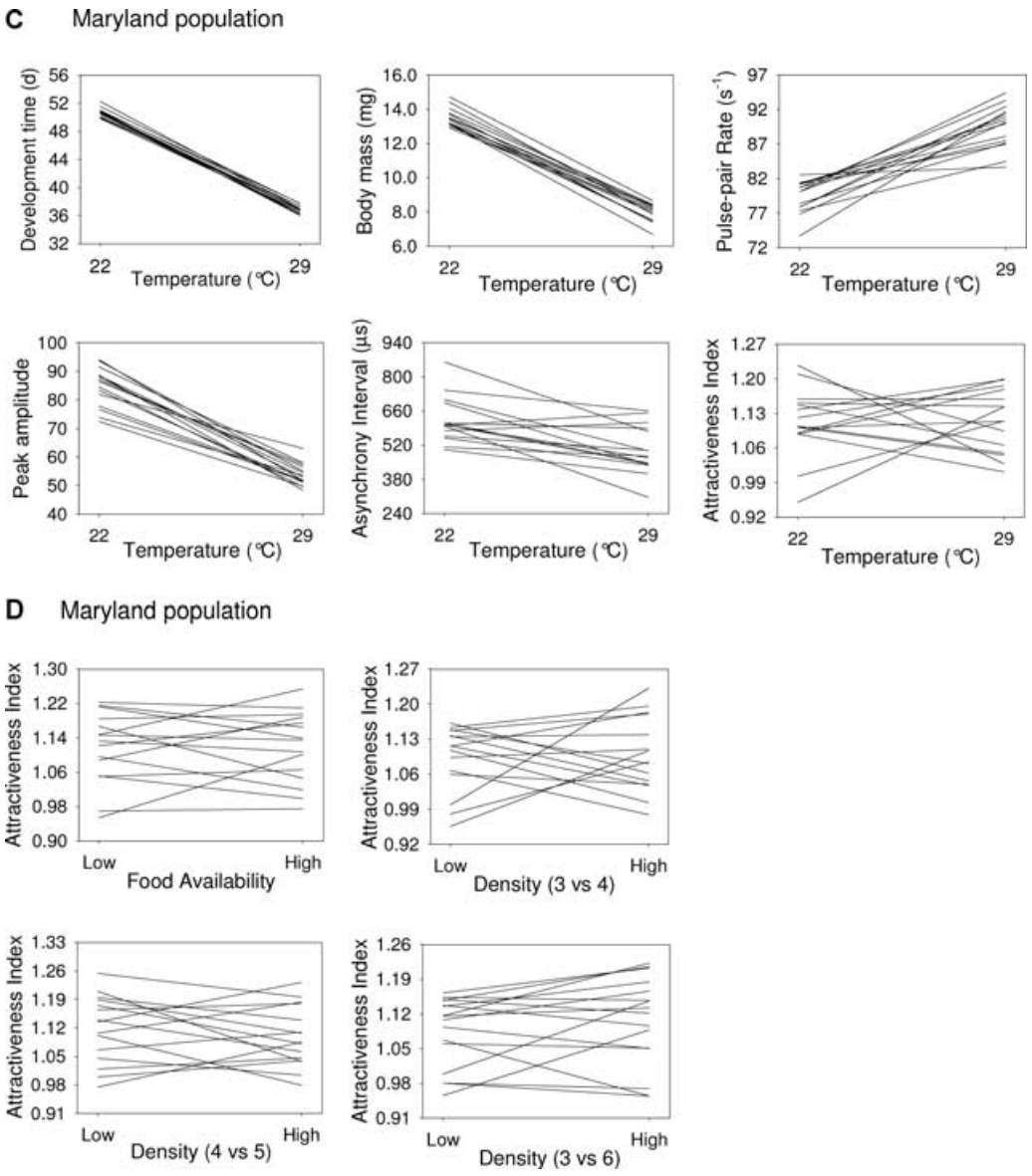

Figure 2. Continued.

partially independent. If a genotype's expression of one signal character, for example peak amplitude, responds to developmental temperature in a different manner from the way its expression of another character, for example pulse-pair rate, responds, an index representing these several characters could show greater diversity of reaction norms than any single character. Using this particular example, we note that peak amplitude and pulse-pair rate have previously been shown to be negatively correlated, presumably a biomechanical outcome of the positive and negative influences of body size (weight) on amplitude and rate, respectively (Brandt and Greenfield 2004). Thus, one might initially expect variation for overall attractiveness of male song to be rather limited. However, the relationships between size and song characters, as well as the influence of developmental temperature on these relationships, may vary genetically. Such possibilities could account for the observed variance in male song attractiveness and the diversity of reaction norms we report in this study. Viewed from another perspective, we also note that the crossover observed for male song attractiveness poses a problem for signal reliability: Are females likely to pair with males such that they produce attractive sons when the environment changes? This conundrum is analyzed in Greenfield and Rodriguez (2004), and potential solutions are indicated (see also Hunt et al. 2004).

Can findings on the reaction norms that we have observed and depicted in Figure 2 help us to predict how these variants would fare in the field? Whereas the environmental gradients we have presented in the laboratory certainly lack the complexity found in the field, we can make some basic inferences from our records of

Table 4. Incidence of ecological crossover for developmental performance indices (DP, development period; BW, body weight at eclosion) and song performance indices (PR, pulse-pair rate; PA, peak amplitude; AI, asynchrony interval; AT, attractiveness index of male song) along three environmental gradients in both Louisiana and Maryland populations of *Achroia grisella*. Values indicate the percentage of all possible pairs of sire families for which reaction norms along a given gradient crossed; parenthetic values are percentages of pairs of sire families for which ecological crossover was statistically significant ($P<0.05$) by the modified Azzalini–Cox rank-order test (Baker 1988).

Environmental gradient	DP	BW	PR	PA	AI	AT
Louisiana population						
Temperature	28.89 (0.00)	31.11 (8.89)	48.89 (0.00)	60.00 (4.44)	64.44 (8.89)	62.22 (22.22)
Food availability	33.33 (0.00)	51.11 (2.22)	13.33 (0.00)	53.33 (6.67)	51.11 (4.44)	62.22 (4.44)
Density[1]	31.11 (0.00)	53.33 (20.00)	40.00 (0.00)	24.44 (0.00)	48.89 (6.67)	48.89 (4.44)
Density[2]	24.44 (0.00)	42.22 (0.00)	35.36 (2.22)	24.44 (4.44)	44.44 (0.00)	46.67 (6.67)
Density[3]	26.67 (0.00)	31.11 (4.44)	26.67 (0.00)	46.67 (11.11)	33.33 (0.00)	51.11 (0.00)
Maryland population						
Temperature	38.10 (1.90)	33.33 (1.90)	62.86 (20.00)	43.81 (6.67)	36.19 (2.86)	53.33 (15.24)
Food availability	40.00 (0.95)	41.90 (0.95)	30.48 (3.81)	49.52 (7.62)	30.48 (0.00)	27.62 (0.95)
Density[1]	46.67 (5.71)	50.48 (2.86)	53.33 (11.43)	60.00 (2.86)	30.48 (0.95)	45.71 (9.52)
Density[2]	49.52 (5.71)	47.61 (1.90)	36.19 (2.86)	40.95 (3.81)	52.38 (5.71)	39.05 (1.90)
Density[3]	20.00 (0.00)	19.05 (0.95)	37.14 (10.48)	28.57 (0.00)	40.95 (4.76)	26.67 (1.90)

[1]Density gradient assessed by comparison of rearing conditions 3 versus 4, Table 1.
[2]Density gradient assessed by comparison of rearing conditions 4 versus 5, Table 1.
[3]Density gradient assessed by comparison of rearing conditions 3 versus 6, Table 1.

field environmental conditions. Temperatures registered in honey bee colonies at both the Louisiana and Maryland sites vary sufficiently over the course of the season of *A. grisella* development (Fig. 1) that different variants could be more attractive to local females at different times of the year. This effect may be further sustained by microclimatic differences between specific locations at these sites, as well as by food availability and population density. The latter two factors are expected to vary seasonally and spatially, and although a high incidence of crossover was not found along these environmental gradients, crossover may conceivably be magnified when food availability and population density vary in conjunction with temperature. Such a joint variation likely affects natural populations. We also note that temperature in the field typically fluctuated 10°C on a daily basis (Fig. 1), but we are unable to predict the influence of variation at this scale on the reaction norms and their crossover. Future studies should confirm whether the predicted changes in relative fitness of reaction norm variants do occur in natural populations.

Our analysis of phenotypic plasticity suggests that the diversity of reaction norms reflects the co-occurrence of relatively canalized and plastic genotypes (sire families). For most of the traits and environmental gradients tested, approximately one half of the sire families could be categorized as canalized by virtue of exhibiting rather horizontal reaction norms, whereas the remaining sire families were significantly plastic. Plasticity was unidirectional for some traits and environmental gradients, but others, such as the attractiveness index of male song, showed bidirectionality wherein some sire families performed significantly better under one rearing condition along the gradient and other families performed better under the other condition. As implied above, this bidirectionality may arise from the complex nature of some traits and how the individual components of this complexity respond to different environmental conditions.

A further inference that can be made from analyzing the phenotypic plasticity data is that the various reaction norms generally represent separate traits. We make this claim based on the absence of any patterns in plasticity between different indices or between different environmental gradients. Thus, a genotype's reaction norm for male song attractiveness and for development appear to be independent responses, as are its reaction norms for one of these indices measured along different gradients.

The ultimate objective of our study was to determine whether GEI can contribute to the maintenance of V_A for male *A. grisella* signal characters in the face of directional selection imposed by female choice. Based on the characters we measured among sire families in two different North American populations of this species, our answer is an equivocal yes. Although we have observed significant ecological crossover for male song attractiveness along a thermal gradient that occurs regularly in the field, various uncertainties remain. To begin, song attractiveness, while representing a major influence on the rate at which a male encounters females, is not the only factor that contributes to his mating and reproductive success. In field populations the location at which a male signals, his survivorship (Bonduriansky and

Brassil 2002, 2005), and chance (see Sutherland 1985) are likely to contribute and can reduce the relative importance of song characteristics. Second, our studies of GEI and ecological crossover analyzed the performances of different genotypes along environmental gradients while each was reared separately from another. However, several genotypes most likely co-occur, interact, and compete in the field, and it is not clear that reaction norms under such circumstances would resemble those reported here (Fig. 2). At this point we cannot predict whether the incidence of ecological crossover under a more realistic, competitive milieu would be higher or lower. Finally, we note that substantial variation occurs among *A. grisella* females for responses to male song. As found in male song characters, this variation is repeatable and has a genetic component (Jang and Greenfield 2000). Moreover, phenotypic plasticity, GEI, and ecological crossover exist for the female response trait (Rodriguez and Greenfield 2003). Consequently, a thorough analysis of the role of GEI in maintaining variance in male song attractiveness must necessarily include a parallel study of female response and preference. Again, without this additional information on the response trait, including the nature of its genetic covariance with male song, we cannot predict whether its consideration would increase or decrease the level of crossover for the male song trait that we indicate now (Table 4).

Perhaps the most robust assessment of the hypothesis that GEI maintains variance in male song and resolves the lek paradox would be an experimental investigation of populations subject to different levels of environmental heterogeneity across space and time. In this context, a finding of higher genetic variance in populations subjected to elevated levels of environmental heterogeneity would support the role of GEI. To date, several studies have approached this question for nonsexual traits by comparing populations of different geographical origins (Hawthorne 1997; Santos et al. 1999; Kassen 2002), but only one has experimentally manipulated environments to vary the heterogeneity to which populations are exposed (Mackay 1981). We recognize that such investigation of sexually selected traits would represent a formidable undertaking, and to our knowledge it has not been done with any natural or laboratory population. Here, we propose this challenge as a means for probing the evolutionary mechanisms underlying the lek paradox under the most natural of circumstances.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Estimated variance components of developmental and song performance indices for Louisiana and Maryland populations of *Achroia grisella*.

Table S2. Estimated causal variance components of developmental and song performance indices for Louisiana and Maryland populations of *Achroia grisella*.

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