

Genotype \times environment interaction for male attractiveness in an acoustic moth: evidence for plasticity and canalization

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Abstract

The lek paradox arises when choosy females deplete the genetic variance for male display traits from a population, yet substantial additive genetic variation (V_A) in male traits persists. Thus, the lek paradox can be more generally stated as one of the most fundamental evolutionary questions: What maintains genetic variation in natural populations? One solution to this problem may be found in the condition-dependent nature of many sexually selected traits. Genotype \times environment ($G \times E$) interactions can maintain V_A under conditions of environmental heterogeneity provided certain restrictions are met, although antagonistic pleiotropy has also been proposed as a mechanism. Here, we provide evidence for $G \times E$ interactions and against the role of antagonistic pleiotropy in the maintenance of V_A for sexually selected traits. Using inbred lines of the lesser waxmoth *Achroia grisella*, we measured V_A for song attractiveness, condition and development rate under different competitive environments and found that genotypes differed in their plasticity. We argue that variation persists in natural populations because $G \times E$ interactions prevent any one variant from producing the optimal phenotype across all environments.

Introduction

Aggregations of sexually displaying males, leks, are some of the most spectacular yet paradoxical phenomena in animal behaviour (Wilson, 1975; Borgia, 1979; Kirkpatrick & Ryan, 1991). Lekking males do not provide direct fitness benefits to their mates or offspring (Andersson, 1994; Höglund & Alatalo, 1995; Shuster & Wade, 2003), and the mechanisms for indirect genetic benefits remain unclear (Lande, 1981; Pomiankowski, 1988; cf. Kokko *et al.*, 2002). Directional selection, as generally imposed by female mate choice, should deplete the additive genetic variance (V_A) for male secondary sexual traits (Taylor & Williams, 1982; Charlesworth, 1987), which in turn should relax selection maintaining female preferences. Nonetheless, many investigations reveal that the level of additive genetic variance for male sexually selected traits can be relatively high in natural populations, comparable with the level observed for nonsexual

traits (Bakker & Pomiankowski, 1995). Thus, an attempt to understand female choice for males displaying at leks begs a classical evolutionary question: What maintains genetic variation in natural populations? (Lewontin, 1974).

Whereas evolutionary geneticists identify no fewer than seven mechanisms that can, in theory, maintain (additive) genetic variance within a population (Barton & Turelli, 1989; Roff, 1997; Lynch & Walsh, 1998), most investigations of sexually selected traits have concentrated on mutation-selection balance, genetic tradeoffs, and environmental heterogeneity. The recognition that the expression of sexually selected traits, particularly male features evaluated by females, often depends on development, energy reserves, or physiological state (David *et al.*, 2000; Scheuber *et al.*, 2003, 2004; but see caveats in Cotton *et al.*, 2004) – qualities collectively termed ‘condition’ – is central to the various hypotheses and studies.

One explanation, the ‘genic capture hypothesis’, argues that substantial additive genetic variance exists for condition (e.g. Merilä *et al.*, 2001) – and hence male secondary-sexual traits. This variance could be

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maintained by mutation-selection balance because genes at many loci throughout the genome are expected to influence condition (Rowe & Houle, 1996; cf. Kotiaho *et al.*, 2001). This explanation has received renewed interest (Tomkins *et al.*, 2004), but several tests of its predictions are equivocal in support.

A second explanation is that a negative genetic correlation, a 'genetic tradeoff', exists between life-history and sexually selected traits (Charlesworth & Hughes, 2000). Antagonistic pleiotropy can maintain additive genetic variance provided appropriate dominance relations among alleles (Curtis *et al.*, 1994; Chippindale *et al.*, 2001) and an adequate mutation rate to counter the loss of alleles because of genetic drift (Lynch & Hill, 1986; Lynch & Walsh, 1998). Traits evolving under antagonistic pleiotropy are not necessarily required to be expressed in both sexes, as argued by Hedrick (1999). Instead, theoretical modelling has shown that sex-limited expression slows the loss of alleles (Reinhold, 2000). A recent study has demonstrated a negative genetic correlation between male attractiveness and survival in guppies and implicated this tradeoff in maintaining genetic variance for attractiveness (Brooks, 2000; cf. Hunt *et al.*, 2004). However, general findings among animals indicate that such tradeoffs are more likely the exception than the rule: the

most attractive males in a population are typically those of superior condition with regard to size, development rate, survival to adulthood, and longevity, while relatively unattractive males are usually found to be in poor condition (Jennions *et al.*, 2001).

A third explanation is based on the expectation that condition-dependent traits, by their very nature, exhibit phenotypic plasticity (see Fig. 1 for a heuristic diagram; see Schlichting & Pigliucci, 1998; e.g. Griffith *et al.*, 1999). Phenotypic plasticity is absent when a genotype exhibits a horizontal reaction norm (Fig. 1a) and present when that reaction norm has a nonzero slope (Fig. 1b). Phenotypic plasticity combined with environmental heterogeneity can sustain genetic variation under the following circumstances. Consider 2 genotypes, A and B, for a male display trait whose mean phenotypic expressions are markedly different along an environmental gradient, illustrating one type of genotype × environment ($G \times E$) interaction (Fig. 1c). When one genotype, A, has the capacity to produce the optimum phenotype across all environments (Fig. 1c), $G \times E$ interactions are unlikely to maintain genetic variation. But when neither genotype exhibits the superior phenotype over the entire gradient, a $G \times E$ interaction known as 'crossover' occurs (see Roff, 1997;

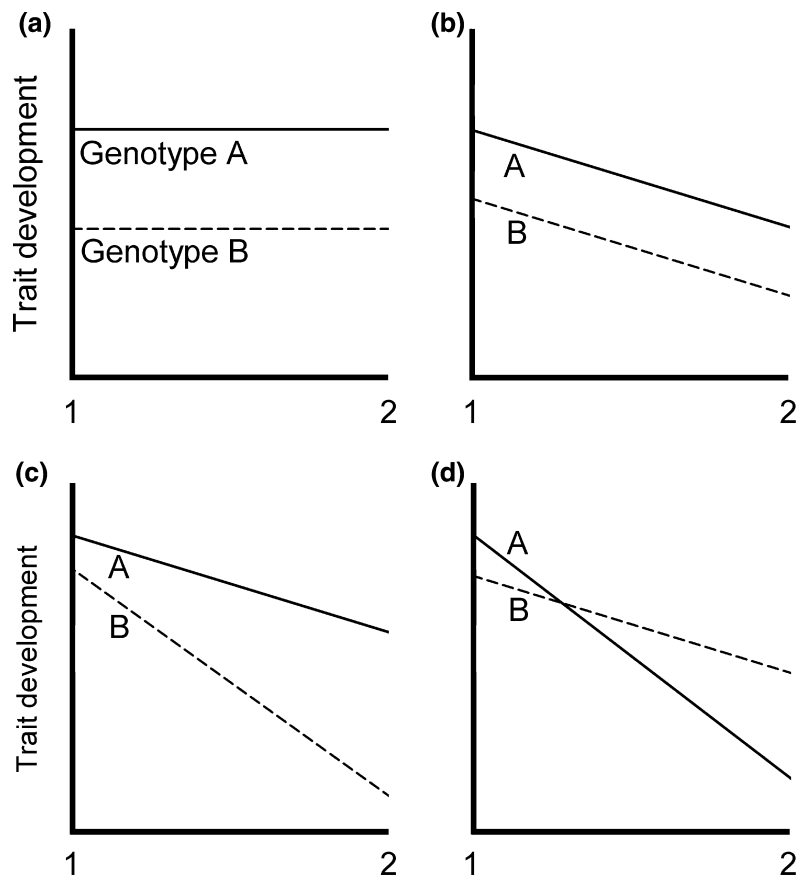


Fig. 1 Heuristic diagram of various forms of phenotypic plasticity. (a) Genotypes A and B exhibit different levels of trait development in both environments 1 and 2, but the levels expressed by both genotypes do not change across environments, i.e. reaction norms (solid and dashed lines) are flat and phenotypic plasticity is absent. (b) Genotypes A and B exhibit comparable reductions in development in environment 2, i.e. reaction norms are steep but parallel, and both genotypes exhibit phenotypic plasticity. (c) Genotypes A and B exhibit different reductions in development in environment 2, i.e. reaction norms are not parallel, and a genotype × environment interaction ($G \times E$) is present. (d) Reaction norms of A and B intersect, i.e. ecological crossover is present, with each genotype exhibiting the greater trait development in only one of the two environments.

Lynch & Walsh, 1998); this designation is used because the reaction norms of the two genotypes intersect (Fig. 1d). Ecological crossover is a necessary first step, but by itself is not sufficient to maintain genetic variation (Via & Lande, 1987; Prout & Savolainen, 1996; Prout, 2000). An under-appreciated point of Levene's (1953) original model is that additional requirements are necessary. Theoretical modelling of the 'environmental heterogeneity hypothesis' has shown that crossover interactions can maintain additive genetic variation (see Felsenstein, 1976; Slatkin, 1978) if (i) environments vary spatially and dispersal occurs among environments (Gillespie & Turelli, 1989), or if (ii) variation is temporal and generations overlap (Charlesworth, 1988; Ellner & Hairston, 1994; Proulx, 2001). Simple one locus-two allele models of $G \times E$ interaction have restrictive requirements for genotypic fitness in each environment, but it is unclear how such models apply to polygenic quantitative traits (Via & Lande, 1987; Gillespie & Turelli, 1989) whose mutational input continually replaces alleles lost to selection or genetic drift (Lynch & Hill, 1986; Lynch & Walsh, 1998). For empirical studies of this general process, see Mackay (1981), Hawthorne (1997), Merilä & Fry (1998), Santos *et al.* (1999), Kruuk *et al.* (2001), Svensson *et al.* (2001), and Kassen (2002).

For sexually-selected traits, several laboratory studies have demonstrated significant $G \times E$ interaction, including crossover, for male signalling and female preference (Jia *et al.*, 2000; Rodríguez & Greenfield, 2003; also see Qvarnström, 1999; Welch, 2003 for $G \times E$ interactions in field populations). In the lesser waxmoth, *Achroia grisella* (Fabricius 1794; Lepidoptera: Pyralidae), high- and low-lines for several male signal characters were created via two-way artificial selection, and these lines showed significant crossover when reared under different environmental regimes (Jia *et al.*, 2000). However, overall male attractiveness in these lines was unclear, and other factors potentially maintaining additive genetic variance were not assessed.

The present study continues this line of inquiry with a quantitative genetic experiment using a set of randomly derived inbred lines from a natural *A. grisella* population. The experiment includes replicate insects from each line, as well as F1 families derived from crosses between inbred lines. The two experimental populations (the lines and F1s) provide complimentary information regarding genetic covariances among traits (potential trade-offs) and genotype by environment interactions. We measure overall male attractiveness in moths reared under different environmental regimes, in addition to measurements of major developmental characters. We test both the genetic tradeoff and environmental heterogeneity hypotheses. The results provide no evidence that any developmental feature offsets the disadvantages that unattractive males experience in mating. However, there is a high incidence of crossover for male attractiveness between lines reared under different environments.

These findings further support the environmental heterogeneity hypothesis that could, at least in part, explain the 'lek paradox' in this species.

Methods

Lek paradox in *A. grisella*

Achroia grisella males display an ultrasonic calling song that is attractive to females up to 1–2 m distant (Spangler *et al.*, 1984). Playback experiments and choice tests have shown that a male's attractiveness and mating success are positively influenced by three song characters: peak amplitude, pulse-pair rate, and the length of silent gaps within pulse-pairs (Jang & Greenfield, 1996, 1998). Despite this mate choice pressure, considerable additive genetic variance remains: all three characters have been found to be both repeatable and heritable within several populations examined (Collins *et al.*, 1999; Brandt, 2003). Selection gradient analysis was used to calculate coefficients for weighting each character in an index of overall song attractiveness (Jang & Greenfield, 1998), which was also found to be heritable (Brandt, 2003).

Signal characters, recording and analysis

Males produce their ultrasonic courtship song by wing-fanning, which causes a pair of tymbals at the forewing bases to buckle in and out (Spangler *et al.*, 1984). A male's tymbals buckle twice during each cycle of wing movement, once on the upstroke and once on the downstroke. Each buckling produces a highly damped 100- μ s pulse of 70–130 kHz sound. Due to the slightly asynchronous movement of the wings, a pair of two sound pulses, one from each tymbal and separated by a brief (100–1200 μ s) silent gap, is usually produced during each upstroke and downstroke. Male songs were recorded in a semi-anechoic chamber during scotophase under temperature and photoperiod identical to that used in rearing. A condenser microphone (ACO Pacifica Model 7016 (Belmont, CA, USA); frequency response ± 2 dB from 10 to 100 000 Hz; ± 6 dB from 10 to 160 000 Hz) recorded the ultrasonic courtship song, which was amplified 40 dB and then digitized at 500 kHz using an anti-aliasing filter (Pettersson Elektronik model F2000, Uppsala, Sweden), an I/O card (National Instruments DAQ-6062E, Austin, TX, USA), and signal processing software (Pettersson Elektronik, Batsound Pro). From a 1-s sample of the digitized male song, the mean peak amplitude (PA), asynchrony interval (AI = interval between onsets of two pulses in a pair), and pulse-pair rate (PR = wingstroke rate) were extracted using a custom computer program adapted from other signal processing software (Cambridge Electronic Design, Spike2, Cambridge, UK). Selection gradient analysis of song characteristics from *A. grisella* males was used to develop an overall index of

attractiveness ($AT = 0.524PA + 0.296AI + 0.117PR$; see Jang & Greenfield, 1998).

Environmental heterogeneity and assay of male condition

Achroia grisella are obligate symbionts of the western honeybee, *Apis mellifera*. The moth larvae feed on honeycomb, stored organic material, and detritus in and around 'weakened' bee colonies that have decreasing numbers of workers (Künike, 1930). Adult moths stay in the vicinity of the natal colony if these nonrenewable food resources are still available, and males display in small leks in, on, or near the colony (Greenfield & Coffelt, 1983). Environmental heterogeneity arises for *A. grisella* because larval density, and thus food resources, vary considerably both between honeybee colonies and within colonies over time as they advance through the normal colony ageing process. Male and female *A. grisella* adults live only 7–14 days and neither feed nor drink, indicating that larval nutrition and growth are the main determinants of condition. Thus, body mass is an accurate measure of energy reserves for a newly eclosed adult male and can serve as a fair proxy for condition.

Derivation of inbred lines and environmental regimes

To examine the mechanism(s) maintaining variation in condition and male song attractiveness in *A. grisella*, we developed random inbred lines from a natural population originally collected in Kansas and kept as an outbred colony since 1997 with an effective population size ≥ 500 . We maintained 11 lines in the laboratory through nine generations of full-sib mating, which is predicted to have reduced heterozygosity to a level below 18% of that found initially (Crow & Kimura, 1970). This breeding procedure, conducted in the absence of any artificial selection, effectively compartmentalized genetic variation present in the population into distinct units, i.e. lines, that could be treated as distinct genotypes. In the ninth generation, we obtained 90 second-instar larvae from each line. We divided each sample of 90 larvae equally among three environmental regimes, 30, 12, and 4 g of artificial diet in a 0.12-L polythene container, and reared them to adult. These regimes, representing nearly *ad libitum* (30 g) to adverse (4 g) nutritional conditions, generated low-, medium-, and high-density competition, respectively, and were designed to resemble the situations that natural populations of *A. grisella* would regularly experience as food resources in ageing bee colonies diminish. We measured the development rate, determined as the reciprocal of the period from oviposition to adult eclosion, and body mass at eclosion of each individual in all 33 samples (i.e. 11 lines in three treatments). The mean sample size per inbred line per treatment is 7.73 ± 0.40 (SE) for a total of 255 male waxmoths. We also recorded each male's song and then

measured the three critical characters and a composite attractiveness index (see below).

Genetic tradeoffs

Life-history traits such as fecundity, lifespan and developmental rate could be in antagonistic pleiotropy with attractiveness. We have previously shown that body size (and fecundity) and lifespan do not exhibit antagonistic pleiotropy with male attractiveness (Brandt & Greenfield, 2004). Increased male attractiveness is related to increased size, greater fecundity and longer lifespan. Development rate is potentially a target trait for antagonistic pleiotropy because there is an intrinsic relationship between song attractiveness and condition (as measured by body mass). Thus, small males, who generally do not sing attractively, might be compensated by fast development. To determine whether antagonistic pleiotropy (i.e. a genetic tradeoff) exists between male song attractiveness and development rate under any of the three rearing environments, we measured all eclosing male individuals from each inbred line and calculated inbred line mean values for the two traits in each environment. Individual attractiveness data were normalized via log transformation prior to analysis. We computed Pearson product-moment correlations, r , of mean log (attractiveness) vs. mean development rate for the inbred lines, separately in each of the three environments. A significantly negative correlation would be interpreted as a genetic tradeoff between the two traits.

Line cross analyses

Because variation in inbreeding depression among lines might have confounded the above analysis of genetic tradeoffs by biasing genetic correlations towards positive values (Lynch & Walsh, 1998), we conducted a second analysis using F1 hybrids. We created eight different hybrid (i.e. outbred) crosses from the 11 tested lines and measured the genetic correlations of F1 progeny among song attractiveness, development rate, and condition for each of the three environmental regimes. As above, we calculated trait mean values for these line cross hybrids under each environment and analysed the correlations among song and development traits.

Phenotypic plasticity

To measure the extent of phenotypic plasticity, we used mixed-model ANOVA to assess the differences in male song attractiveness, body mass, and development rate across the three rearing environments and also within inbred lines.

G × E interactions

To measure the extent of G × E interactions, we first used mixed-model ANOVA and examined the significance of

interaction effects (Fry, 1992; Lynch & Walsh, 1998) for male song and life-history traits. We then calculated the cross-environment genetic correlation (Lynch & Walsh, 1998), ρ_x , which implicitly assumes that a trait's expression in two environments represents two different traits. Using the variances and covariances of inbred line mean values, the additive genetic correlation across environments can be estimated as:

$$\rho_x = \frac{\sigma_G^2 - \sigma_I^2}{\sqrt{(\sigma_G^2 + \sigma_I^2)^2 - 4\sigma_{G,I}^2}}$$

where σ_G^2 , σ_I^2 and $\sigma_{G,I}$ denote the genotypic variance, the interaction variance and the covariance between them respectively. When $G \times E$ interactions are absent (Fig. 1a,b), $\sigma_I^2 = \sigma_{G,I} = 0$ and $\rho_x = 1$ (conversely, when there is a negative correlation between genotypic rank in the two environments such that all inbred lines have the same mean rank across environments, $\sigma_G^2 = \sigma_{G,I} = 0$ and $\rho_x = -1$; see Lynch & Walsh, 1998). Therefore, $G \times E$ interactions are more likely when $\rho_x \ll 1$. In order to determine whether the ρ_x values reported are significantly different from +1, we used a bootstrap procedure to generate a distribution of estimates of ρ_x (resampling program written for SAS by L.M. Meffert). Finally, we measured the incidence of crossover interaction for a trait as the number of pairwise comparisons of inbred lines whose reaction norms intersected (Fig. 1d) divided by the total number of pairwise comparisons.

Statistical considerations

Significance levels for all tests were corrected for multiple tests via the Holm procedure (see Holm, 1979; Krauth, 1988). Power analyses were conducted on nonsignificant results by the method of Zar (1999).

Results

Genetic tradeoffs

We detected no significant correlations between song attractiveness and development rate in our lines within any of the three rearing environments for tests on either the inbred lines or their hybrids (Fig. 2). Moreover, no significant shift towards negative genetic correlations between development rate and song attractiveness was found in the hybrids (Fig. 2b,d,f).

Phenotypic plasticity

We found significant differences in male song attractiveness and body mass across the three rearing environments within inbred lines (Table 1), demonstrating phenotypic plasticity for these traits. Developmental rate, however, showed no such effect. Importantly, there was appreciable among-line variation in the level of plasticity

(Fig. 3). For example, some lines exhibited extensive plasticity, whereas other lines exhibited a canalized response across rearing environments (e.g. compare lines a and b, respectively, in Fig. 3a). Significant variation for all three traits was also found across inbred lines within the same environment (Table 1), consistent with estimates for V_A and heritabilities reported previously (Collins *et al.*, 1999; Brandt & Greenfield, 2004).

G \times E interactions

Our preliminary estimation of $G \times E$ interactions via mixed-model ANOVA revealed that song attractiveness had a marginally significant $G \times E$ interaction (Table 1) which did not persist after the Holm (1979) correction for multiple tests. Neither log body mass nor development rate exhibited a $G \times E$ interaction in the mixed-model ANOVA, so $G \times E$ interactions were initially considered to be weak or absent for these two traits. However, a more specific measure, the cross-environment genetic correlation ρ_x , was significantly < 1 for all traits (Table 2), implying significant $G \times E$ interaction (see Fry, 1992) for song attractiveness, body mass and development rate. In agreement with the mixed-model ANOVA results, song attractiveness had the largest ρ_x values. Consistent with these ρ_x values, a high incidence of crossover interactions was observed between inbred lines for song attractiveness (33% of all pairwise comparisons), body mass (39%), and development rate (55%; Fig. 3, Table 2). We also found significantly positive correlations among inbred lines ($r = 0.08$ – 0.57), and among hybrids ($r = 0.30$ – 0.66), between male song attractiveness and body mass in each of the three rearing environments, corroborating previous findings on the genetic covariance of these traits (Brandt & Greenfield, 2004).

Discussion

When combined with data concerning the life history and ecology of *A. grisella*, our crossover data offer strong support for a role of $G \times E$ interactions in maintaining male trait variation, a first step towards the persistence of V_A for male attractiveness. In general, these data agree with previous findings from experiments with artificially selected lines in *A. grisella* (Jia *et al.*, 2000). This study however, provides more robust tests because they focus on overall male attractiveness, not individual song characters, and are based on a set of random inbred lines that collectively represent genetic variance in the population. Further, the absence of negative correlations between attractiveness and development indicate that antagonistic pleiotropy is unlikely to maintain the observed variation. This inference is consistent with previous findings that genetic correlations between song attractiveness and adult body mass, longevity or number of nights or hours per night of singing in *A. grisella* are largely positive (Brandt & Greenfield, 2004). One might

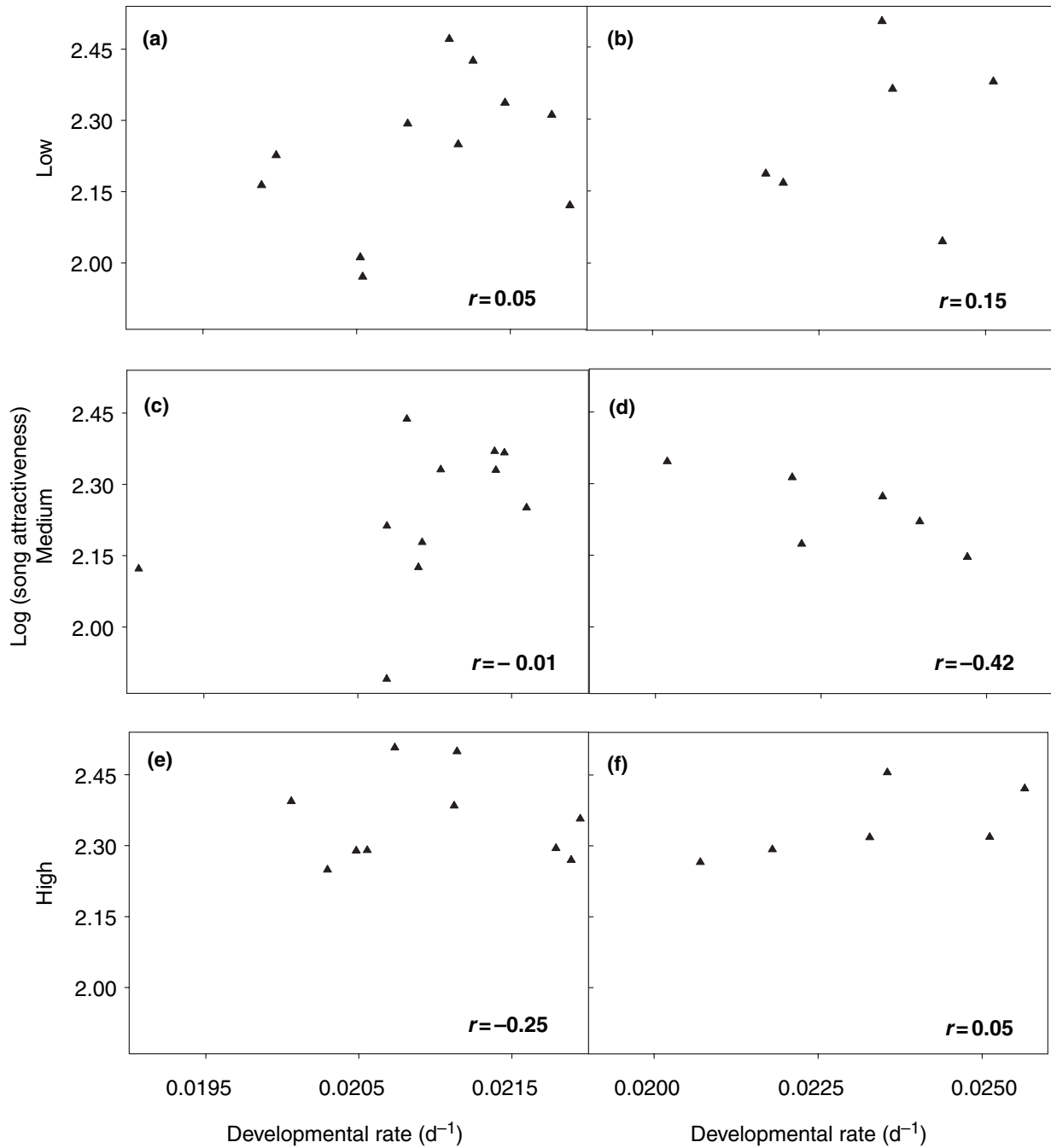


Fig. 2 Correlation between male song attractiveness (log transformed) and development rate under three different rearing environments, designated as low, medium, and high amount of diet. (a, c, e): Random inbred lines. (b, d, f): Hybrid F₁ progeny (see Methods). Symbols represent mean values of the individual lines and their hybrids; Pearson product-moment correlations are shown for each comparison. None of the correlations are significant. Power analyses for a one-tailed test of H_0 (that r is not less than zero) for each correlation coefficient were fairly high for all three environmental treatments (P -values ≥ 0.25 , power range 0.82–0.96; P -values ≥ 0.15 , power range 0.79–0.97, for the inbred lines and their hybrids, respectively; Zar, 1999). Additionally, a one-tailed test of the H_0 that the correlation coefficient for the hybrids is not less than the correlation coefficient for the inbred lines yielded P -values > 0.25 for all three environmental treatments.

suggest that measuring a different suite of traits would be more conducive to uncovering antagonistic pleiotropy, but the most obvious candidates – fecundity, lifespan and

developmental rate – exhibit no genetic tradeoffs with attractiveness. In summary, attractive males are in superior condition: they are larger, do not take longer

Table 1 Analysis of variance summary for male song and life-history traits.

Trait	Source	d.f.	F	P
Log (song attractiveness)	Genotype	10, 252	2.25	0.016*
	Environment	2, 244	4.45	0.013*
	G × E	20, 222	1.70	0.035
Log (body mass)	Genotype	10, 252	2.34	0.012*
	Environment	2, 244	12.56	<0.0001*
	G × E	20, 222	1.45	0.100
Development rate	Genotype	10, 252	1.06	0.398
	Environment	2, 244	0.36	0.699
	G × E	20, 222	1.10	0.350

Analysis of variance (mixed-model) was performed using a general linear model to accommodate unbalanced design. Male song attractiveness and body mass were normalized via log transformation in order to meet the requirement of homogeneous variances (Levene's test: see Brown & Forsythe, 1974). For analysis, data subjected to ANOVA were restricted to genotype (inbred line) samples in which $n \geq 5$ in a given rearing environment. Rearing environment (fixed factor) and line (random factor) statistics were determined prior to inclusion of the interaction. *P*-values reflect a correction for multiple tests using the Holm (1979) method for each trait (* $P < 0.05$ following Holm correction).

to develop, survive longer, and spend more time singing on nightly and lifetime bases (cf. Jennions *et al.*, 2001).

Examination of reaction norms (Fig. 3) reveals that crossover interactions among our *A. grisella* lines arise because some genotypes are relatively plastic and others more canalized. The plastic lines express enhanced condition under favourable nutrition and density but are markedly inferior under stress. The canalized lines generally express modest condition and suffer only slight or no decreases under stress. The more plastic genotypes exhibit environmental sensitivity characterized by a sloping reaction norm (see Fig. 1b–d) and can yield a variety of phenotypes, in contrast to the average phenotype expressed by the more canalized genotypes which are buffered from the environment and characterized by a relatively horizontal reaction norm (Fig. 1a). Importantly, no genotypes exhibited maximum attractiveness and condition under both favourable and stressful environments (Fig. 3). We suggest that underlying physiological or developmental limitations may preclude any one genotype from achieving superior condition across all environments. The large proportion of crossover interactions amongst the 11 inbred lines mitigates against the possibility of random error measurement generating these data. Measurement error could just as easily lead to a reduction in the proportion of crossover as to an increase, given that there are 11 inbred lines and 55 potential crossovers.

Our experimental design, using both inbred lines and F1 families, was developed from considerations of genetic models for both antagonistic pleiotropy and G × E interactions. Antagonistic pleiotropy models suggest that traits should exhibit substantial dominance variance, V_D , at

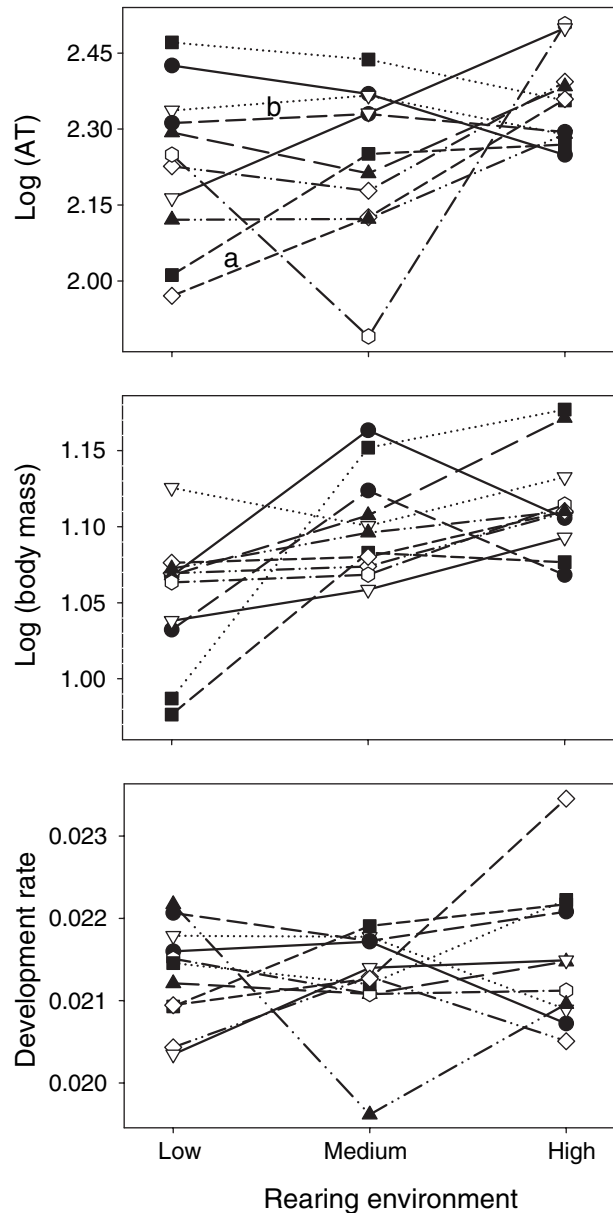


Fig. 3 Male song attractiveness (log transformed), body mass (log transformed), and developmental rate of 11 inbred lines developing under three different rearing environments. Reaction norms of the individual lines are shown. Intersections of reaction norms indicate incidence of G × E interactions. A highly plastic response to rearing environments is characterized by line a, while line b represents a canalized response. Rearing environments are designated as low, medium and high amount of diet.

equilibrium (Lynch & Walsh, 1998). This dominance variance will contribute to the variation within and among outbred F1 families and thus potentially confound estimates of V_A or additive genetic covariances among traits (Falconer & Mackay, 1996). However, V_D does not contribute to the genetic variance among fully

Table 2 Summary of G × E crossover interactions for male song and life-history traits.

Trait	ρ_x values			Proportion crossover
	Low	Medium	High	
Log (song attractiveness)				
Low	–			0.33
Medium	0.49	–		
High	–0.08	–0.45	–	
Log (body mass)				
Low	–			0.39
Medium	–0.09	–		
High	0.30	0.25	–	
Development rate				
Low	–			0.55
Medium	–0.29	–		
High	–0.09	0.19	–	

ρ_x , cross-environment additive genetic correlation (Lynch & Walsh, 1998).

ρ_x values significantly <1 were found in all comparisons. The standard errors for functions based on variance components, like ρ_x , are unknown for complicated designs when data are unbalanced (see Via, 1984; Sokal & Rohlf, 1995), so a bootstrap procedure was used to identify significant results (resampling program written for SAS by L.M. Meffert). The 95% confidence interval for all three traits, for all three comparisons, ranged from a low value of – 0.66 to a high value of 0.63, clearly excluding both +1 and – 1 from the interval. The incidence of G × E crossover interactions is calculated as the number of pairwise comparisons of lines exhibiting intersecting reaction norms (see Fig. 1d) divided by the total number of pairwise comparisons.

inbred lines, and only minimally to the variance among highly inbred lines (as used in this experiment), even when dominance is present at the loci affecting a trait. Instead, the variance among inbred lines is a function of several 'inbreeding dominance components' that are quite different from V_D (Cockerham & Weir, 1984; Kelly, 1999). If alternative alleles segregate at intermediate frequencies, as is likely with antagonistic pleiotropy, then these inbreeding components are likely to be small even when V_D is large (see Cockerham & Weir, 1984 for equations expressing variance components as functions of gene effects and allele frequencies).

A potential difficulty associated with variances and covariances among inbred lines is that rare recessive (or partially recessive) alleles make a disproportionate contribution to the inbreeding dominance components (Charlesworth & Hughes, 2000; Kelly, 2003). Imagine that inbred lines are fixed for differing numbers of deleterious mutations, and that these mutations reduce both attractiveness and condition. The contribution of these loci could induce a positive correlation between attractiveness and condition, obscuring possible antagonistic pleiotropy expressed at other loci. This effect of variation in 'genetic load' is far less pronounced within outbred F1 families. In this way, our separate analyses of

inbred lines and F1 families are complimentary, each obviating different sorts of genetic bias.

The existence of crossover is a necessary, but not sufficient, condition for the maintenance of V_A for male attractiveness. In addition to crossover as demonstrated in the laboratory, the requisite environmental variation must exist in the field. Unpredictable environmental fluctuations are present in the life history of *A. grisella*. A single mated female can disperse to found a new population in an unaffected beehive. As food resources decline and more individuals infest the hive, fluctuations in resource quality and quantity occur as competition for these resources increases. Larval diapause is also exhibited by *A. grisella*, adding temporal variation beyond that found in the seasonal environments they often inhabit. All of the above factors could lead to an overlapping of generations and environmental heterogeneity, as demanded by theory.

Extrapolating our laboratory findings to the field, we speculate that the environmental heterogeneity and dispersal that natural populations of *A. grisella* experience may potentially allow coexistence of various reaction norms (genotypes). Each genotype would have the capacity to produce the optimal phenotype in some environment, but as one or more environmental factors change, the genotype expressing optimal condition – and male signalling – would shift. Because these environmental changes might be rapid and unpredictable, males with suboptimal phenotypes, representing genotypes that had performed optimally at another time or place, would continue to appear. Thus, additive genetic variance could be retained and the lek paradox resolved in this system.

Confirmation that environmental heterogeneity actually maintains additive genetic variance would require some indication (i) that the distribution of reaction norm variants observed in the laboratory reflects that found in a natural population(s), (ii) that environmental fluctuations and dispersal which could potentially generate crossover interactions do occur in the field, and (iii) that natural populations subject to greater environmental heterogeneity exhibit higher levels of additive genetic variance. To date, these data do not exist for *A. grisella*, or for any other animal species to our knowledge.

The existence of widespread G × E interactions strong enough to maintain V_A would seem to undermine the reliability of signal traits exhibiting such ecological crossover (see Greenfield & Rodriguez, 2004). For a lekking species with female choice, like *A. grisella*, the male signal would become an unreliable indicator of future offspring success once the environment changes. If the environment changes unpredictably and multiple genotypic variants exist, choosing a different (unattractive) mate will not necessarily benefit the female. There are multiple developmental routes to male unattractiveness, one such path being the expression of deleterious mutations. Under such circumstances, one might expect females to 'hedge their bets' and mate with multiple

males. But, *A. grisella* females almost always mate singly, implying that there may be a fitness cost to mating with multiple males in the field.

An additional factor that may contribute to maintenance of additive genetic variance in *A. grisella*, and in other species, is variation in female response. Natural populations may exhibit genetic variation in female preferences (Butlin, 1993; Gerhardt *et al.*, 1996; Ritchie, 1996, 2000; Shaw, 2000; Ritchie *et al.*, 2001; Coleman *et al.*, 2004). Artificial selection on female response has revealed significant additive and nonadditive genetic variance for female preferences (Meffert *et al.*, 2002; Meffert & Regan, 2002). Female *A. grisella* exhibit repeatable variance in their preference function for, and response threshold to, male song (Jang & Greenfield, 2000; Rodriguez & Greenfield, 2003; Greenfield & Rodriguez, 2004; Greig & Greenfield, 2004). Further tests revealed heritability, phenotypic plasticity, and G × E interactions for these sexually selected female traits. Because the reaction norm for female response may influence the variance in male song in a population, we propose that future investigations of the lek paradox – which should consider natural populations and field environments – include thorough examination of female preference and response traits alongside analyses of male signalling.

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References

- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Bakker, T.C.M. & Pomiankowski, A. 1995. The genetic basis of female mate preferences. *J. Evol. Biol.* **8**: 129–171.
- Barton, N.H. & Turelli, M. 1989. Evolutionary quantitative genetics: how little do we know? *Ann. Rev. Genet.* **23**: 337–370.
- Borgia, G. 1979. Sexual selection and the evolution of mating systems. In: *Sexual Selection and Reproductive Competition in Insects* (M. S. Blum & N. A. Blum, eds), pp. 19–80. Academic Press, New York.
- Brandt, L.S.E. 2003. Evolutionary origins and consequences of female mate choice in an ultrasonic moth, *Achroia grisella*. PhD Dissertation, University of Kansas, Lawrence, KS.
- Brandt, L.S.E. & Greenfield, M.D. 2004. Condition-dependent traits and the capture of genetic variance in male advertisement song. *J. Evol. Biol.* **17**: 821–828.
- Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**: 67–70.
- Brown, M.B. & Forsythe, A.B. 1974. Robust tests for the equality of variances. *J. Am. Stat. Assoc.* **69**: 364–367.
- Butlin, R.K. 1993. The variability of mating signals and preferences in the brown planthopper, *Nilaparvata-lugens* (Homoptera, Delphacidae). *J. Insect Behav.* **6**: 125–140.
- Charlesworth, B. 1987. The heritability of fitness. In: *Sexual Selection: Testing the Alternatives* (J. W. Bradbury & M. B. Andersson, eds), pp. 21–40. Wiley, New York.
- Charlesworth, B. 1988. The evolution of mate choice in a fluctuating environment. *J. Theor. Biol.* **130**: 191–204.
- Charlesworth, B. & Hughes, K.A. 2000. The maintenance of genetic variation in life-history traits. In: *Evolutionary Genetics: From Molecules to Morphology*, Vol. 1 (R. S. Singh & C. B. Krimbas, eds), pp. 369–392. Cambridge Univ. Press, Cambridge.
- Chippindale, A., Gibson, J.R. & Rice, W. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **98**: 1671–1675.
- Cockerham, C.C. & Weir, B.S. 1984. Covariances of relatives stemming from a population undergoing mixed self and random mating. *Biometrics* **40**: 157–164.
- Coleman, S.W., Patricelli, G.L. & Borgia, G. 2004. Variable female preferences drive complex male displays. *Nature* **428**: 742–745.
- Collins, R.D., Jang, Y., Reinhold, K. & Greenfield, M.D. 1999. Quantitative genetics of ultrasonic advertisement signalling in the lesser waxmoth, *Achroia grisella* (Lepidoptera: Pyralidae). *Heredity* **83**: 644–651.
- Cotton, S., Fowler, K. & Pomiankowski, A. 2004. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution* **58**: 1038–1046.
- Crow, J.F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*. Harper and Row Publishers, New York.
- Curtsinger, J.W., Service, P.M. & Prout, T. 1994. Antagonistic pleiotropy, reversal of dominance, and genetic polymorphism. *Am. Nat.* **144**: 210–228.
- David, P., Bjorksten, T., Fowler, K. & Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* **406**: 186–188.
- Ellner, S. & Hairston, N.G. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* **143**: 403–417.
- Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction To Quantitative Genetics*. Prentice Hall, London.
- Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration. *Annu. Rev. Genet.* **10**: 253–280.
- Fry, J.D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution* **46**: 540–550.
- Gerhardt, H.C., Dyson, M.L. & Tanner, S.D. 1996. Dynamic properties of the advertisement calls of gray treefrogs: patterns of variability and female choice. *Behav. Ecol.* **7**: 7–18.
- Gillespie, J.H. & Turelli, M. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics* **10**: 253–280.
- Greenfield, M.D. & Coffelt, J.A. 1983. Reproductive behaviour of the lesser waxmoth, *Achroia grisella* (Pyralidae: Galleriinae): signalling, pair formation, male interactions, and mate guarding. *Behaviour* **84**: 287–315.

- Greenfield, M.D. & Rodriguez, R.L. 2004. Genotype × environment interaction and the reliability of mating signals. *Anim. Behav.* **68**: 1461–1468.
- Greig, E.I. & Greenfield, M.D. 2004. Sexual selection and predator avoidance in an acoustic moth: discriminating females take fewer risks. *Behaviour* **141**: 799–815.
- Griffith, S.C., Owens, I.P.F. & Burke, T. 1999. Environmental determination of a sexually-selected trait. *Nature* **400**: 358–360.
- Hawthorne, D.J. 1997. Ecological history and evolution in a novel environment: habitat heterogeneity and insect adaptation to a new host. *Evolution* **51**: 153–162.
- Hedrick, P.W. 1999. Antagonistic pleiotropy and genetic polymorphism: a perspective. *Heredity* **82**: 126–133.
- Höglund, J. & Alatalo, R. 1995. *Leks*. Princeton University Press, Princeton, NJ.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**: 65–70.
- Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussière, L.F. 2004. High-quality male crickets invest heavily in sexual display but die young. *Nature* **432**: 1025–1027.
- Jang, Y. & Greenfield, M.D. 1996. Ultrasonic communication and sexual selection in waxmoths: female choice based on energy and asynchrony of male signals. *Anim. Behav.* **51**: 1095–1106.
- Jang, Y. & Greenfield, M.D. 1998. Absolute versus relative measurements of sexual selection: assessing the contributions of ultrasonic signal characters to mate attraction in Lesser Wax Moths, *Achroia grisella* (Lepidoptera: Pyralidae). *Evolution* **52**: 1383–1393.
- Jang, Y. & Greenfield, M.D. 2000. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. *Heredity* **84**: 73–80.
- Jennions, M.D., Møller, A.P. & Petrie, M. 2001. Sexually selected traits and adult survival: a meta-analysis. *Q. Rev. Biol.* **76**: 3–36.
- Jia, F.Y., Greenfield, M.D. & Collins, R.D. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype × environment interaction. *Evolution* **54**: 953–967.
- Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.* **15**: 173–190.
- Kelly, J.K. 1999. An experimental method for evaluating the contribution of deleterious mutations to quantitative trait variation. *Genet. Res.* **73**: 263–273.
- Kelly, J.K. 2003. Deleterious mutations and the genetic variance of male fitness components in *Mimulus guttatus*. *Genetics* **164**: 1071–1085.
- Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of female mating preferences and the paradox of the lek. *Nature* **350**: 33–38.
- Kokko, H., Brooks, R., McNamara, J.M. & Houston, A.I. 2002. The sexual selection continuum. *Proc. R. Soc. Lond. B* **269**: 1331–1340.
- Kotiaho, J., Simmons, L. & Tomkins, J. 2001. Towards a resolution of the lek paradox. *Nature* **410**: 684–686.
- Krauth, J. 1988. *Distribution-Free Statistics: an Application-Oriented Approach*. Elsevier, Amsterdam.
- Kruuk, L.E.B., Merilä, J. & Sheldon, B.C. 2001. Phenotypic selection on a heritable size trait revisited. *Am. Nat.* **158**: 557–571.
- Künike, G. 1930. Zur biologie der kleinen wachsmotte, *Achroia grisella* Fabr. *Z. Angew. Entomol.* **16**: 304–356.
- Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. U.S.A.* **78**: 3721–3725.
- Levene, H. 1953. Genetic equilibria when more than one ecological niche is available. *Am. Nat.* **87**: 331–333.
- Lewontin, R.C. 1974. *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.
- Lynch, M. & Hill, W.G. 1986. Phenotypic evolution by neutral mutation. *Evolution* **40**: 915–935.
- Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Inc., Sunderland, MA.
- Mackay, T.F.C. 1981. Genetic variation in varying environments. *Genet. Res. Camb.* **37**: 79–93.
- Meffert, L.M. & Regan, J.L. 2002. A test of speciation via sexual selection on female preferences. *Anim. Behav.* **64**: 955–965.
- Meffert, L.M., Hicks, S.K. & Regan, J.L. 2002. Nonadditive genetic effects in animal behavior. *Am. Nat.* **160**: S198–S213.
- Merilä, J. & Fry, J.D. 1998. Genetic variation and causes of genotype-by-environment interaction in the body size of Blue Tit (*Parus caeruleus*). *Genetics* **148**: 1233–1244.
- Merilä, J., Kruuk, L.E.B. & Sheldon, B.C. 2001. Natural selection on the genetical component of variance in body condition in a wild bird population. *J. Evol. Biol.* **14**: 918–929.
- Pomiankowski, A.N. 1988. The evolution of female preferences for male genetic quality. *Oxford Surv. Evol. Biol.* **5**: 136–184.
- Proulx, S. 2001. Female choice via indicator traits easily evolves in the face of recombination and migration. *Evolution* **55**: 2401–2411.
- Prout, T. 2000. How well does opposing selection maintain variation? In: *Evolutionary Genetics: From Molecules to Morphology* (R. S. Singh & C. B. Krimbas, eds), pp. 157–181. Cambridge University Press, New York.
- Prout, T. & Savolainen, O. 1996. Genotype-by-environment interaction is not sufficient to maintain variation: Levene and the leafhopper. *Am. Nat.* **148**: 930–936.
- Qvarnström, A. 1999. Genotype-by-environment interactions in the determination of the size of a secondary sexual character in the collared flycatcher (*Ficedula albicollis*). *Evolution* **53**: 1564–1572.
- Reinhold, K. 2000. Maintenance of a genetic polymorphism by fluctuating selection on sex-limited traits. *J. Evol. Biol.* **13**: 1009–1014.
- Ritchie, M.G. 1996. The shape of female mating preferences. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 14628–14631.
- Ritchie, M.G. 2000. The inheritance of female preference functions in a mate recognition system. *Proc. R. Soc. Lond. B.* **267**: 327–332.
- Ritchie, M.G., Saarikettu, M., Livingstone, S. & Hoikkala, A. 2001. Characterization of female preference functions for *Drosophila montana* courtship song and a test of the temperature coupling hypothesis. *Evolution* **55**: 721–727.
- Rodriguez, R.L. & Greenfield, M.D. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution* **57**: 1304–1313.
- Roff, D. 1997. *Evolutionary Quantitative Genetics*. Chapman and Hall, New York.
- Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition-dependent traits. *Proc. R. Soc. Lond. B* **63**: 1415–1421.

- Santos, M., Eisses, K.T. & Fontdevila, A. 1999. Competition and genotype-by-environment interaction in natural breeding substrates of *Drosophila*. *Evolution* **53**: 175–186.
- Scheuber, H., Jacot, A. & Brinkhoff, M.W.G. 2003. The effect of past condition on a multicomponent sexual signal. *Proc. R. Soc. Lond. B* **270**: 1779–1784.
- Scheuber, H., Jacot, A. & Brinkhoff, M.W.G. 2004. Female preference for multiple condition-dependent components of a sexually-selected signal. *Proc. R. Soc. Lond. B* **271**: 2453–2457.
- Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic Evolution: a Reaction Norm Perspective*. Sinauer, Sunderland, MA.
- Shaw, K.L. 2000. Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution* **54**: 1303–1312.
- Shuster, S.M. & Wade, M.J. 2003. *Mating Systems and Strategies*. Princeton University Press, Princeton, NJ.
- Slatkin, M. 1978. Spatial patterns in the distribution of polygenic characters. *J. Theor. Biol.* **70**: 213–228.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. W.H. Freeman & Co., New York.
- Spangler, H.G., Greenfield, M.D. & Takessian, A. 1984. Ultrasonic mate calling in the lesser wax moth. *Physiol. Entomol.* **9**: 87–95.
- Svensson, E., Sinervo, B. & Comendant, T. 2001. Condition, genotype-by-environment interaction, and correlational selection in lizard life-history morphs. *Evolution* **55**: 2053–2069.
- Taylor, P.D. & Williams, G.C. 1982. The lek paradox is not resolved. *Theor. Popul. Biol.* **22**: 392–409.
- Tomkins, J.L., Radwan, J., Kotiaho, J.S. & Tregenza, T. 2004. Genic capture and resolving the lek paradox. *Trends Ecol. Evol.* **19**: 323–328.
- Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore. I. Genotype-environment interaction in larval performance on different host plant species. *Evolution* **38**: 881–895.
- Via, S. & Lande, R. 1987. Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype-environment interaction. *Genet. Res. Camb.* **49**: 147–156.
- Welch, A.M. 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. *Evolution* **57**: 883–893.
- Wilson, E.O. 1975. *Sociobiology: the New Synthesis*. Harvard University Press, Cambridge, MA.
- Zar, J.H. 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.

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