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Condition-dependent traits and the capture of genetic variance in male advertisement song

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Abstract

The occurrence of additive genetic variance (V_A) for male sexual traits remains a major problem in evolutionary biology. Directional selection normally imposed by female choice is expected to reduce V_A greatly, yet recent surveys indicate that a substantial amount remains in many species. We addressed this problem, also known as the 'lek paradox', in *Achroia grisella* (Lepidoptera: Pyralidae), an acoustic moth in which males advertise to females with a pulsed ultrasonic song. Using a standard half-sib/full-sib breeding design, we generated F_1 progeny from whom we determined V_A and genetic covariance (COV_A) among seven traits: three song characters, an overall index of song attractiveness, nightly singing period, adult lifespan, and body mass at adult eclosion. Because *A. grisella* neither feed nor drink as adults, the last trait, eclosion body mass, is considered a measure of 'condition'. We found significant levels of V_A and narrow-sense heritabilities (h^2) for all seven traits and significant genetic correlations ($= COV_{Ai,j} / \sqrt{(V_{A\,i} \cdot V_{A\,j})}$) between most pairs of traits (i, j). Male attractiveness was positively correlated with body mass (condition), adult lifespan, and nightly singing period, which we interpret as an energy constraint preventing males in poor condition from singing attractively, from singing many hours per night, and from surviving an extended lifespan. The positive genetic correlation ($r = 0.79$) between condition and attractiveness, combined with significant levels of V_A for both traits, indicates that much of the variation in male song can be explained by V_A for condition. Finally, we discuss the morphological and physiological links between condition and song attractiveness, and the ultimate factors that may maintain V_A for condition.

Introduction

A growing body of systematic observations and experimental findings firmly establish that intersexual selection by female choice is a major process in evolution (Andersson, 1994; also see Cronin, 1991 for historical perspective). However, the evolutionary mechanisms underlying advantages accrued by males displaying the particular features attractive to females are seldom clear. This problem has been most severe in those species where females acquire no unambiguous 'direct' benefits, factors influencing their survival or fecundity, from mating with one male over another. Such cases are most striking among, although not limited to, lekking species, and this observation led to coining the phrase 'paradox of the lek' (Borgia, 1979). As originally used, the paradox referred to the problem of intense choice of mates by females that were obtaining no apparent benefit from their preferences (Kirkpatrick & Ryan, 1991).

Population and quantitative genetic modelling (Lande, 1981; Kirkpatrick, 1982) has confirmed Fisher's (1930) verbal argument that in the absence of direct benefits, female choice can still evolve because it may increase the reproductive success of progeny. That is, by choosing 'aesthetically attractive' males, females may produce sons displaying superior attractiveness who can achieve

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greater success in the 'mating arena' (arbitrary, or Fisherian, process). Alternatively, a choosy female may produce offspring of superior viability by mating with males who bear viability as well as attractiveness alleles that are inherited by both sons and daughters (indicator, or 'good-genes', process; Pomiankowski, 1988). Recent laboratory and field studies provide evidence supporting the arbitrary and indicator sexual selection processes in various invertebrate and vertebrate species (e.g. Jones *et al.*, 1998; Møller & Alatalo, 1999).

Both 'indirect' sexual selection mechanisms, the arbitrary and indicator processes, depend on substantial additive genetic variance for male features and female preferences and predict genetic covariance between these male and female traits; the covariance is assumed to arise from linkage disequilibrium generated by mate choice. However, a special conundrum arises because the directional selection typically imposed by female mate choice is expected to deplete most additive genetic variance as genes for attractive male features become fixed (see Charlesworth, 1987). In turn, the preference behaviour responsible for female mate choice may be gradually eliminated. Nonetheless, female mate choice based on indirect, genetic benefits does occur (see above references for arbitrary and indicator selection processes), and a survey has revealed substantial amounts of additive genetic variance among sexually selected male traits – as much as generally found for non-sexual characters (Bakker & Pomiankowski, 1995). Some authors (e.g. Taylor & Williams, 1982; Kotiaho *et al.*, 2001) have applied the 'paradox of the lek' phrase to the problem of how such variance is maintained in natural populations.

Among the various factors and mechanisms that have been proposed for maintaining additive genetic variance in sexually selected traits (see Jia *et al.*, 2000; also see Roff, 1997), the purported condition dependence of such traits merits especial attention. Rowe & Houle (1996) suggested that variation in sexually selected male traits results from a positive correlation between these traits and male development, energy reserves or physiological state, qualities otherwise known as 'condition'. Because additive genetic variance for condition may be high, variance for male traits follows. Recent experimental studies (David *et al.*, 2000; Kotiaho *et al.*, 2001) and a meta-analysis of data gleaned from the literature (Jennions *et al.*, 2001) support this contention: contrary to an expectation that trade-offs exist between male attractiveness and life history traits such as survival (e.g. Brooks, 2000), in many species those males who exhibit the most attractive displays also tend to enjoy the greatest longevity, possibly as a result of superior condition.

Here, we report a study testing the condition-dependence hypothesis for maintenance of variation in sexually selected traits in an acoustic moth, *Achroia grisella* (Lepidoptera: Pyralidae). Male *A. grisella* gather in small leks and attract females with an ultrasonic calling song. Previous work demonstrated that female choice imposes directional selection on several male song characters (Jang & Greenfield, 1996), that males vary considerably in calling song attractiveness (Jang & Greenfield, 1998) and that additive genetic variance comprises a substantial proportion of this variation (Collins *et al.*, 1999). To determine whether condition dependence and additive genetic variance for condition were responsible for variation in *A. grisella* male song characters, we used a half-sib/full-sib breeding design and measured sexual and life-history traits among male offspring. These measurements, arranged in a variance/covariance matrix (*G* matrix), confirmed that genetic variance in condition and condition-dependence of male signal traits allowed for the capture of genetic variance in those traits. Moreover, our study indicates the specific morphological and physiological links between condition and signal attractiveness. Whereas these findings in themselves do not identify what maintains genetic variance for condition, they point future investigations in a fruitful direction for addressing that ultimate question, the lek paradox.

Materials and methods

A. grisella natural history and acoustic communication

A. grisella (lesser waxmoth) are obligate symbionts of the honeybee, *Apis mellifera*. The moth larvae feed on honeycomb, stored organic material, and detritus in and around weakened or dying bee colonies, those with declining numbers of workers (Künike, 1930; Milum, 1940). Adult moths stay in the vicinity of the natal honeybee colony if these non-renewable food resources are still available and adult males display in small leks in, on, or near the colony (Greenfield & Coffelt, 1983). Female and male adults live only approximately 7 and 14 days, respectively, and neither feed nor drink. Thus, larval nutrition and growth are by far the main determinants of male condition. Accordingly, a newly eclosed adult male is a closed system, and its body mass is an accurate measure of energy reserves and may be considered a fair proxy for condition (see Jakob *et al.*, 1996 for general discussion).

Unlike most moth species, wherein pair formation is facilitated by female olfactory signalling and male searching (Silberglied, 1977; Greenfield, 1981), *A. grisella* males produce an ultrasonic advertisement signal that attracts receptive females within a radius of several metres (Spangler *et al.*, 1984). The male signals are generated by wing-fanning, which causes a pair of tymbals on the tegulae, small sclerites at the bases of the forewings, to buckle inward or outward. During a given wing stroke, a highly-damped 100-μs sound pulse is produced by each wing. Because wing movement is slightly asynchronous in most males, a single wing stroke ordinarily generates a pair of pulses separated by a brief

(100–500 μs) silent gap. Consequently, a male fanning his wings at 45 strokes s^{-1}, an average rate at 25 °C, normally produces 90 pulse pairs s^{-1}. The sound pulses include frequencies ranging from 70 to 130 kHz, and the mean broadcast amplitude is 95 dB peSPL (Spangler *et al.*, 1984). Individual males may continue signalling without interruption for 6–10 h each night, and many do so on each night until death (Greenfield & Coffelt, 1983).

Laboratory playback experiments and choice tests using live moths have shown that a male's attractiveness and mating success are influenced by three song characters: amplitude, pulse-pair rate (PR), and the length of silent gaps within pulse pairs (Jang & Greenfield, 1996). Females prefer male songs that are higher in amplitude, delivered at a faster PR, and include longer silent gaps within pulse pairs. All three of these song characters were found both repeatable and heritable within several populations studied (Jang *et al.*, 1997; Collins *et al.*, 1999; Jia *et al.*, 2000). Selection gradient analysis has been used to calculate coefficients for weighting each of these characters in an index of overall attractiveness (Jang & Greenfield, 1998). Because *A. grisella* females seldom re-mate (Greenfield & Coffelt, 1983), the attractiveness index may be a reliable predictor of a male's reproductive success as well as his mating success.

Population studied

We used a laboratory population of *A. grisella* derived from several hundred insects collected at infested honeybee colonies near Lawrence, KS in July 1997. A random breeding protocol practised every generation retained genetic variance in this stock population. The population was kept in an environmental chamber maintained at 25.5 °C and under a 12 : 12 L : D photoperiod. A standard diet (Dutky *et al.*, 1962), consisting of wheat, corn and oat flours, glycerine, Brewer's yeast, beeswax, honey and water, was provided to the larvae.

We used virgin, individually reared adults for all experiments to ensure the standardization of environmental conditions. Virgin adults were obtained by placing second-instar larvae in individual 30-ml cups, each supplied with diet *ad libitum* (1 g). These insects developed, pupated, and eclosed within their individual cups. The date of eclosion was designated as day 0 of the insect's adult lifespan.

Breeding design

Our half-sib/full-sib breeding design included 50 sires, each mated to a minimum of four dams. Sires and dams were randomly sampled from the stock population, and each was paired first on day 0 of its adult lifespan. Pairs were held in a 30-ml plastic cup for 24 h, during which mating almost invariably occurred. The sires were paired with another virgin female every 2 days until day 10, provided they survived to that age. Between pairings, sires were kept isolated from all other moths.

Following the 24-h interval allotted for pairing, we removed the dam to another 30-ml cup lined with pleated wax paper, in which eggs were normally deposited. Three days following pairing we placed the egg clutch from each successfully mated dam on 100 g of standard diet in a plastic container (16 × 12 × 6.5 cm), transferred the container to an environmental chamber kept under the same conditions as the stock population, and allowed larvae to hatch and develop. To maintain a balanced breeding design, we randomly chose four of the successful pairings of each sire and discarded the larval containers from the remaining one or two pairings. Thus, we established 200 (= 50 × 4) full-sib families.

At 25 days following pairing, we chose a random sample of 30 pupae or late-instar larvae from each full sibship container and placed them in individual 30-ml cups supplied with 1-g diet. We checked these cups twice daily for adult eclosion, removed the males, weighed (± 0.005 mg) them on day 0, recorded their calling songs later that same day (day 0), monitored overall calling activity (day 1 and later), and documented their lifespan. To ensure that these data were not biased toward the earliest sons to eclose, we measured no more than three eclosing males per full sibship per day until a total of 10 sons from that full sibship was achieved or all male offspring within that sibship had eclosed. Thus, we would have measured a total 2000 (= 50 × 4 × 10) F_1 males had sex ratios within all of the 30-insect samples been approximately 50 : 50 and survival been uniformly high, but our actual total equalled 1957 owing to skewed sex ratios and mortality in some of the sibship samples.

Measurement of song characters and singing activity

To record signals, we transferred males following weighing to individual screen cages (1.5-cm height, 1-cm diameter) and placed these at separated, acoustically insulated cells within a semi-anechoic room (3 × 3 × 2.5 m; see Jang & Greenfield, 1998) also kept at 25.5 °C and a 12 : 12 L : D photoperiod. Recordings were made with a condenser microphone (model 7016, ACO Pacific, Belmont, CA; frequency response ± 6 dB from 10 to 200 000 Hz) during the first 2 h of scotophase. The microphone output was amplified 40 dB and sampled at 298 kHz with a personal computer fitted with an analog : digital soundcard (8-bit A : D converter; SoundFX, Engineering Version; SiliconSoft; San Jose, CA) and custom digital signal processing software. From each male we saved a 65-kB file that represented a 220-ms sample of its song. We subsequently analysed these files and measured the PR, the mean peak amplitude (PA) of the sound pulses, and the mean asynchrony interval (AI) within pulse pairs, defined as the time elapsing between onsets of the two pulses. Using a weighted linear formula developed from selection gradient analysis (Jang & Greenfield, 1998), we calculated an attractiveness index (AT = 0.117 PR + 0.524 PA + 0. 296 AI) of each male's song.

On day 1 we determined the duration of each male's singing activity throughout the 12-h scotophase. We left males in their screen cages and transferred them to an environmental chamber (25.5 °C; 12 : 12 L : D photoperiod) fitted with separated, acoustically insulated cells as in the recording room. This feature ensured that males would not perceive their neighbours' songs as loud and adjust their own singing accordingly (see Jia *et al*., 2001). Using a bat detector (model S25, UltraSound Advice, London, UK), a device that converts ultrasound to audible frequencies, we checked whether each male was singing at 30-min intervals beginning 1 h prior to scotophase onset and lasting until 2 h after the last observation of singing. Based on previous observations showing that isolated males usually sang continuously once they began at the beginning of the night, we equated the total number of checks during which a male sang as the duration of his nightly singing period. Because a previous study also showed that a male's nightly singing duration was relatively consistent throughout his adult lifespan (Brandt, 2003), we did not monitor the males' singing activity throughout the night at later ages. We did, however, observe males twice daily until death to document their lifespan and confirm that they sang on each night.

Estimation of genetic parameters

We estimated the additive genetic variance (V_{A}) for each of seven traits, five song traits, body mass at eclosion, and adult lifespan, and the genetic covariances ($\mathrm{COV}_{\mathrm{A}}$) between those traits. The five song traits included peak amplitude, pulse-pair rate, asynchrony interval, overall attractiveness, and nightly singing duration. V_{A} and $\mathrm{COV}_{\mathrm{A}}$ values were estimated from the 1957 F_1 male offspring using a two-level nested analysis of variance (model II, for random factors) for unequal sample sizes (Becker, 1984; Sokal & Rohlf, 1995). Owing to approximate homogeneity of variance and normality, we used untransformed data throughout our analyses. Estimates of narrow-sense heritability (h^2) and genetic correlations were determined from the V_{A} and $\mathrm{COV}_{\mathrm{A}}$ estimates per Becker (1984). Standard errors for these genetic parameters were obtained via jack-knife resampling (Efron & Tibshirani, 1986; Meyer *et al*., 1986).

Anatomical measurements

Because our quantitative genetic estimates revealed significant correlations between song characters and body mass (see Results), we made further anatomical measurements to pinpoint the source of these correlations. Specifically, we had found that body mass was positively correlated with PA but negatively correlated with PR. To examine the relationship between body mass and PA, we obtained a sample of 33 males from the stock population, weighed and recorded the song of each individual, and measured the major axes (± 0.005 mm) of both tymbals (Fig. 1) and computed the mean of the two measurements. We focused on tymbal size because sound is radiated from these structures and larger tymbals might be expected to buckle over a greater displacement distance, thereby generating sound waves of higher amplitude (Greenfield, 2002). In effect, we tested whether tymbal size was commensurate with body mass.

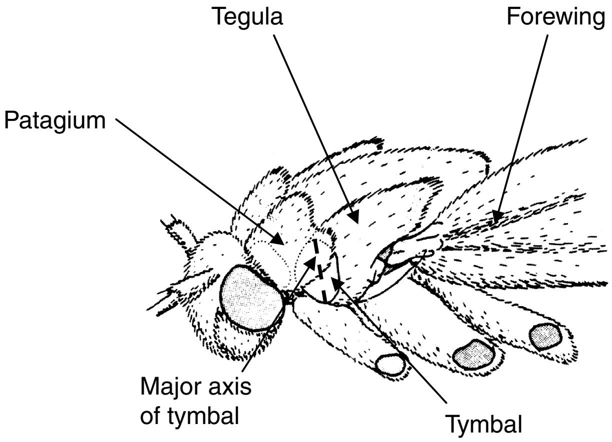

Fig. 1 Location of the sound-producing tymbal on the front of the tegula (sclerite covering base of fore-wing) of male *A. grisella,* with major axis of the tymbal indicated. Drawing modified from Spangler *et al.* (1984).

To examine the relationship between body mass and pulse-pair rate, we determined the second moment of inertia of the wings of the 33 males. This measurement describes how wing mass is distributed around the axis perpendicular to the body and accounts for shape as well as size of the wings. Wings with higher second moments of inertia are expected to require more energy to move and, consequently, to be stroked at a slower rate (see Alexander, 1983). Analogous to the above examination, we tested whether the second moment of inertia was commensurate with body mass.

To determine second moments of inertia, we removed both fore and hind left wings from each male, mounted them on a microscope slide, scanned the slide with a digital scanner and saved a digitized image at a standard magnification. We then printed the image on paper and cut transverse sections of equal width from the printed image of the wings beginning at the base and progressing distally (Fig. 2). Second moments of inertia (I) were then calculated from these transverse paper sections following a simplified formula, $I = \sum (A_i \cdot x_i^2)$, where A_i is the area of the ith transverse section and x_i the distance along wing axis from wing base to midline of that transverse section (see Alexander, 1983, pp. 28–29).

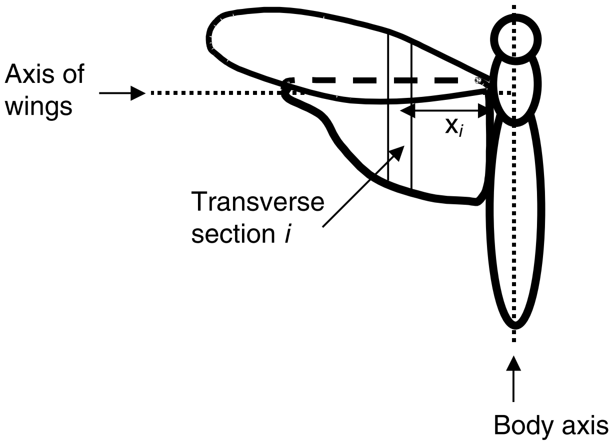

Fig. 2 Position of fore- and hind-wing with respect to wing and body axes. Representative transverse section used in determining second moment of inertia ($I = \sum (a_i \cdot x_i^2)$, where A_i is the area of the *i*th transverse section and x_i the distance along wing axis from wing base to midline of that transverse section) of left wings is shown.

Results

Phenotypic variation and evolvability

Means and variances for all seven song and life history traits measured are presented in Table 1. Overall, our results are consistent with those reported in previous studies of *A. grisella* (Jang & Greenfield, 1996; Jang *et al.*, 1997). Mean values for these traits varied considerably between full- and half-sib families, suggesting that the variances included significant genetic components. This suggestion is supported by finding substantial coefficients of additive genetic variance ($CV_A = \sqrt{V_A}/\bar{x}$, where $\bar{x}$ = phenotypic mean) and narrow-sense heritability (h^2) estimates for most traits, both values indicating the potential for response to selection (see Houle, 1992). Again, our CV_A and h^2 estimates for the various traits are consistent with those reported in previous studies (Collins *et al.*, 1999; Jia *et al.*, 2000). The one inconsistency is the heritability of asynchrony interval, which was previously reported as insignificant (Collins *et al.*, 1999). Possibly, high environmental variance accounted for low heritability in the previous study.

Genetic correlation

We arranged our variance and covariance values in a standard *G* matrix, presented in Table 2, which also includes genetic correlations ($= COV_{A\ i,j} / \sqrt{(V_{Ai} \cdot V_{Aj})}$). The matrix indicates that genetic correlations between all song and life history traits are significant save between adult lifespan and two song traits, PR and the nightly singing duration. The strength of these genetic correlations indicates that any one of the seven traits may influence fitness.

Importantly, all significant genetic correlations were positive except those involving PR. That is, those males who were more attractive (and sang with higher peak amplitudes and longer asynchrony intervals) were larger individuals who tended to sing for longer nightly periods and to enjoy longer adult lifespans; we found that all males did sing on every night throughout their lives. Because adult *A. grisella* neither feed nor drink, only those males in superior condition at the time of eclosion may have the energy reserves needed for extended longevity and for sustaining lengthy periods of singing.

Anatomical correlates of song

Figure 3 shows the least-squares linear regressions of peak amplitude on tymbal axis and of tymbal axis on eclosion body mass. As expected, we found that larger moths did have larger tymbals and that moths with larger tymbals sang at higher peak amplitudes. Similarly, Fig. 4 shows the regressions of PR on the second moment of inertia of the wings and of the second moment of inertia on eclosion body mass. Again, we found that larger moths did have higher second moments of inertia and that moths with higher second moments of inertia sang at slower PRs.

Table 1 Phenotypic mean (x), phenotypic variance, coefficient of additive genetic variance ($CV_A = \sqrt{V_A}/x$), narrow-sense heritability (h^2 ± SE), and *P*-values of h^2 for seven song and life history traits in male *A. grisella*. See text for trait definitions and calculation of coefficients.

	Phenotypic mean	Phenotypic variance	CV_A	h^2 ± SE;	*P*-value
Pulse-pair rate (s^{-1})	90.41	184.19	0.11	0.56 ± 0.01;	$\ll$0.001
Peak amplitude*	85.74	372.63	0.13	0.36 ± 0.11;	0.002
Asynchrony interval (μs)	666.38	154194.60	0.12	0.19 ± 0.08;	$\ll$0.001
Attractiveness index†	252.75	14178.52	0.21	0.21 ± 0.02;	$\ll$0.001
Eclosion body mass (mg)	14.66	6.16	0.09	0.31 ± 0.11;	$\ll$0.001
Adult lifespan (days)	15.31	15.93	0.26	1.00 ± 0.19;	$\ll$0.001
Nightly singing period (h)	10.44	0.64	0.04	0.23 ± 0.07;	0.001

*Peak amplitude scaled linearly in units proportional to microphone voltage output.
†Attractiveness index given in units determined by calculations from selection gradient analysis; see text.

Table 2 Genetic variance/covariance (G) matrix for song and life history traits in male *A. grisella*. Values were determined from a half-sib/full-sib breeding experiment including 50 sires, four dams per sire, and 10 sons per dam. Boldface values in diagonal cells are additive genetic variances (V_A), values below and to the left of the diagonal are genetic covariances (COV_A), and values above and to the right of the diagonal are genetic correlations ($= COV_{A\,i,j} / \sqrt{(V_{A\,i} \cdot V_{Aj})}$) ± SE. See text for definitions of traits.

	PR	PA	AI	AT	EBM	NSP	AL
Pulse-pair rate (PR)	**104.37**	−0.75 ± 0.11†	−0.62 ± 0.13†	−0.57 ± 0.18*	−0.74 ± 0.09†	−0.97 ± 0.06†	−0.07 ± 0.15
Peak amplitude (PA)	−88.55	**132.96**	0.80 ± 0.07†	0.94 ± 0.05†	0.81 ± 0.09†	0.81 ± 0.10†	0.59 ± 0.14†
Asynchrony interval (AI)	−1077.10	1569.73	**29290.96**	0.60 ± 0.02†	0.38 ± 0.07†	0.56 ± 0.03†	0.30 ± 0.02†
Attractiveness index (AT)	−30.20	56.22	43.22	**26.94**	0.79 ± 0.11†	0.83 ± 0.10†	0.64 ± 0.09†
Eclosion body mass (EBM)	−10.40	12.92	88.82	5.65	**1.90**	0.81 ± 0.08†	0.63 ± 0.12†
Nightly singing period (NSP)	−3.79	3.54	36.50	1.64	0.42	**0.15**	0.14 ± 0.12
Adult lifespan (AL)	−2.67	27.15	206.23	13.31	3.51	0.21	**16.08**

*$P < 0.05$.

†$P < 0.05$ after Bonferroni correction for multiple tests.

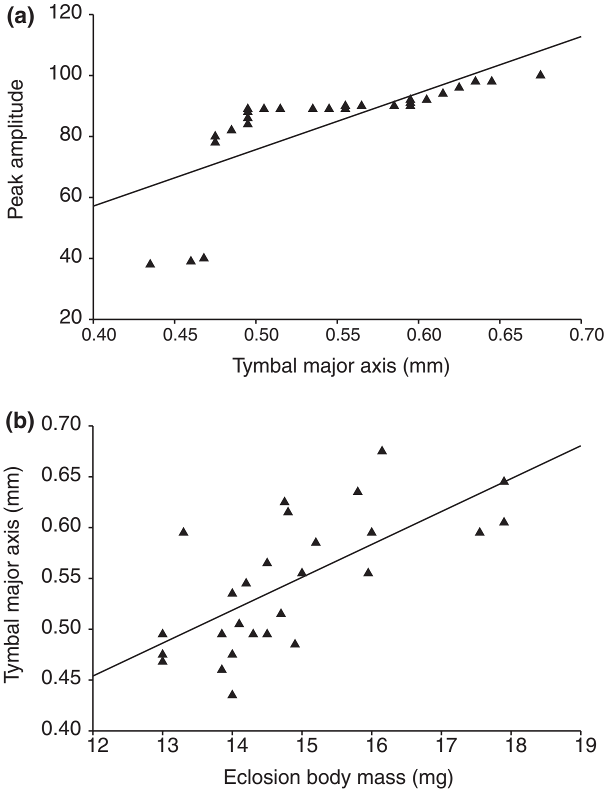

Fig. 3 Least-squares linear regressions of (a) song peak amplitude vs. mean length of tymbal major axis ($y = -17.0 + 185\,x$, $r^2 = 0.507$, $t = 5.07$, $P < 0.001$; after removal of three outliers at lower left: $y = 44.6 + 81.0\,x$, $r^2 = 0.823$, $t = 9.94$, $P < 0.001$) and (b) tymbal major axis vs. eclosion body mass ($y = 0.066 + 0.0324\,x$; $r^2 = 0.468$; $t = 4.69$; $P < 0.001$) in male *A. grisella*.

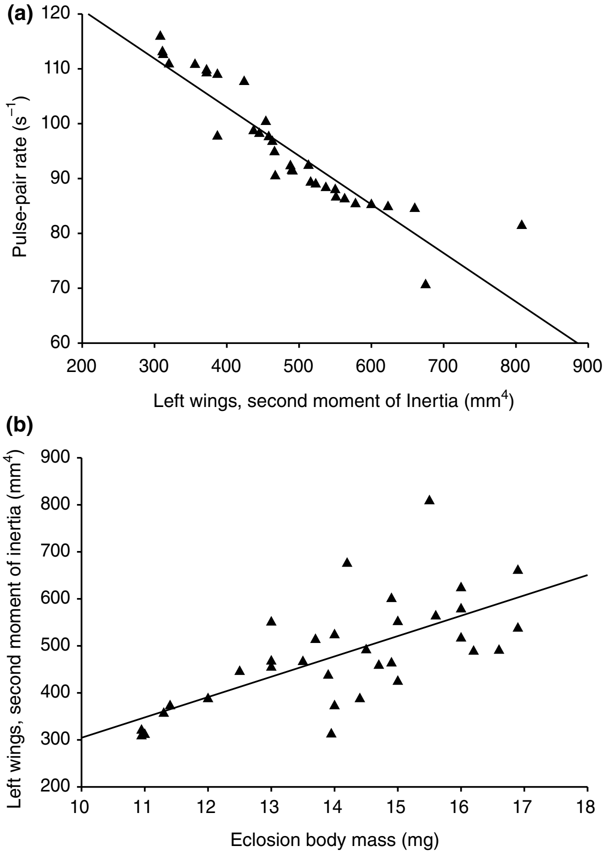

Fig. 4 Least-squares linear regressions of (a) song pulse-pair rate vs. second moment of inertia of left wings ($y = 138 - 8.86\,x$; $r^2 = 0.841$; $t = -12.81$; $P < 0.001$) and (b) second moment of inertia vs. eclosion body mass ($y = -1.28 + 0.433\,x$; $r^2 = 0.445$; $t = 4.99$; $P < 0.001$) in *A. grisella*.

Discussion

Previous and current findings demonstrate considerable phenotypic variance for condition, song characters and adult lifespan in male *A. grisella* and substantial additive genetic components of these variances. Thus, we judge these traits as evolvable. Our current findings of additive genetic variance for condition and a significant genetic correlation between condition and male song attractiveness support the hypothesis that variance in sexually-selected traits stems from variance in condition (see Rowe & Houle, 1996). In *A. grisella,* this relationship reflects the large contribution of PA to song attractiveness and the influence of body size on amplitude. Although larger males generally sing at slower – less attractive – PRs, this factor is overridden by the two other critical song characters, asynchrony interval and peak amplitude (see Jang & Greenfield, 1998). We must note that the attractiveness index calculation is based on analysis of a different population than the one studied here, and relative contributions of the various song characters to overall song attractiveness might differ between populations. Nonetheless, positive influences of PR, asynchrony interval, and PA on attractiveness have been found in several *A. grisella* populations (cf. Jang & Greenfield, 1996, 1998), and we perceive no reason to doubt the basic relationship, and genetic correlation, between body size and song attractiveness in our current study.

Contrary to expectations based on energy constraints, we found no evidence for trade-offs between song attractiveness and other parameters enhancing fitness in *A. grisella*. Those males who sang with greater attractiveness – which does consume more energy (Reinhold *et al.*, 1998) – were not only larger, but they also sang for more hours per night and, given that they survived longer, for more nights. Moreover, another recent study (A. Danielson-François, unpublished data) demonstrated that no trade-off exists between song attractiveness and development rate in *A. grisella*. Thus, we claim that variation in condition, combined with the correlation between condition and song, is the primary factor yielding variation in male song attractiveness. It is conceivable that a trade-off between song attractiveness and predation risk occurs in the field, where louder songs might be more apparent to natural enemies, e.g. substrate gleaning bats (Greenfield & Baker, 2003). However, a field survey of a natural *A. grisella* population did not reveal reduced longevity among attractive males (Brandt, 2003).

A corollary of the hypothesis that variance in sexually selected traits stems from variance in condition is that additive genetic variance for these traits should increase as they evolve toward greater condition dependence (Rowe & Houle, 1996): Under such dependence, only males in maximum condition can exhibit the trait in exaggerated form. Evidence for this expectation has been found in a comparative, phylogenetic analysis of stalk-eyed fly species (Wilkinson & Taper, 1999). In *A. grisella,* we note that CV_A values for the three song characters as well as overall song attractiveness are each higher than CV_A for condition, a relationship that is consistent with the corollary. But, further comparative evidence would be necessary to substantiate the inferred response, that additive genetic variance for sexually-selected traits increased as their condition dependence evolved.

At an ultimate level, the findings we report on the condition-dependence of sexually selected traits really just shift our key question – the lek paradox – to another level: determining what maintains additive genetic variance for condition. In formulating their condition-dependence hypothesis, Rowe & Houle (1996) proposed that this variance component can be maintained when a large number of genes, each experiencing high mutation rates, influence condition. Unfortunately, our inability to estimate mutation rates renders this mutation-selection balance explanation currently untestable. On the other hand, a recent study of *A. grisella* points toward genotype × environment interaction as the critical evolutionary mechanism maintaining additive genetic variance for condition (A. Danielson-François unpublished data). That study reports that certain genotypes exhibit superior condition and song attractiveness as long as the environmental regime is favourable but decline markedly in condition and attractiveness when stressed by density and insufficient food. Alternatively, other genotypes never exploit favourable environments very effectively, but they do not suffer greatly under stress. The co-occurrence of such genotypes means that no single genotype will perform maximally in all environments, a phenomenon known as ecological crossover. Theoretically, ecological crossover is capable of retaining additive genetic variance in a population (Gillespie & Turelli, 1989; Ellner & Hairston, 1994), and our ongoing investigations of *A. grisella* are aimed at experimentally verifying this potential in natural populations.

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