Different sexual traits show covariation among genotypes: implications for sexual selection

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An unresolved question in sexual selection research is whether different secondary sexual traits are developmentally independent or instead whether their degree of expression is a manifestation of a general resource pool (i.e., condition) within the organism. If degree of expression of different sexual traits reflects ability to accumulate condition, then covariation should exist across genotypes in the expression of these traits, even if they are very different in kind. Here we present evidence for predicted covariation between morphological (sex comb size) and behavioral (courtship song) sexual traits among genetic lines of Drosophila bipectinata Duda extracted from a natural population. There is evidence that both these traits in Drosophila are condition dependent and subject to sexual selection. We detected significant body size–independent differences in comb size among 32 lines. Replicate lines exhibiting relatively high and low values of comb size were then subjected to analyses of courtship song. High sex comb lines exhibited shorter mean burst period and shorter mean burst duration than low sex comb lines. These song differences occurred only during the distant pursuit phase of male courtship and existed despite factoring out individual variations in sex comb size, the trait on the basis of which test lines were originally chosen. The results verify the prediction of an association between condition-dependent secondary sexual traits across genotypes and, therefore, support the existence of an overall genetic quality related to condition acquisition. Key words: condition, courtship song, Drosophila bipectinata, genetic quality, secondary sexual traits, sex comb. [Behav Ecol 18:311–317 (2007)]

Several leading models describing the evolution of secondary sexual traits require that sexual trait expression covaries positively with overall genetic “quality” of individuals (Zahavi 1975; Kodric-Brown and Brown 1984; Grether 1996; Andersson et al. 2002; Kokko et al. 2003). Genetic quality may be envisioned as a genotype’s ability to acquire limiting resources from the environment, so that individuals of higher quality come to accumulate a greater pool of resources (i.e., condition) available for allocation among competing physiological functions (Rowe and Houle 1996; Bonduriansky and Rowe 2005).

An important prediction of the hypothesis that variation in sexual trait expression reveals differences in genetic quality as defined above, but one that has received little attention, concerns covariation in expression among different condition-dependent sexual traits of individuals. If the different traits are expressions of a common underlying genetic property conferring condition (Møller and Pomiankowski 1993; Johnstone 1996; Møller and Mousseau 2003), and given sufficient variation in genetic quality or condition, there should exist a positive relationship in expression between the sexual traits (Kodric-Brown and Brown 1984; Johnstone 1995b; Candolin 2003), even if they are very different in kind (e.g., morphological vs. behavioral). Houle (1991) provided theoretical rationale for this prediction by showing that if certain reasonable assumptions concerning the genetic architecture of resource acquisition are met, the equilibrium additive genetic covariation among condition-dependent traits can be positive.

The few empirical studies that have tested for covariation among secondary sexual traits have focused on the phenotypic level and have yielded mixed support for this prediction (Andersson et al. 2002; Møller and Petrie 2002; Jawor and Breitwisch 2004; Moczek et al. 2004). Noteworthy, therefore, is a study of guppies (Poecilia reticulata), showing no consistent trend toward positive genetic covariation among different ornamental traits of males, suggesting that collectively these traits do not reveal a common genotypic property of males (Brooks and Endler 2001). Similarly, Bonduriansky and Rowe (2005) found that out of all possible genetic correlations between 4 sexually selected, condition-dependent traits in the piophilid fly, Prochyliza xanthostoma, only one correlation was significantly positive. Interestingly, this significant correlation occurred between head length and antenna length, the 2 traits exhibiting strongest condition dependence.

Here we test for an association among genotypes between 2 very different sexual traits of male Drosophila bipectinata: sex comb size and elements of courtship song. The sex comb consists of stout teeth arranged in rows on the first and second tarsal segments of the male front legs (Figure 1A). Polak and Starmer (2005) have shown that a 4 °C increase in developmental temperature increases comb fluctuating asymmetry (a putative measure of developmental instability) and reduces both comb tooth number and adult body size, suggesting that comb expression is condition dependent. Comb size (i.e., tooth number) is inheritable and under positive sexual selection in natural populations in northeastern Australia (Polak et al. 2004).

Courtship songs in Drosophila are the product of wing beats produced by contractions of the insect’s flight musculature (Ewing 1979). As such, courtship songs are energetically expensive to produce and thus have the potential to exhibit condition dependence (Hoikkala and Isoherranen 1997; Hoikkala et al. 1998; Talyon and Dowse 2004). Drosophila song consists of bursts (trains) of sound pulses (Figure 1B), and studies have shown that certain features of this structure can be direct targets of mate choice. For example, in Drosophila montana, males producing songs with shorter pulses and
higher carrier frequency are preferred by females (Aspi and Hoikkala 1995; Ritchie et al. 1998; Hoikkala and Suvanto 1999) and produce progeny with elevated survival (Hoikkala et al. 1998). In Drosophila mojavensis, burst duration (BD) differs geographically and parallels differentiation in epicuticular hydrocarbons that serve as contact pheromones during courtship (Etges et al. 2006), suggesting that BD is under sexual selection in this species. Finally, in Drosophila melanogaster, females prefer more pulses per unit time up to a threshold but show no preference for any particular number of pulses per burst (Talyn and Dowse 2004).

Although not previously studied in D. bipectinata, there is compelling evidence for condition-dependent expression of some song features in Drosophila. In both D. montana and Drosophila littoralis, overwintering, field-caught males show high repeatability for pulse length, suggesting condition-dependent variation of this trait (Hoikkala and Isoherranen 1997). Pulse number (a determinant of BD) also shows evidence of condition-dependent expression (as revealed by significant repeatability after cold stress) and exhibits significant heritability in laboratory and field populations of D. montana (Aspi and Hoikkala 1993; Hoikkala and Isoherranen 1997). Here we examine variation across genotypes within an Australian population of D. bipectinata in BD and burst period (BP) against a backdrop of variation in sex comb size.

We extracted genotypes (isofemale lines) from a natural population and chose lines with largest and smallest mean sex comb size for analysis of male courtship song. If degree of investment into the sex comb reflects genetic quality, as defined above, that is shared at least in part with that of song, we made the specific prediction that individuals from lines developing larger combs should on average express shorter BPs and BDs. This prediction is reasonable because song consisting of higher burst frequency (number bursts per unit time) should be more energetically costly than the same amount of song consisting simultaneously of longer BDs and longer intervals between bursts. Because shorter values of BD and BP result in a greater rate of burst production, BDs and BPs should reflect male stamina and physiological recovery rate.

Because some song components were significantly correlated with comb size among individuals, we tested for song differences among lines while factoring out sex comb size variation in order to control for scaling between these traits. This correction is necessary for establishing whether song differences reveal properties other than scaling with sex comb size (the trait used to originally choose the lines). Figure 2A illustrates the hypothetical situation wherein differences between observed song means (Y1 and Y2) occur entirely as a result of scaling (and see Cotton et al. 2004, p. 781). We envision that such scaling could arise because a small subset of loci coding specifically for the sex comb exerts pleiotropic effects on song attributes and not because of genotypic effects. Thus, once adjusted to a common sex comb size (X0), line means (T1 and T2) cease to differ. In Figure 2B, however, song means differ despite the correction. We envision that this alternative outcome could arise if the condition-dependent sexual traits reflect an inherent property of the lines: the effects of loci throughout the genome that collectively contribute to the genetic component of condition.

MATERIALS AND METHODS

Isofemale lines

A total of 32 lines were established, each from a single copulating pair of D. bipectinata captured in the field. Individual pairs were gently aspirated directly into 35-ml vials with food from the surface of exposed flesh of jackfruit, Artocarpus heterophyllus Lam. (Moraceae), from 11–14 January 2004 at the Cape Tribulation Farmstay, northeastern Queensland, Australia. Pairs were captured between 5:15 and 6:30 AM, coinciding with the narrow period of peak mating activity for this species (Polak et al. 2004). Each newly captured female was allowed to oviposit for 3 days into a 35-ml food vial and transferred to a fresh vial for another 3 days for a total of 2 vials per line. The food consisted of 1.7 g instant Drosophila medium (Carolina Supply Co.), 8 ml water, and 1 ml of crushed banana mixed with live yeast slurry. A general stock of flies was established from 2 pairs and each newly captured pair was added to this stock. On January 16, flies were brought to a laboratory at Macquarie University, Sydney, Australia and maintained in an environmental room at 24–26 °C and 60–70% RH, and at a 12:12 h light:dark photoperiod (lights came on and off at 6:00 AM and 6:00 PM, respectively).

Sex comb size and thorax length (estimate of body size) were determined for 8–10 F1 flies emerging from each replicate vial per isofemale line. Flies were killed with ethylene dichloride fumes and their foretarsi placed on the surface of double-sided transparent tape. For each male, the numbers of teeth in the sex comb segments on each foretarsus were counted under an Olympus SZX12 microscope, and thorax length was measured using an ocular micrometer of a WILD
M4A microscope. We define “comb size” as the number of teeth summed across the first and second segments of the comb (i.e., C1 + C2) (Figure 1A); C1 and C2 constitute the major elements of the comb and are positively correlated genetically (Polak et al. 2004).

We used an analysis of covariance (ANCOVA), with line and vial (nested within line) as factors and thorax length as the covariate, to test for comb size differences among lines. Four test lines were chosen for acoustic analysis: 2 lines each exhibiting relatively high and relatively low mean comb size. Lines were cultured for another generation under conditions specified above, and F2 flies from each line were characterized for comb size as above. ANCOVA tested whether the differences in comb size between lines persisted among the F2 flies (i.e., exhibit cross-generational stability in expression). F2 and F3 flies were subjected to acoustic analysis.

Recording fly song and behavior

The courtship songs of D. bipectinata are produced at very low amplitude, so all audio and video recordings were made in a sound-attenuating chamber (2.38 m wide × 2.15 m high × 2.38 m deep; Amplusilence S.p.a., Robassomero, Italy), lined with 10-cm “Sonex” foam baffles (Ilbruck Inc., Bad Wildungen, Germany) on the side walls and 15-cm baffles on the ceiling. To further reduce external noise, a double-walled rectangular sound-attenuating box was constructed. The test arena was mounted within this box and illuminated by a 15-Watt incandescent lamp.

Flies were confined within a cylinder (diameter 22 mm, height 4 mm) of soft clear plastic and a floor of fine PVC-coated fiberglass mesh (Cyclone MINIwave 0.25-mm yarn, 7.2 × 12 strands/cm). The top of the cylinder was sealed with a glass microscope slide, above which was mounted a video camera (Panasonic WV-BP550) with a wide-angle lens. A Sennheiser MKH-40 microphone was clamped immediately below the arena floor, facing upward. Flies were inserted through a 40-mm-long tube (3 mm internal diameter) joined to a port in the arena wall.

Video signals were recorded on a digital VCR (Sony DSR-20P) in PAL DVCAM format (resolution 576 × 720 pixels, 50 fields/s). The microphone was connected to a Behringer MIC-100 preamplifier, which also provided 48V phantom power. To obtain additional gain, the output signal was then fed through an amplifier/mixer (Behringer Eurorack MX602A), which also converted the balanced microphone output to an unbalanced line-level signal suitable for the digital VCR. Audio levels were metered at every step in the recording pathway to ensure that clipping did not occur. Sound was digitized at 44.1 kHz, 16 bits. This system has a flat frequency response (±1 dB) from 50 Hz–20 kHz, with no wow or flutter. The video and sound tracks were synchronized using a common time code.

Sound recordings were made between 5:30 and 8:00 AM. Female subjects were 3 days old and had been separated from males for at least 24 h. Flies were kept in the dark inside a box within the environmental room overnight until immediately prior to testing; peak courtship activity of this species in the field begins at first light and runs to ≈ 1 h after sunrise (Polak et al. 2004). A female was transferred with an aspirator from her holding vial to the arena through a tube. A barrier of clear polyacetate film was inserted through a slit in this tube close to the arena wall to prevent her escape. A male fly was then aspirated into the tube, and the open end of the tube was plugged with a dowel. After 5 min of acclimation, the barrier was removed and the dowel was gently pushed into the tube, thus encouraging the male to enter the arena. No fly of either sex was anesthetized on the morning of its use in recordings. Recordings lasted 13 min for each male.

During recording sessions, we watched interactions on an external video monitor and listened to the recorded song with headphones. Video time code was continuously displayed to track recording duration. The temperature of the sound-attenuating chamber was noted at the beginning and end of each recording.

Analyses of song recordings

Recordings from the digital Sony VCR were transferred to a computer (Macintosh G4, Apple Computer) via IEEE 1394 “firewire,” using iMovie software. Original DV files of 2–5 GB each were then MPEG-4 compressed at 80% quality to generate QuickTime files of typically 0.5 GB using Cleaner software 6 (Discreet). The video stream was deinterlaced and noise reduced, but the sound track was left un compressed to avoid the introduction of artifacts. Sections containing multiple
bouts of male courtship were then isolated and saved as self-contained Quicktime movies. Copies of the sound track were created as mono audio (.aif) files. These were then further processed and analyzed using Canary v. 1.2.4 (Cornell Bioacoustics, Ithaca, NY).

To remove background sounds that masked songs in the time domain, all audio files were first high-pass discrete Fourier transform filtered in Canary, completely attenuating frequencies below 80 Hz. Preliminary analyses had revealed that most of the noise was within the filter stopband; later checks of song fundamental frequency confirmed that no song components were affected by the filter.

Within the 13 min of video recording, males typically engaged in multiple bouts of courtship. A bout of courtship began when a male engaged in courtship and ended when he mounted or when the pair separated by >2.5 body lengths. During courtship, males produce bursts (or trains) of sound pulses. We measured BD and BP from oscillograms generated by Canary (Figure 1B) for songs produced during a male’s first courtship bout (and see Crossley [1986] for similar terminology). Song measurements were made without knowledge of the identity of the singing male (i.e., to which line it belonged). For some courtship bouts, song was of insufficient amplitude and therefore not clearly discernable because males were too far from the microphone. In these cases, subsequent bouts were sampled. The courtship bout from which temporal measurements were obtained (1, 2, 3, or 4) for a given male was coded in preliminary analyses to check whether bout explained variation in male courtship (see next section). BD and BP were distinguished according to the following behavioral phases of courtship. During “distant pursuit,” males chase females from 2.5–1.5 body lengths away, whereas during “close pursuit” males chase females from a distance of ~ 2 body lengths to the tip of her wings. During “near contact,” females are typically stationary and males court from a distance of 1 body length to a head-under-her-wings position. When mounting, a male climbs or lunges on top of a female, grasps her with his foretarsi (at which point the combs come into contact with the female’s abdomen) and curls his abdomen downward. A successful mounting results in copulation. A mounting, whether successful or not, ends a bout of courtship. We distinguished song BP and BD according to these 3 courtship phases in order to refine our analysis of song variation. Males produce a long song (many pulses [e.g., 25] per burst) during distant pursuit, whereas during close pursuit and near contact, males predominantly sing a short song (few [e.g., 3] pulses per burst) (Crossley 1986). It was important to distinguish these song types because song parameters, such as the BP, can vary significantly between them (Crossley 1986).

The song of a total of 79 males (n = 38 and 41 from high and low comb size lines, respectively) was analyzed. However, we were unable to acquire BP and BD for all phases of courtship for all males either because males did not sing during a particular phase or because song was of insufficient amplitude to be confidently discerned (see above). Moreover, distant pursuit occurred especially infrequently, so sample sizes are least for this phase of courtship. Multiple BPs and BDs for a given phase were measured per courtship bout of a male, and a mean for that male was calculated and entered into MANOVAs, below. Number of males, and mean number of measurements for a given song trait made per male, are reported.

**Song differences among lines**

Multiple analyses of covariance (MANCOVAs) with comb size as the covariate were conducted to discriminate between high and low sex comb lines with respect to BP and BD in the different courtship phases. Although the comb size covariate was significant only for BD (see Results), comb size was entered as a continuous variable in all analyses for consistency among traits. In a first MANCOVA, we tested for effects of line on 4 dependent variables: BD and BP during near contact and close pursuit. A second MANCOVA tested for line effects on these same traits but during distant pursuit. Separate MANCOVAs were necessary because distant pursuit occurred less frequently than either the close pursuit or near contact courtship phases. Roy’s greatest root $F$ statistic was used to derive statistical conclusions in all MANCOVAs. Squared canonical correlations ($r^2$) are reported to describe between-line variation collectively explained by the response variables.

“Protected” univariate ANCOVAs (Scheiner 1993) were conducted subsequent to detecting significant line effects with either MANCOVA. The homogeneity of slopes assumption across replicate lines was verified by testing for an interaction between line and comb size; in no case was this interaction significant. Distributions of residuals were visually inspected to check for normality and for constancy of error variances across groupings. The influence of conspicuous outliers was quantitatively assessed by the DFFITS statistic (SAS 2002).

The addition of male thorax length, female thorax length, temperature, and bout as covariates in preliminary MANCOVAs revealed that none of these variables had significant effects on the dependent variables; these variables were thus excluded from the reported analyses. Significant effects of temperature on *Drosophila* song parameters are known (Ritchie and Kyriacou 1994; Noor and Aquardo 1998), and their absence here may be attributable to the particular song parameters measured and, perhaps, partly also to the narrow range of temperatures recorded in the sound-attenuating chamber (range: 25–27.5 °C, $X \pm SD$: 26.1 ± 0.85 °C).

### RESULTS

#### Choosing high and low sex comb lines

ANCOVA, with line and vial (nested within line) as factors and male thorax length as the covariate, revealed significant differences in comb size (i.e., C1 + C2, Figure 1A) among the set of lines originally surveyed ($F_{2,7} = 1.76, P = 0.05$). Four lines were chosen, based on mean comb size, to represent the experimental lines: 2 lines with the lowest mean comb size and 2 lines with the highest mean comb size (Table 1).

A second ANCOVA on F2 flies, with line and male thorax length as the covariate, revealed that the comb size differences among high and low lines persisted in the subsequent generation ($F_{3,71} = 31.31, P < 0.0001$). The high lines maintaining a higher comb size than the low lines (Table 1). Thus, these results demonstrate cross-generational stability in the expression of trait differences and confirm previous work on the genetic basis of variation in sex comb size (Polak et al. 2004).

<table>
<thead>
<tr>
<th>Line</th>
<th>Generation</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>27.0 ± 0.43</td>
<td>28.38 ± 0.43</td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>26.2 ± 0.47</td>
<td>27.16 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>24.4 ± 0.47</td>
<td>22.88 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>22.9 ± 0.47</td>
<td>24.04 ± 0.44</td>
<td></td>
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</tbody>
</table>
Testing for song differences among lines

MANCOVA, with comb size as the covariate, on BP and BD during close pursuit and near-contact variables, revealed a significant overall effect of line ($F_{3,53} = 2.50, P = 0.069$), near-contact BD ($F_{3,61} = 0.34, P = 0.80$), and close pursuit BD ($F_{3,41} = 1.40, P = 0.26$). Least-squares means for all song traits are provided in Table 2. Only in the case of close pursuit BP was the line effect significant. However, 3 outliers with considerable influence were detected at the high end of the close pursuit BP distribution. The removal of these outliers from the ANCOVA normalized the residuals but eliminated the significant effect of line on close pursuit BP ($F_{3,35} = 0.42, P = 0.74$) (Table 2). In an alternative approach using the full data set with these outliers included, we performed an ANCOVA on rank-transformed (Conover and Iman 1981) close pursuit BP values, which normalized the data, and likewise found no effect of line on close pursuit BP ($F_{3,38} = 1.52, P = 0.25$). The comb size covariate was not significant in any of the above ANCOVAs ($P$ values: 0.26–0.97).

The second MANCOVA revealed a strong effect of line on the song variables expressed during distant pursuit ($F_{3,15} = 15.39, P < 0.0001, r^2 = 0.75$). The comb size covariate was significant in the analysis of BP ($F_{3,17} = 5.36, P = 0.05$) but not for BD ($P = 0.995$) (Table 2). Protected ANCOVAs showed significant line effects on both BP ($F_{3,15} = 5.82, P = 0.0069$) and BD ($F_{3,17} = 4.47, P = 0.017$) (Figure 3). Thus, genotypes that develop a large body size–specific sex comb also express a shorter BP and BD, but only during distant pursuit courtship.

DISCUSSION

Our initial screening of 32 genetic isolates of *D. bipunctata* extracted from a natural population in northeastern Australia revealed significant among-line variation in sex comb tooth number (comb size), corroborating the results of a previous study showing significant heritable variation underlying comb size in the same population (Polak et al. 2004). We used replicate lines from either extreme of the comb size distribution and showed that these differences persisted through to F2 progeny, thus confirming their genetic basis.

These replicate lines chosen from either extreme of the comb size distribution exhibited the predicted difference in 2 out of 6 components of male courtship song. This finding suggests genetic covariation between these very different classes of secondary sexual traits. By using such maximally diverged lines in regard to sex comb size, we effectively increased the sensitivity of our assays for detecting correlated differences in the second trait. Specifically, males from the high sex comb lines produced songs with significantly shorter mean BP and mean BD during the distant pursuit phase of courtship than did males from the genetic lines developing smaller combs. The reason for why these differences are restricted to distant pursuit courtship song is unknown. It may be, for example, that song production during this phase of courtship is relatively more expensive energetically in order to accommodate the greater distance the signal must travel, thus only revealing differences in physiological condition among competing genotypes at this phase. Indeed, during distant pursuit males produce bursts of song that consistently contain more pulses per burst than during other phases of courtship (Crossley 1986). If more pulses per burst and a shorter latency between bursts require greater energy expenditure and/or shorter recovery times, then song production during distant pursuit may be pushing males especially close to their physiological limits, thus revealing quality differences among them at this phase.

Because both the sexual traits we measured are likely to be condition dependent (see Introduction), their coordinated expression at the genotypic level provides support for a key prediction of the "good genes" hypothesis that degree of sexual trait expression reveals variation in intrinsic quality (condition acquisition ability) within a population (Kodric-Brown and Brown 1984; Andersson 1994; Johnstone 1996; Candolin 2003). It follows that comb size and song may be acting as a suite of traits from which females may acquire reinforcing information regarding the quality of a potential mate. However, although males with larger combs enjoy a higher probability of copulating in at least one field population (Polak et al. 2004), whether either of these traits in *D. bicinctata* are the direct targets of female choice remains to be explored.

The present study is unique because it reports an association across genotypes between 2 very different sexual traits, one being morphological and the other behavioral. A genetic origin of this coordinated expression is further supported by our having reared the lines under common environmental conditions, a procedure that minimized the possibility of

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**Table 2**

<table>
<thead>
<tr>
<th>Courtship phase/song trait</th>
<th>Line</th>
<th>H1</th>
<th>H2</th>
<th>L1</th>
<th>L2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>$0.323 \pm 0.054$ (16, 18.3)</td>
<td>$0.321 \pm 0.054$ (12, 12.4)</td>
<td>$0.474 \pm 0.046$ (17, 13.3)</td>
<td>$0.332 \pm 0.067$ (13, 8.9)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>$0.114 \pm 0.018$ (19, 16.4)</td>
<td>$0.0997 \pm 0.018$ (14, 13.4)</td>
<td>$0.123 \pm 0.016$ (19, 14.3)</td>
<td>$0.122 \pm 0.024$ (14, 9.4)</td>
</tr>
<tr>
<td>Close pursuit</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>BP</td>
<td>$0.358 \pm 0.045$ (12, 7.9)</td>
<td>$0.353 \pm 0.044$ (11, 6.4)</td>
<td>$0.421 \pm 0.050$ (8, 6.8)</td>
<td>$0.393 \pm 0.060$ (9, 4.5)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>$0.180 \pm 0.040$ (14, 8.9)</td>
<td>$0.097 \pm 0.042$ (11, 6.1)</td>
<td>$0.150 \pm 0.048$ (8, 7.3)</td>
<td>$0.221 \pm 0.055$ (10, 4.5)</td>
</tr>
<tr>
<td>Distant pursuit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>$0.260 \pm 0.014$ (7, 4.4)</td>
<td>$0.383 \pm 0.18$ (4, 4.0)</td>
<td>$1.27 \pm 0.19$ (3, 4.3)</td>
<td>$0.873 \pm 0.16$ (7, 3.4)</td>
</tr>
<tr>
<td></td>
<td>BD</td>
<td>$0.106 \pm 0.025$ (7, 4.4)</td>
<td>$0.0956 \pm 0.033$ (4, 3.8)</td>
<td>$0.243 \pm 0.030$ (4, 3.8)</td>
<td>$0.218 \pm 0.029$ (7, 3.3)</td>
</tr>
</tbody>
</table>

Number of males and mean number of measurements per male are given in parentheses for each trait.

* Means for this trait do not include outliers (see text for details).
environmentally induced covariation among the traits, which could arise from shared sensitivity of independent developmental pathways to a common environmental agent(s) (Klingenberg 2003). The likely involvement here of largely separate pathways is suggested by the different nature of these traits: the sex combs, which occur on the anterior-ventral portion of the front tarsi of males, are modified mechanosensory bristles (Kopp and True 2002), whereas courtship songs are the result of wing movements generated by the neuromusculature within the thoracic cavity (reviewed in Gleason 2005).

We also considered the possibility that the observed associations arose because of a direct interaction between the different developmental networks producing these traits, a recognized cause of covariation among traits of individuals (Cheverud 1982; Nijhout and Emlen 1998). Under such a “local effects” model, the observed differences in sex traits among lines would merely reflect scaling between the traits and not differences in overall genetic quality (Figure 2). Such scaling could arise in cases where natural allelic variation at a relatively small subset of developmental loci of the sex comb were to differentially influence the development of song-producing neuromuscular systems. The existence of such “local effects” is feasible because in at least one case (BD during distant pursuit) song was significantly correlated with comb size at the individual level. Thus, we accounted for the scaling possibility by correcting song features for interindividual differences in comb size using ANCOVA. This approach of statistical control was applied to factor out any effects of scaling between song and sex comb size (Figure 2). A nonsignificant line effect in our ANCOVAs would have supported the local effects hypothesis, as occurred in a previous study of the relationship between a measure of developmental instability and mating behavior (Polak and Stillabower 2004), an outcome receiving its own theoretical support (Klingenberg and Nijhout 1999). In the present study, however, strong differences in song structure between the genetic isolates persisted despite the correction. Hence, our data support the hypothesis that these different secondary sexual traits of D. bipectinata tap a general property of the genotypes we surveyed, namely, that which confers overall genetic quality related to the acquisition of condition.

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REFERENCES


Figure 3

Least-squares mean duration of the BP and BD during distant pursuit in male Drosophila bipectinata for high (H) and low (L) sex comb genetic lines extracted from a natural population. Error bars represent ±1 standard error.