Indirect genetic effects and the lek paradox: inter-genotypic competition may strengthen genotype × environment interactions and conserve genetic variance

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Abstract Understanding the evolutionary mechanisms that maintain genetic variation in natural populations is one of the fundamental goals of evolutionary biology. There is growing evidence that genotype-by-environment interaction (G × E) can maintain additive genetic variance (V_A), but we lack information on the relative performance of genotypes under the competitive situations encountered in the field. Competing genotypes may influence each other, and this interaction is also subject to selection through indirect genetic effects (IGE). Here, we explore how genotypes perform when interacting and evaluate IGE in order to understand its influence on V_A for sexually-selected traits in the lesser waxmoth, *Achroia grisella*. We found that inter-genotype differences and crossover interactions under joint rearing are equal to or greater than values when reared separately. A focal genotype exhibited different performances when jointly reared with various genotypes—suggesting that IGE may be responsible for the increased levels of crossover and differences in performance observed. We suggest that some genotypes are superior competitors for food acquisition in the larval stage, and that these differences influence the development and evolution of other genotypes through IGE. We reaffirm the role of G × E in maintaining V_A and note the general importance of IGE in studies of evolutionary mechanisms.

Keywords Acoustic communication · Courtship song · Mate choice · Sexual selection · Signal evolution

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Introduction

Identifying and understanding the evolutionary mechanisms that maintain genetic variation in natural populations is one of the fundamental goals of evolutionary biology (Lewontin 1974). Populations undergoing directional selection are expected to experience a reduction in genetic variation, and it is therefore puzzling when additive genetic variance (V_A) persists in spite of such selection pressure. The problem is particularly evident in sexual selection, where male traits are often subject to strong directional selection imposed by female choice yet continue to exhibit substantial levels of V_A. This perplexing situation is commonly known as the 'paradox of the lek' (Borgia 1979; Taylor and Williams 1982; Kirkpatrick and Ryan 1991), a reference to the intense sexual selection that females typically exert on males at lekking aggregations, and its resolution has broad implications for the evolution of traits under directional selection.

Within the domain of evolutionary theory, a multitude of mechanisms that could maintain V_A in natural populations under various circumstances are currently proposed (Roff 1997; Lynch and Walsh 1998). However, empirical investigations have been somewhat limited, and in the context of sexual selection the few studies that have been done focused on four possibilities: frequency-dependent selection (e.g., Fitzpatrick et al. 2007), genetic trade-offs or antagonistic pleiotropy (see Rose 1982; Curtsinger et al.

1994), mutation-selection balance (see Tomkins et al. 2004), and genotype × environment interaction (G × E) (see Felsenstein 1976; Gillespie and Turelli 1989). Whereas some evidence has been found in support of each of these four, no single mechanism appears to represent a universal explanation. Moreover, such supporting evidence is generally incomplete. Relevant to the absence of universality, we note that several studies have reported genetic trade-offs between male sexual attractiveness and survivorship which may contribute to V_A in particular species (e.g., Brooks 2000), but an extensive survey reveals that this situation is more of an exception than a rule: In many species, males that exhibit superior attractiveness are in good 'condition', and as a consequence they perform well by most measures, including survivorship (Jennions et al. 2001; e.g., Brandt and Greenfield 2004). Regarding the incompleteness of evidence, we note the growing evidence that G × E can sustain V_A when the environment is heterogeneous across space or time and G × E is sufficiently strong that no one genotype produces the superior genotype across all environments. This situation, an ecological crossover of reaction norms, has been reported for several invertebrate (e.g., Jia et al. 2000) and vertebrate (e.g., Qvarnström 1999; Welch 2003; Mills et al. 2007) species, but we typically lack information on the actual diversity of reaction norms found in natural populations. Of equal importance, these studies of G × E have tested each genotype's performance separately in the various environments, which leaves their relative performances under the competitive situations that inevitably occur in the field unknown. While recent population and quantitative genetic models (Harris et al. 2008; Wolf et al. 2008) have indicated a role for competition in maintaining genetic variance within populations, empirical studies of the lek paradox have not approached this issue.

One way to address the question of competition described above would be to test G × E under conditions in which unrelated genotypes are allowed to interact. Here, interactions may be expected to be non-altruistic, although we suggest that even related genotypes might forgo altruism under heightened competition (cf. Queller 1994; West et al. 2002). As the phenotypes produced by different genotypes interact, a complex relationship comes into play: A focal individual's genotype, in conjunction with the environment, produces its phenotype, but that individual also produces environmental effects and thereby represents selection pressure on nearby conspecifics, and vice-versa. These influences have been termed 'indirect genetic effects', and they may influence the same and/or different traits in neighbors (Dawkins 1982; Moore et al. 1997). In the present context we consider the possibility that indirect genetic effects may influence the relative performances of interacting genotypes such that the level of G × E, and the

ecological crossover of reaction norms in particular, differ from the level found where given genotypes are reared and tested singly. We entertain this possibility based on evidence that traits expressed during interactions may evolve differently from other traits and depend on the genotypes of the interacting individuals (Meffert 1995; Rice 1996; Hughes 1989; Casares et al. 1993; Bleakley et al. 2007). And when multiple genotypes interact in a natural population, the potential exists for evolutionary change to occur more rapidly than otherwise as a result of indirect genetic effects (Wolf 2000, 2003). Thus, a recent study has noted a specific mechanism by which indirect genetic effects may influence condition and secondary sexual traits as they relate to the paradox of the lek (Miller and Moore 2007; also see Petfield et al. 2005).

Here, we continue our previous studies on the role of G × E in maintaining V_A for sexually-selected traits in the lesser waxmoth, *Achroia grisella* (Lepidoptera: Pyralidae) by examining the potential contributions of indirect genetic effects. Male *A. grisella* attract females with an ultrasonic advertisement song (Spangler et al. 1984), and recordings and playback experiments have shown that considerable variation exists among individual males in the several song characters that influence male attractiveness (Jang and Greenfield 1996) as well as in overall attractiveness of male song (Jang and Greenfield 1998; Reinhold et al. 1998). These song characters and attractiveness are repeatable within individual males (Jang et al. 1997), and breeding experiments have confirmed their heritability (Collins et al. 1999; Brandt and Greenfield 2004). Thus, the persistence of variation in male song represents the paradox of the lek in *A. grisella*. Further breeding experiments using artificially selected (Jia et al. 2000) and random inbred lines (Danielson-François et al. 2006) developed from natural populations indicated the presence of different reaction norms that might, in part, resolve the paradox: Certain genotypes are markedly plastic in their response to conditions along an environmental gradient, exhibiting superior performance under favorable conditions but suffering marked reductions under stress, whereas other genotypes are more canalized, showing rather consistent performance across the gradient (Danielson-François et al. 2006). In the present paper we explore how these separate genotypes perform when interacting, and we analyze these data to evaluate whether any indirect genetic effects that arise under competition might expand or diminish the role of G × E in maintaining V_A.

We approached the investigation of indirect genetic effects in *A. grisella* by measuring the song and developmental traits of genotypes reared jointly and comparing these performances with traits expressed by these genotypes when reared separately. Measurements of developmental traits were made for females as well as

males, and we replicated the measurements within each pair of genotypes that were jointly reared. To afford repeatability for these measurements, we represented different genotypes by highly inbred lines. As there is no current consensus on quantitative treatment of indirect genetic effects, and given the constraints of our limited sampling of *A. grisella* genotypes, we developed a set of ad hoc calculations to estimate these effects and their influences on ecological crossover. Overall, we report that inter-line differences and crossover interaction under joint rearing are equal to or greater than the values observed when lines were reared separately. Moreover, the different performances of a focal genotype jointly reared with various other genotypes suggest that indirect genetic effects occur and may be responsible for the elevated level of inter-line differences and crossover. While our research does not directly address the specific nature of the inter-actions leading to indirect genetic effects, potential sources are presented in the discussion. These findings strengthen the claim that G × E can maintain V_A in male attractive-ness, and they underline the importance of considering indirect genetic effects in studies of evolutionary mechanisms.

Methods

Ecology and acoustic behavior of the lesser waxmoth

Achroia grisella are obligate symbionts of western hon-eybee (*Apis mellifera*) colonies, where the moth larvae feed on honeycomb and other organic material (Künike 1930). The moths typically infest honeybee colonies that are characterized by declining numbers of workers and hence a non-renewing food supply. Mating occurs within, on, or near the colony, and females subsequently oviposit there provided that some food remains. Male and female adults are short-lived, respectively surviving only 10 and 5 days on average, and they neither feed nor drink (Greenfield and Coffelt 1983). Consequently, body mass at adult eclosion is a valid estimate of 'condition', and it influences a male's song characters and overall attractiveness (Brandt and Greenfield 2004) and a female's fecundity (see below).

Male *A. grisella* sing while remaining stationary on the substrate and beating their wings, and they typically do so uninterruptedly for 6–10 h each night until death (Spangler et al. 1984). Wing-beating causes a pair of small tymbal organs situated at the base of the forewings to resonate, which generates a train of paired pulses of high-frequency sound. Females generally prefer male songs in which the pulses are relatively intense, delivered at fast repetition rates, and include lengthy 'asynchrony intervals' separating the two pulses within a pair (Jang and Greenfield 1998).

Experimental design

We studied reaction norms of genotypes represented by inbred lines randomly extracted from a natural population of *A. grisella* collected at Lawrence, Kansas. Each line was bred from a single brother–sister mating, which we con-tinued every generation. We reared the larvae on a synthetic diet (see Jang and Greenfield 1996) and main-tained them within environmental chambers at 25°C and a 12:12 L:D photoperiod. Comparisons with outbred *A. gri-sella* derived from the same natural population indicated inbreeding depression of approximately 5% for body mass, development rate, and male song characters.

Ten generations following initial establishment of the inbred lines, at which juncture we estimated that hetero-zygosity had been reduced >85% (Crow and Kimura 1970), we chose five lines (line numbers 25, 39, 41, 50, and 78) for our tests of indirect genetic effects. We specifically chose lines in which the generations happened to be syn-chronized such that some females in each line could mate and then oviposit on the same day. Thus, we could pair equal-aged offspring from different lines and observe their performance following joint-rearing in which the two genotypes competed. An additional criterion, satisfied in four (line numbers 25, 39, 41, and 78) of the five lines, was divergent developmental and male song features among the lines as observed in measurements made one generation earlier. For each of the five lines, we mated a pair of recently eclosed adults, collected all of the eggs that the female deposited following mating, and maintained the larvae on 1–2 g of synthetic diet in a 30-ml cup. We ensured that neither the male nor female had previously mated, and we noted that each of the five females, one from each line, had deposited several hundred eggs during the same 2-days interval, nearly all of which hatched. When these offspring had attained the 2nd larval instar, we ini-tiated the joint-rearing procedure by transferring 30 larvae total, 15 from each of two different lines, to 0.12 l poly-ethylene containers supplied with synthetic diet. We tested three different joint rearings of the five lines (lines 25 and 39, lines 41 and 50, and lines 39 and 78). Thus, one line (line number 39) was tested in two joint rearings, once with each of two different lines (line numbers 25 and 78). The three different joint rearings were replicated between five and nine times depending on the number of offspring available from the full-sib families. Each replicate of 30 larvae was kept in a separate 0.12 l rearing container.

We determined reaction norms of each of the five lines along the food availability gradient by providing one half of the replicates of each of the three different joint rearings with 8 g diet (low availability; high competition) and one half with 12 g (moderate availability; moderate competi-tion). We chose this environmental gradient and the

specific levels based on the measurements made of these lines, reared singly, during the previous generation. These measurements revealed that the *A. grisella* population generally exhibited phenotypic plasticity in response to these levels of diet, but that the several lines tested here differed in their specific responses. Moreover, survival to the adult was generally high at these values of diet availability and larval density (see "Results" below). To ensure standardization and repeatability, we homogenized the ingredients during preparation of the diet and used the same batch for the tests described here as had been used for the previous generation.

We noted the date of eclosion of each adult in the rearing containers, weighed them to the nearest 0.01 mg 24 h afterwards, and then recorded the song of the males (see below). Both males and females were saved for genotyping (see below), which provided the line identity of each eclosing individual. These data allowed us to determine reaction norms for body mass, developmental rate (reciprocal of developmental period from oviposition to adult eclosion), and male song characters and overall attractiveness. We also used these data to calculate the survivorship (2nd instar larva to adult) of each line under the two environmental conditions.

To determine whether the body mass at eclosion of female *A. grisella* predicted parameters related to fitness, we tested the fecundity of a sample of 81 females randomly chosen from eight inbred lines. We mated each female when aged 1 day to a different male from the same population and then noted the number of eggs she deposited on a standard substrate within an individual cup. Pertinent to the potential correlation between female body mass and fecundity, we note an earlier study (Brandt and Greenfield 2004) that reported positive correlations between male body mass at eclosion and various song and developmental parameters, including adult longevity.

Male song recording and analysis

As in our previous studies of *A. grisella* (Jang and Greenfield 1996, 1998) we recorded songs during the initial 6 h of the night in a semi-anechoic chamber maintained at 25°C. We used a condenser microphone (ACO Pacifica Model 7016; frequency response ±2 dB from 10 to 100,000 Hz and ±6 dB from 10 to 160,000 Hz; Belmont, CA, USA) whose output 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 (Batsound Pro, Pettersson Elektronik). From a 1-s sample of the digitized male song, we determined the mean peak amplitude (PA), the average asynchrony interval (AI) between paired pulses (see Jang

and Greenfield 1996), and the repetition rate of pulse pairs (PR). These measurements were accomplished with a custom program modified from commercially available signal processing software (Spike2, Cambridge Electronic Design, Cambridge, UK) by L. Brandt. We estimated the overall attractiveness of each male's song via a linear predictive model ($AT = 0.524PA + 0.296AI + 0.117PR$) generated from selection gradient analysis (see Jang and Greenfield 1998).

Genotyping

Our experimental design required us to identify the line (genotype) of each adult that emerged from the containers in which two different lines were reared jointly. Because no physical marker was available for this purpose, we relied on amplified fragment length polymorphism (AFLP) markers to determine the line identity of each eclosed moth.

Genomic DNA was extracted (DNAeasy Tissue Kit, Qiagen) from adult whole specimens stored at −80°C, and AFLP markers (Vos et al. 1995) were developed with the restriction enzymes *Eco*RI and *Mse*I (NEB, Beverly, MA) using the AFLP plant-mapping kit for small genomes (50–500 Mb; Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The *Eco*RI selective primer was fluorescently labeled to allow detection of fragments on an ABI 3700 automated sequencer (Applied Biosystems). AFLP fragments were visualized and analyzed in GENESCAN (v. 3.1 ABI) and GENOTYPER (v. 2.1 ABI). For each selective primer pair, this process generated between 50 and 100 detectable AFLP bands that represented fragments ranging in size from 50 to 500 bp. After screening insects of known line identity with 64 selective primer combinations, we found that one primer pair, *Eco*RI-TC/*Mse*I-CTG, generated unique sets of bands for each of the two lines in a given joint rearing in nearly all cases. For the few moths that could not be identified with this primer pair, we found that a subsequent pair, either *Eco*RI-TG/*Mse*I-CTT or *Eco*RI-TG/*Mse*I-CTA, resolved the ambiguity. We then used one or more of these primer pairs to genotype each eclosed adult in our joint rearing procedure. An individual was identified as belonging to a given line of the two possibilities if it possessed the bands found only in that line, rather than on the basis of an absence of bands.

Analysis of crossover and indirect genetic effects

To evaluate crossover interactions within the three different joint rearings tested in generation 10, we determined the mean performances of both genotypes in each joint-rearing container (replicate) for the various parameters

measured. From these mean values, we noted the presence or absence of crossover for each pairwise combination of rearing containers, one container supplied with 8 g diet and the other with 12 g diet, for a given joint rearing and parameter. Here, crossover was designated when the difference between the mean performance of genotype i and genotype j under environment 1 (8 g diet) and the difference between the two mean performances under environment 2 (12 g diet) were opposite in sign. We then tabulated the proportion of all pairwise combinations of replicates for a given joint rearing in which crossover was observed, and we considered this proportion to represent the expected level of crossover for that joint rearing and parameter along the 8–12 g environmental gradient. This procedure was applied to two developmental parameters (body mass at eclosion, developmental rate) and four song parameters (repetition rate of PR, PA, AI, and AT) for males and to the two developmental parameters for females.

We assessed the influence of competition between genotypes on crossover by comparing the above results, obtained from generation 10 insects, with crossover values obtained in generation 9, where genotypes had been reared singly. We note that the environmental gradient tested in generation 9 had included 4 and 12 g diet per 30 individuals. Thus, our comparison offers a conservative estimate of potential effects of inter-genotype competition on crossover, since the environmental gradient tested under competition in generation 10 was narrower, being only 8 vs. 12 g diet per 30 individuals. We used the 8 g diet treatment in generation 10 to ensure equivalent survival under both diet treatments (8 and 12 g); survival under the 4 g treatment in generation 9 had been slightly lower than that under the 12 g treatment.

In an additional comparative analysis of data from generations 9 and 10, we examined the relationship between a genotype's plasticity along an environmental gradient and its crossover interactions with other genotypes under competition. For each developmental and song measure, we evaluated plasticity along the diet availability gradient (4, 12, and 30 g diet per 30 individuals) via least-square linear regression applied to data from generation 9 males. Thus, a line was designated as plastic (regression slope $\neq 0$; $P < 0.05$) or canalized (regression slope did not differ from 0; $P > 0.15$) for each of the six parameters. We then considered our crossover data from generation 10 in light of these designations.

We estimated the potential influence of indirect genetic effects on development and song by analyzing the performances of generation 10 insects from the genotype (line 39) that had been jointly reared with either of two different genotypes (lines 25 or 78) within the rearing containers. For each of the two developmental and four song

parameters evaluated and under both conditions (8 g and 12 diet) tested along the environmental gradient, we determined whether the line identity of the genotype with which line 39 insects were reared had a consistent influence on their performance. For the 12-g condition, we also included the performance of line 39 insects from generation 9 in the comparison. Thus, we contrasted the situation of inter-genotype competition occurring in a given rearing container (generation 10) with that where competition was only intra-genotypic (generation 9).

Results

Genetic variance, phenotypic plasticity and ecological crossover

Survival to the adult was generally high (60–70%) within replicates among the five lines and two environmental conditions tested, and we observed no significant phenotypic plasticity for survivorship between the 8 and 12 g diets within any line (Mann–Whitney U-test; $P > 0.21$) or significant differences in survivorship between the lines under either diet condition (1-way ANOVA, Holm-Sidak method for multiple pairwise comparisons; $P > 0.05$). However, significant differences were observed between lines for the two developmental parameters in both males (1-way ANOVA; $P < 0.01$) and females ($P < 0.01$), as well as for all of the four male song parameters ($P < 0.02$) save pulse-pair rate (PR) under the 8 g diet and asynchrony interval (AI) and attractiveness index (AT) under the 12 g diet. Relevant to our measurements of the body mass of females, least-squares linear regression indicated a significant correlation ($r = 0.345$, $P = 0.002$, $t = 3.27$) between mass at eclosion and fecundity. We note that analyses of male data from the 12-g diet condition in generation 9 only revealed significant differences (Kruskal–Wallis 1-way ANOVA on ranks; $P < 0.01$) among these several lines for two male song parameters, AI and the AT.

The incidence of crossover interactions between pairs of replicates, one reared under the 8 g diet condition and the other under the 12 g condition, ranged between 0 and 67% among the parameters measured and the three different joint rearings (Table 1). The highest values were found for female body mass in the joint rearing of lines 41 and 50 and for male body mass, male developmental rate, and pulse-pair rate of male song in the joint rearing of lines 25 and 39. Overall, the median incidence of crossover interaction was 41%. The actual reaction norms, including their variance, from which we made the above assessment of crossover interactions are depicted in Fig. 1.

A comparison of the incidence of crossover interactions observed in the joint rearing of lines 39 and 25 and in the

Table 1 Incidence of crossover interaction between inbred lines of *A. grisella*

Sex	Parameter	Joint rearing—generation 10			Line pairing—generation 9	
		39/25	39/78	41/50	39/25	39/78
Male	Body mass	0.57	0.32	0.00	0	1
	Developmental rate	0.51	0.32	0.33	0	1
	Song pulse pair rate	0.51	0.44	0.50	1	0
	Song peak amplitude	0.43	0.44	0.33	0	0
	Song asynchrony interval	0.41	0.40	0.39	0	0
	Song attractiveness index	0.41	0.40	0.39	0	0
Female	Body mass	0.40	0.44	0.67	–	–
	Developmental rate	0.41	0.20	0.28	–	–

Values are given for two developmental parameters for males and females and for four male song parameters. In both generation 10, where lines were reared jointly, and in generation 9, where they were reared separately, crossover was designated when the mean performance of a given line was superior to that of the other line under one rearing condition but inferior under the other condition. For the three joint rearings in generation 10, the values represent the proportion of all binary combinations of replicates, one reared under the 8-g diet and the other reared under the 12-g diet, in which crossover was observed. For the line pairings in generation 9, during which samples were not replicated, 1 indicates that crossover was observed between the two lines and 0 indicates its absence. Data for the pairing of lines 41 and 50 in generation 9 are not given because line 50 was not measured
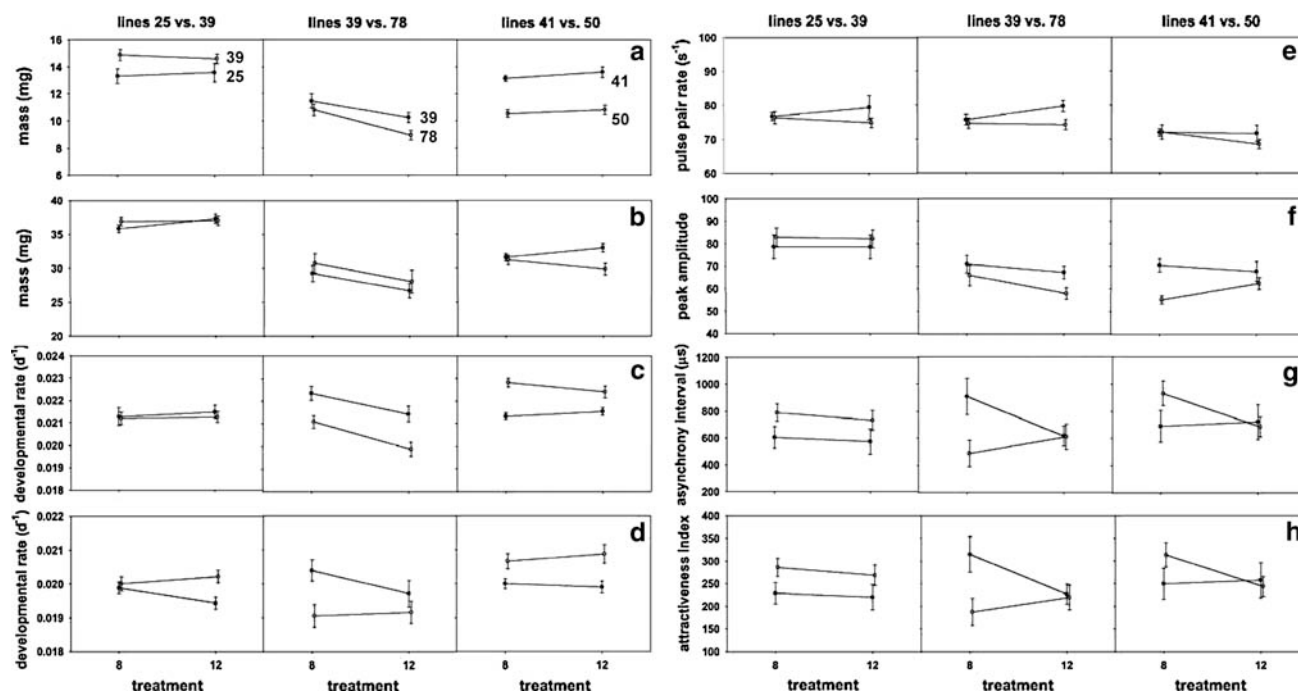

Fig. 1 Reaction norms showing performance of the 3 joint rearings of lines in generation 10 under 2 treatments (8 and 12 g diet). Data are given for 8 indices of performance: (**a**) male body mass, (**b**) female body mass, (**c**) male developmental rate, (**d**) female developmental rate, (**e**) male song pulse pair rate, (**f**) male song peak amplitude (arbitrary units, on linear scale), (**g**) male song asynchrony interval, and (**h**) male song attractiveness index (arbitrary units). Numbers adjacent to reaction norms within panel (**a**) distinguish the two lines within a joint rearing; these designations, open and solid symbols, are used for all 8 performance indices. Vertical bars indicate standard error of the mean performance value of the various replicates of a given line and treatment; error bars of the two lines of a given joint rearing and treatment are offset horizontally so that both remain visible. Incidences of crossover interaction reported in Table 1 and in the text are reflected by the levels of intersection of reaction norms and of the overlap of error bars

joint rearing of lines 39 and 78 with crossover data obtained for these combinations of lines in generation 9 showed that crossover under the competition in joint rearings was at least as great as that found under the separated rearing procedure in generation 9. For lines 39 and 25, crossover in generation 9 was observed only for male PR, and for lines 39 and 78 it was observed only for male body mass and developmental rate (Table 1). We note that line 50 had not

been tested in generation 9, precluding a comparison for the joint rearing of lines 41 and 50. Moreover, measurements of lines were not replicated in generation 9, and data were not obtained from females in all lines.

Analyses of generation 9 male data revealed phenotypic plasticity ($P < 0.05$; least squares linear regression, t-test for slope) across diet conditions for body mass and PA in line 39 (slope for body mass remained significant following Holm correction for multiple tests) and for developmental rate, AI and AT in line 78 (slopes for AI and AT remained significant following Holm correction). On the other hand, line 25 males were designated as canalized ($P > 0.16$) for all six parameters. For the three parameters where line 78 had regression slopes greater than 0 ($P < 0.05$), a comparison of slopes of lines 25 and 78 showed that line 78 values were significantly ($P < 0.05$) greater. We note that in the joint rearings in generation 10 that included line 39 (Table 1), a higher (but not significant; $P = 0.06$, t-test) incidence of crossover among the various parameters was observed in the joint rearing with line 25 (relatively canalized) than in the joint rearing with line 78 (relatively plastic).

Indirect genetic effects

A comparison of performances observed in the joint rearing of lines 39 and 25 with those observed in the joint rearing of lines 39 and 78 indicated significant genotypic influences on several parameters. For males, body mass and developmental rate of line 39 differed significantly ($P = 0.001$, 0.02; Mann–Whitney U-test; for body mass, $P < 0.05$ following Holm correction for multiple tests) between the two joint rearings under the 8-g diet condition, and body mass, pulse-pair rate, and PA differed ($P = 0.001$, 0.03, 0.002; t-test; for body mass and PA, $P < 0.05$ following Holm correction) under the 12-g diet (Figs. 2, 3). For females, body mass of line 39 differed significantly ($P = 0.001$, 0.001; Mann–Whitney U-test; $P < 0.05$ following Holm correction) between the two joint rearings under both diet conditions. We note that body mass and PA of line 39 males reared separately (generation 9) under the 12-g diet were intermediate between values observed in the two joint rearings (generation 10; Fig. 3). For other parameters, however, the performance of line 39 males reared separately was either similar to that observed in the joint rearing of lines 39 and 25 or below both levels seen in the joint rearings.

Discussion

Our results from the joint rearing experiment reported here, taken in conjunction with previous findings on the same

genetic lines reared separately, suggest that crossover increases under inter-genotype competition. Owing to the smaller samples of data in that earlier rearing procedure, it is difficult to provide formal tests of this claim. Nonetheless, in comparing males from the two data sets, one observes that the median proportion of crossover between lines 39 and 25 was 0.47 among all six measured parameters in generation 10, whereas crossover was found for only one of these six parameters in generation 9. Similarly, the median proportion of crossover between lines 39 and 78 was 0.40 in generation 10, while crossover was found for only two of six parameters in generation 9. That a wider difference separated the two (diet) environments tested in generation 9 than in generation 10 strengthens the claim that the incidence of crossover increased under the rearing procedure in generation 10. We emphasize that environmental conditions in the two generations were otherwise equivalent.

Within the generation 10 data, one observes further that the level of crossover in the joint rearing of lines 39 and 25 exceeded that in the joint rearing of lines 39 and 78 for six of the eight parameters measured among males and females (Table 1). Data from generation 9 indicate that line 25 is relatively more canalized than the other two lines for the parameters measured, and we suggest that the higher incidence of crossover observed for line 25 reflects an interaction between plastic and canalized genotypes. That is, line 39 tended to outperform line 25 under favorable conditions (12-g diet), but performed more poorly when under greater stress (8-g diet). But when two relatively plastic genotypes, lines 39 and 78, were jointly reared, crossover was reduced because both genotypes responded to the environmental gradient in the same fashion.

A second, related observation made from the generation 10 data is that the performance of line 39 insects was influenced by the identity of the other line with which they were jointly reared (Figs. 2, 3). We interpret these findings as indirect genetic effects of the various *A. grisella* genotypes. Our data suggest that these indirect genetic effects occur in both males and females and that their strongest influence is on body mass and secondarily on parameters of male song (pulse-pair rate, PA) known to be correlated with body mass (Brandt and Greenfield 2004). But we also observe that significant indirect genetic effects did not arise for attractiveness index, the song parameter that purportedly would have the greatest influence on mating success and fitness.

How may indirect genetic effects arise in *A. grisella*, and how might these effects influence genetic variance within a population? Because the strongest effects appear to be acting on body mass, it is likely that they arise from differential ability to compete for food or to assimilate it (see Leroi et al. 1994a, b and Shiotsugu et al. 1997 for studies on this genre of genetic variance in *Drosophila*).
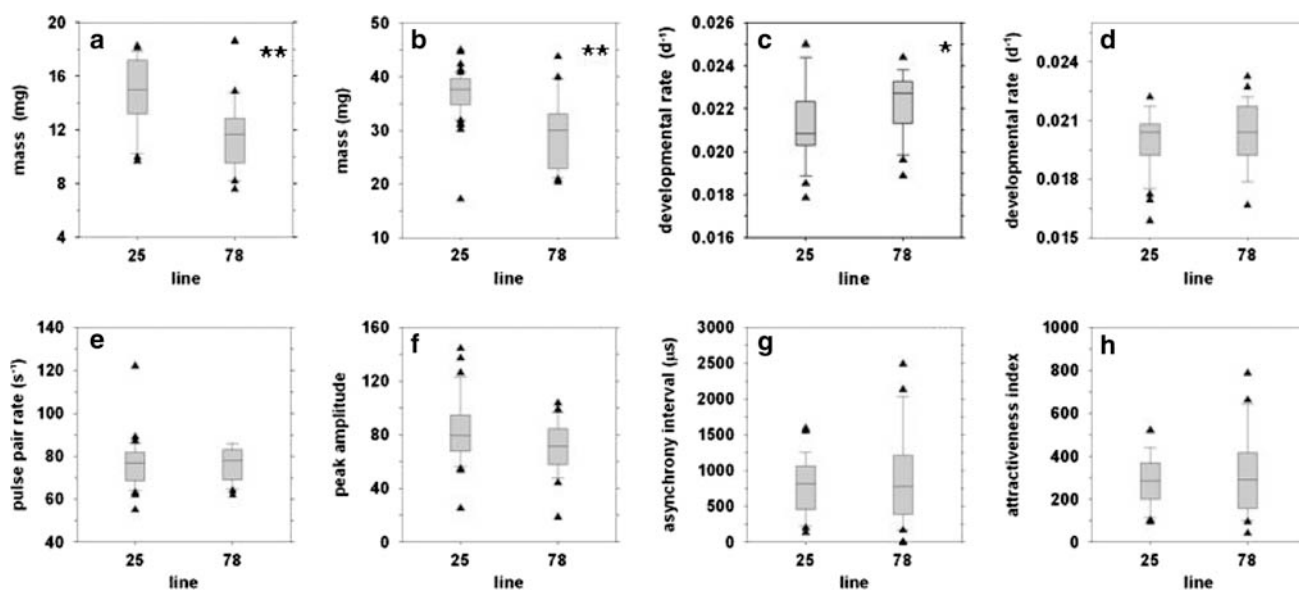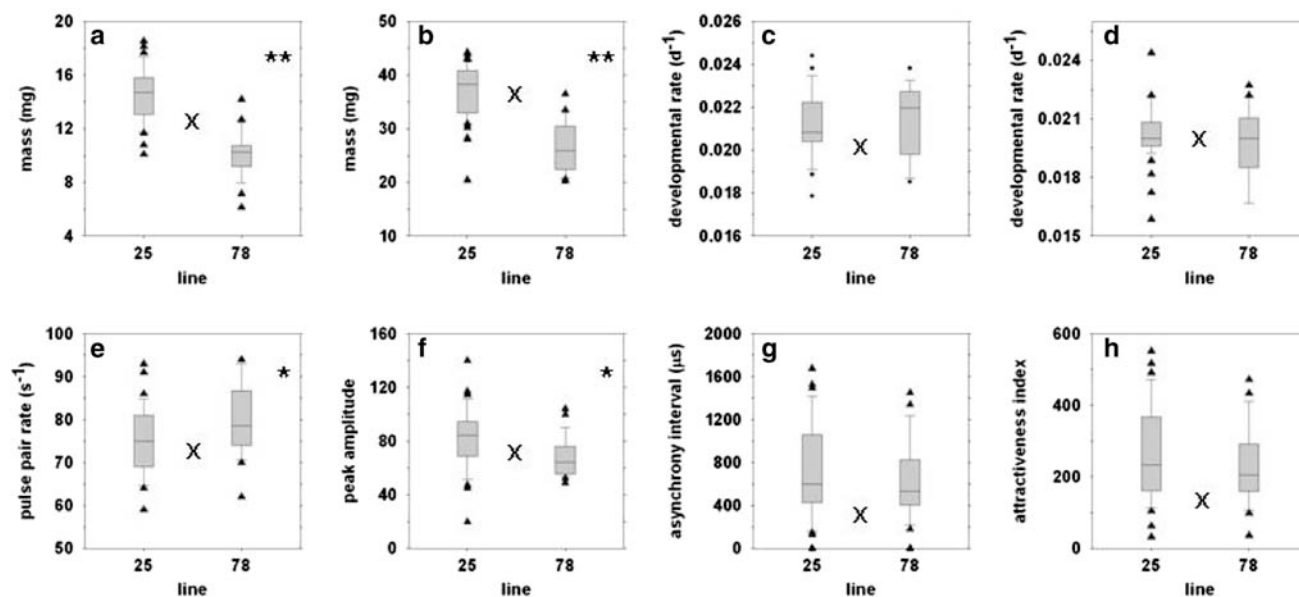
Fig. 2 Performance of line 39 insects when reared jointly with line 25 or line 78 insects; a joint rearing included 15 insects of each line on 8 g diet. Box plots show median performance, 25–75% and 10–90% limits, and outliers for data pooled from all nine replicates of the joint rearing with line 25 and all five replicates of the joint rearing with line 78. Performance values are for (**a**) male body mass, (**b**) female body mass, (**c**) male developmental rate, (**d**) female developmental rate, (**e**) males song pulse pair rate, (**f**) male song peak amplitude (arbitrary units, on linear scale), (**g**) male song asynchrony interval, and (**h**) male song attractiveness index (arbitrary units). * Performance of line 39 differs between the 2 joint rearings ($P < 0.5$, Mann–Whitney U-test); ** $P < 0.05$, Mann–Whitney U-test, adjusted for multiple tests via the Holm correction

Fig. 3 Performance of line 39 insects when reared jointly with line 25 or line 78 insects; a joint rearing included 15 insects of each line on 12 g diet. Box plots represent data as described in Fig. 1. X indicates median performance of line 39 insects when reared separately; i.e. 30 line 39 insects on 12 g diet. * Performance of line 39 differs between the 2 joint rearings ($P < 0.5$, Mann–Whitney U-test); ** $P < 0.05$, Mann–Whitney U-test, adjusted for multiple tests via the Holm correction

Whereas the various genotypes may differ in their ability to feed and develop, particularly when the availability of food is limited, such differences may be magnified when these genotypes compete directly. Thus, slight discrepancies in food acquisition and subsequent development under the separate rearing procedure may become significant differences when a superior genotype is jointly reared with an inferior one. These basic differences in size may then translate to the observed differences in male song and inferred differences in female fecundity. As these

differences may be opposite in sign under relatively favorable versus stressful environments, an elevated level of crossover can result and lead, in turn, to greater maintenance of genetic variation.

The scenario we present above is but a glimpse of the potential outcomes of inter-genotypic competition that may occur in natural populations. Our data represent findings from a proportion of the genetic lines that had been developed from an *A. grisella* population, and the comparison with insects reared in the absence of intergenotypic competition is constrained by the limited sampling taken in this latter situation. Understandably, testing other genotypes could yield somewhat different outcomes from the ones we present here. Rather than making a global statement on the nature and extent of inter-genotypic competition in *A. grisella*, we intend that this study serve as an indication of the potential role of indirect genetic effects in mediating competition and ultimately influencing the genetic variance of a population. That is, evolutionary genetic studies on genotype × environment interaction need to consider other genotypes as part of the environment that a focal genotype is interacting with. We anticipate that this more complete consideration of the meaning of environment may contribute, in some species and circumstances, to progress in resolving the paradox of behavior at leks and of the general dilemma that sexually selected traits often present.

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