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Original Article

Differential genotypic effects of sexual trait size on offspring mating success and viability

Michal Polak,[a] Kerry V. Fanson,[b] Phillip W. Taylor,[b] and Sarsha Yap[b]

[a]Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006, USA and
[b]Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia

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Indicator models of sexual selection predict that females mating with the most ornamented males should produce offspring with enhanced expression of fitness-related traits, such as overall vigor and viability. Empirical support for this prediction, however, is limited. We quantified the effects of a heritable and condition-dependent secondary sexual trait on offspring performance traits in *Drosophila bipectinata* Duda (Diptera: Drosophilidae). Forty-eight genetic (isofemale) lines were extracted from a natural population, reared in a common environment, and characterized in terms of sex comb size. We measured pupal viability and adult mating success among the progeny of the 5 lines with the largest combs (high line category) and the 5 lines with the smallest combs (low line category). The high line category produced offspring that were significantly more viable than the low line category, and this advantage held across 2 developmental temperatures. In contrast, there was no effect of line category on male mating success, although at the individual-level, comb size was significantly positively correlated with mating success. Our results indicate that the relative size of the *D. bipectinata* sex comb taps genotypic properties that enhance offspring fitness in a trait-specific manner. Thus, distinct proximate mechanisms likely underlie relationships between secondary sexual trait expression and different performance traits in offspring, offering a possible explanation for inconsistent support for the existence of indirect benefits in sexual selection.

Key words: **good genes sexual selection, indicator models, mating success, offspring viability, sex combs.**

INTRODUCTION

Indicator models of sexual selection predict that secondary sexual traits signal genetic quality (Zahavi 1977; West-Eberhard 1979; Andersson 1982; Kodrick-Brown and Brown 1984). A multitude of genetic loci are assumed to influence genetic quality, with each locus contributing a small effect to an organism's overall health and physiological condition (Andersson 1982; Kodrick-Brown and Brown 1984; Rowe and Houle 1996; Tomkins et al. 2004). New alleles with potential to influence fitness-enhancing traits appear frequently and ubiquitously throughout the genome, providing the means for persistent positive covariation between genetic quality and expression of secondary sexual traits (Tomkins et al. 2004). Females mating with the most ornamented males should therefore gain indirect benefits through acquisition of good genes for offspring, with both male and female offspring expected to inherit the fitness-enhancing qualities of their ornamented male parent (Johnstone 1995; Kokko et al. 2006).

Indicator models have received considerable, though not ubiquitous, support (Kokko et al. 2003). Møller and Alatalo (1999) performed a meta-analysis of the relationship between male sex trait expression and offspring viability that included 22 estimates of both vertebrate and invertebrate animals, and found an overall weighted mean effect size of 0.122. This effect was significantly different from 0 and suggests that a good genes mechanism, though weak overall, may be taxonomically widespread. In contrast, a subsequent meta-analysis of an expanded data set failed to replicate this significant effect on offspring survivorship (Prokop et al. 2012). Thus, the available data addressing good genes effects in sexual selection are heterogeneous, and their relative importance in sexual selection has been debated (Neff and Pitcher 2005; Kotiaho and Puurtinen 2007; Slatyer et al. 2012). Whereas a growing number of studies examining effects of secondary sexual trait size on offspring viability are available, perhaps because survivorship effects are more often readily amenable to assessment (Møller and Alatalo 1999), studies examining indirect effects on offspring mating success are relatively rare, and studies that examine both simultaneously are rarer still. This limitation restricts our ability to assess the relative importance of sexual trait size on different classes of indirect benefit in sexual selection (Neff and Pitcher 2005). Thus, there is a need for studies that examine different classes of indirect benefits simultaneously in species where direct benefits, such as male parental care, are minimal or absent.

Here, we test the hypothesis that genotype-specific expression of a male secondary sexual trait predicts offspring performance,

Address correspondence to M. Polak. E-mail: polakm@ucmail.uc.edu. K.V.F. Coauthor is now at School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria 3216, Australia

focusing on 2 fitness traits in a common experiment: juvenile survivorship and adult mating success. The secondary sexual trait is the male sex comb in *Drosophila bipectinata* Duda (Diptera: Drosophilidae), a species in which males provide no material benefits to females other than what may be present in the ejaculate. Sex comb size is both heritable and condition dependent (Polak et al. 2004; Polak and Starmer 2005; Polak and Taylor 2007) and has been shown to be under directional sexual selection for increasing size in the Cape Tribulation (Australia) population under study here (Polak et al. 2004). In the present study, we used field-fresh genetic (isofemale) lines, each established with a single wild-caught female (Parsons and Hosgood 1967; David et al. 2005) from the Cape Tribulation population. We then grouped lines according to body size-specific sex comb size and established large- and small-combed categories. We evaluated effects of divergent categories of lines and 2 developmental temperatures on progeny survival and male mating success. The temperatures we used are within the range experienced by developing flies in the field (Polak and Starmer 2005), and they do not differ in terms of pupal mortality they induce (Stanforth A, Polak M, unpublished data; this study). The use of these temperatures thus avoids the potential problem of environmentally induced developmental selection, which, by disproportionately eliminating low-quality individuals from the population under heightened stress conditions, may confound efforts to estimate the strength of selection and its genetic consequences (Møller 1997; Møller and Cuervo 2003; Polak and Tomkins 2013). In the present study, temperature-induced developmental selection operating early in development (such as at the embryo stage) against low-quality individuals could attenuate genotypic effects of sex comb size on the performance traits measured in the (surviving) offspring.

METHODS

Establishment of lines and general culture

Our approach is based on the use of isofemale (genetic) lines, with each line initiated with 1 wild-caught inseminated female (Parsons and Hosgood 1967; David et al. 2005). Lines are then raised for 1 or more generations under similar conditions in order to minimize environmental variation for particular traits of interest, so that among-line variation can in large part be attributed to genotypic effects (Hoffmann and Parsons 1988; Falconer and Mackay 1996; David et al. 2005). In our study, we established lines with wild-caught copulating pairs of *D. bipectinata* (F0 adults) captured by aspiration from the surface of exposed flesh of jackfruit, *Artocarpus heterophyllus* Lam. (Moracea). Pairs were captured between 4 and 11 January 2011 on the grounds of the Cape Tribulation Farmstay, Northeastern Australia (16°5′6.00″S, 145°27′46.83″E). Each pair was placed in a 35-mL clear polystyrene "food vial" that contained oviposition substrate and larval food comprising 1.7-g instant *Drosophila* medium (Carolina Biological Supply Co.), 8-mL water, and a 0.5-mL slurry of banana and active yeast applied to the surface of the medium. Females were allowed to oviposit for 3 days in their respective vials and transferred to fresh food vials for an additional 3 days. A general culture of flies was established with approximately 100 additional females collected at the same location and time that pairs were captured. This general culture, bred in 12–15 food vials per generation, was the source of all females used in the mating success assays, described below. The lines and general culture were brought to Macquarie University in Sydney, Australia, on 18 January 2011, where they were maintained in a controlled environmental room at 24–25.5 °C, and a 12:12 h light–dark photoperiod.

Characterization and choice of lines

Next generation (F1) adults were harvested from the 2 vials per line within 6 h of emergence and sorted by sex. The number of sex comb teeth in both major rows of the sex comb (C1 and C2) on each foretarsus of males (Figure 1), and thorax length were determined for 5 randomly selected F1 males from each line; females do not possess sex combs. TOTC1 and TOTC2 refer to the total number of teeth in C1 and C2, respectively, summed across tarsi. TOTC1,2 refers to the total number of teeth in both rows (i.e., TOTC1 + TOTC2). We tested for differences among lines in TOTC1,2 by fitting a restricted maximum likelihood (REML) mixed model using JMP statistical software (SAS 2012), with Line, Vial (nested within Line and treated as random), and Thorax Length (as the covariate). Vial was not significant as a random effect (95% confidence interval: −0.397, 1.200) and was excluded. A total of 10 test lines were chosen for use in the temperature experiment: the 5 high lines with the greatest number of teeth and the 5 low lines with the least number of teeth. In this way, we created 2 Line Categories, high and low, respectively.

Temperature treatments

Temperature treatments were administered to these 10 lines after they were allowed to undergo 2 generations of laboratory culture. Temperature treatments were administered to pupae produced by the F2 adults. For each line, 2 food vials were each seeded with exactly 8 females and 5 males, and females allowed to deposit eggs for 48 h. When pupae first became visible in these vials, a pupation substrate, consisting of loosely rolled-up tissue paper, was inserted into the food substrate. After 12 h of allowing larvae to populate the tissue and pupate, the tissue was gently removed from the vial with forceps and cut into approximate halves with scissors, taking care not to contact any pupae. Each half of the tissue was placed into a separate 35-mL polystyrene "holding vial." The tissue from the second food vial of each line was likewise cut into halves and each half placed into one of the 2 holding vials. Thus, there were 2 holding vials per line, each containing 2 pieces of tissue originating from a different food vial. Holding vials were stoppered with a dry

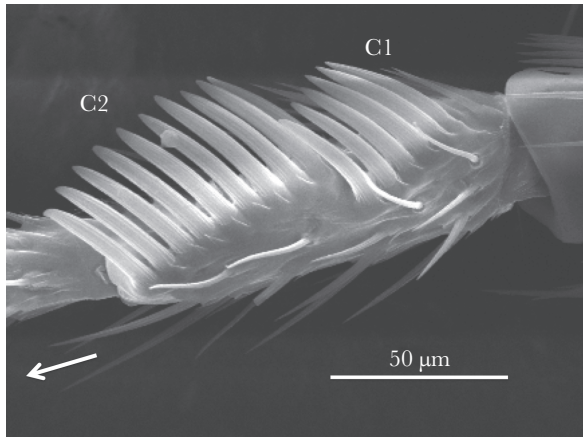

Figure 1
Scanning electron micrograph (×500) of the *Drosophila bipectinata* sex comb, showing foretarsal comb segments C1 and C2. Arrow points toward the distal end of the tarsus.

cotton ball and sealed with Parafilm®. The parafilm was perforated with 6 pinholes for gas exchange. The seal maintained high humidity (>75% relative humidity, verified using Hygrochron temperature and humidity iButtons [Embedded Data Systems]) within vials during the temperature treatments. The holding vials were each placed in an Octagon Pro 20 Precision Incubator (Brinsea Products, UK), one set at 25 °C and the other at 31 °C. Holding vials were placed in their respective incubators for 4 h each day between 8 AM and 12 PM for 3 consecutive days. When not in incubators, vials were held at ambient room temperatures (24–25.5 °C). In sum, the design consisted of 2 replicate holding vials for each Line (n = 10) × Temperature (n = 2) combination (total n = 40 vials).

Pupal mortality

Adult flies that emerged from holding vials were harvested, and the males (as virgins) were set aside for subsequent competitive mating success assays (see Competitive mating success for details). When emergences ceased, all pupal cases were individually teased free of the tissue, and each was examined under a stereomicroscope to determine whether the pupal case was empty (in which case the adult had emerged) or contained a dead fly. For each vial, the proportion of pupae that died was calculated as number dead/total number pupae. Prior to analysis we used an arcsine (square-root) transformation to normalize the data (post-transformation, Shapiro–Wilk W = 0.980, P = 0.70). We used a REML mixed model to analyze the data in JMP (SAS 2012), where the fixed factors were Line Category (high and low) and Temperature (25 and 31 °C). Line (1–5 for each Line Category) was nested within Line Category and treated as a random effect. By nesting replicate lines within Line Category (here and in subsequent analyses of mating success), the analysis accounted for variation in mortality that could arise, for example, due to maternal effects (Sheldon 2000; Qvarnström and Price 2001).

Competitive mating success

After exposure to 25 and 31 °C, 5 pairs of lines were created by randomly assigning 1 high line to 1 low line. Males of each pair of lines competed directly for mates in population cages under ambient environmental room conditions (24−25.5 °C). To acquire the experimental males, flies emerging from their holding vials were separated by sex under light CO_2 anesthesia and held in sex-specific food vials in groups of 20 per vial for 1–2 days. We administered distinctive bristle clips to the males with microscissors under a stereomicroscope to distinguish them by Line and Temperature treatment. Because there were 4 groups (2 lines × 2 temperatures), 4 clip patterns were used: 2–3 bristles were clipped on the right or left sternopleuron and 2–3 bristles were clipped on the right or left dorsal surface of the thorax. After clipping, males were held in food vials for an additional 2–4 days prior to being introduced to a clear acrylic mating cage. All males within a given cage were the same age and carried clips. Cages were 11.5 cm high × 12 cm wide × 24 cm long and contained multiple ports (2-cm diameter holes) in ceiling and side panels for the aspiration of flies in and out of the cages. When not being used to transfer flies, the ports were loosely plugged with cotton wool to prevent flies from escaping. The 2 ends of each cage were sealed with fine mesh for ventilation. Each mating cage contained 3 slices of ripened papaya, each 6–8 cm in diameter.

All females for this experiment were sourced from the general culture. These females were collected within 24 h of emergence and held with general culture males at a 1:1 sex ratio in food vials for 3 days. Females were then separated from males under light CO_2 anesthesia and held for an additional 5–6 days without males in groups of 20–25 flies per yeast-supplemented food vial prior to use (Polak and Simmons 2009).

The evening prior to a competitive mating success assay, and after lights were turned off, flies of both sexes were introduced into their respective mating cage (1 or 2 cages were run on each morning) by gently aspirating the flies through a side port of the cage under dim red light. Forty males were aspirated into each cage, that is, 10 males from each Line Category (low or high) × Temperature (25 or 31 °C) combination. A total of 40 females that had been denied access to males for 5–6 days prior to the assay were aspirated into each cage simultaneously with the males (producing a 1:1 sex ratio). Flies of both sexes remained quiescent on or near the fruit substrate without mating prior to dawn of the next day.

Beginning 20 min before dawn on the next morning, 2 observers aided by headlamps emitting red light continuously monitored the cages for copulating pairs for up to 90 min, coinciding with the window of courtship and mating activity in nature (Polak et al. 2004). As copulating pairs formed, typically on the surface of the fruit substrate, they were aspirated from the cage and placed into a labeled vial for later processing. We continued to collect pairs from a given cage until approximately 50% of the males had mated. Within 60 min of ceasing to collect pairs from a cage, all remaining flies from that cage were recovered. For each mated and single male, we ascertained its line and temperature of origin from bristle clip patterns and determined its thorax length and tooth number in C1 and C2. Two replicate cages were run for each line pair, except for 1 line pair for which only 1 cage could be run owing to time constraints. In total, mating assays were conducted on the mornings of 4 days, with 1–2 cages run each morning. Although a total of exactly 40 males were aspirated into each cage (see above), the number of males recovered from a cage was occasionally less than 40 because some males were lost, damaged, or died between the time they were introduced to a cage and recovered after the mating assay (modal number males recovered = 39, range 37–40, n = 9 cages). Of the total 360 males aspirated into cages, 346 were recovered. Of these 346 males, 170 were mated and 176 were single.

Comb size traits (TOTC1, TOTC2, and TOTC1,2) were analyzed using REML mixed models in JMP (SAS 2012), with Thorax Length as a covariate, and the following fixed terms: Line Category (i.e., low vs. high comb size), Temperature, and the Line Category × Temperature interaction. Line was treated as random and nested within Line Category. All comb traits were close to normally distributed (TOTC1, Shapiro–Wilk W = 0.953, P < 0.05); TOTC2, W = 0.967, P < 0.05; TOTC1,2, W = 0.985, P < 0.05), so were not transformed. Thorax Length data were analyzed similarly but without a covariate in the model. Thorax Length data were close to normally distributed (W = 0.955, P < 0.05) and were likewise not transformed.

Mating success was measured on a binary scale, that is, mated (1) or unmated (0), and analyzed using multiple logistic regression (Hosmer and Lemeshow 1989). We first analyzed mating data without Line Category in the model and tested for the effects of Temperature, Line, Thorax Length, TOTC1, and TOTC2. The Line × Temperature effect was not significant (see Results for details) but was retained in the reported model because its effect is of focal interest to the study.

In a second approach, we employed 2 logistic regression models (Model 1 and Model 2), both of which included Line Category as

a categorical term. Model 1 assessed the effects of Temperature, Line Category, Line, the Line Category × Temperature interaction, Thorax Length, TOTC1, and TOTC2. Line was nested within Line Category and treated as a random effect. We tested for interaction effects between each covariate (TOTC1, TOTC2, and Thorax Length) and all categorical terms in stepwise fashion; because none of these terms were significant, they were excluded from the reported model. We also checked for the effects of Line Pair and Cage (with Cage nested within Line Pair and treated as a random effect); as these terms were not significant, they were likewise excluded.

Model 2 was identical to Model 1 except that we excluded TOTC1 and TOTC2 from consideration. The purpose here was to ascertain whether removal of these variables would alter the effect (or lack of effect) of Line Category: Because Line Category was defined on the basis of comb size, one might expect synergistic effects between them. All logistic regression models were fitted using the PROC GLIMMIX platform in SAS (SAS 2013), which allows random nested effects and reports F statistics for tests of significance. Estimated odds ratios were obtained by exponentiating parameter estimates.

RESULTS

Choice of lines

Sex comb size (as TOTC1,2) differed significantly among the 48 genetic lines established from field-caught pairs ($F_{47,191} = 1.86$, $P = 0.0019$) and was positively related to Thorax Length ($\hat{b} \pm$ standard error [SE], 10.310 ± 4.514; $F_{1,191} = 5.22$; $P = 0.023$). The 5 lines exhibiting the greatest average Thorax Length-corrected comb size (high lines) and the 5 lines exhibiting the smallest average comb size (low lines) were chosen for exposure to temperature treatments, and subsequent assays of pupal survivorship and adult mating success (below). Mean comb size of high lines ($\bar{x} \pm$ SE, 26.73 ± 0.947) was on average 17.7% greater than low lines (22.70 ± 0.948). This significant difference between line categories reported here on F1 adults was confirmed in analyses of the mating cage data on F3 adults, described below. Such cross-generational stability of genotypic differences in sex comb size has been demonstrated previously (Polak et al. 2004; Cooperman et al. 2007; Polak and Simmons 2009). To further confirm the stability of the sex comb size phenotypes across the generations of the present study, we tested whether sex comb size of our 10 test lines differed between the F1 and F3 generations, which it did not: Analysis of covariance showed that whereas the effect of Line ($F_{9,200} = 13.89$, $P < 0.0001$) and Thorax Length ($F_{1,200} = 11.19$, $P = 0.0010$) on TOTC1,2 was significant, the effect of Generation ($F_{1,200} = 0.370$, $P = 0.544$) and the Generation × Line interaction ($F_{9,200} = 0.843$, $P = 0.577$) was not significant.

Pupal mortality

We found a significant effect of Line Category on pupal mortality (Table 1), with high lines exhibiting lower mortality than low lines at both temperatures (Figure 2). The effects of Temperature and the Line Category × Temperature interaction were not significant (Table 1).

Sex comb size and thorax length

The effect of Temperature on all comb size traits and Thorax Length was not significant (Table 2), and the Line Category × Temperature interaction was not significant for any of these response variables (Table 2). This analysis also demonstrated significant separation in comb size between Line Categories (Table 2; Figure 3), thus confirming the difference identified in the F1 generation reported above (see Choice of lines for details).

Competitive mating success

As a first step in our assessment, we analyzed mating probability using logistic regression without Line Category in the model, which revealed significant effects of Line, but not of Temperature or of the Line × Temperature interaction (Table 3). There was a significant negative effect of male Thorax Length on mating probability (range odds ratio = 0.0981). In contrast, there was a strong positive effect of TOTC2 on mating probability (Table 3), such that an increase of one tooth in this comb size covariate resulted in approximately 37% increased mating probability (unit odds ratio = 1.367). There was no significant effect of TOTC1 on mating probability (Table 3).

In our second approach, we modeled Line Category and Line. Here, Line was nested within Line Category and treated as a random effect. In Model 1, Line Category was not significant (Table 4); high line males exhibited a 51.4% (89/173) mating probability, and low line males exhibited a 46.8% (81/173) mating probability. The Line, Temperature, and Line Category × Temperature interaction

Table 1

Results of REML mixed model on pupal mortality

Fixed effect	Numerator df	Denominator df	F	P
Temperature	1	8	0.119	0.739
Line Category	1	8	14.652	0.0050
Temperature × Line Category	1	8	0.277	0.613

Data (as proportion pupae that died) were arcsine (square-root)-transformed prior to analysis. Line is nested within Line Category and treated as a random effect; Line (var [SE] = 0.001378 [0.001443]; 95% CI: −0.00145, 0.00421) and the Line × Temperature interaction (var [SE] = 0.000044 [0.00168]; 95% CI: −0.00324, 0.00333) were not significant. CI, confidence interval; df, degrees of freedom.

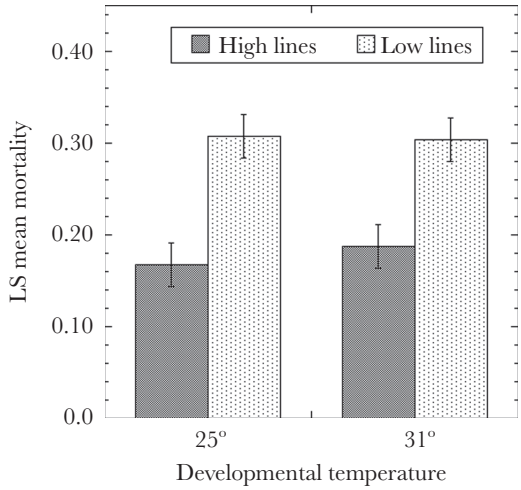

Figure 2

Least-squares mean (±1 SE) mortality of high (large-combed) and low (small-combed) lines across temperatures. Data (as % mortality) were arcsine (square-root)-transformed prior to analysis.

Table 2

Results of REML mixed models on total comb size (TOTC1,2), size of comb segments 1 (TOTC1) and 2 (TOTC2), and Thorax Length

Trait/source	Numerator df	Denominator df	*F*	*P*
TOTC1,2				
Thorax Length	1	152.8	19.139	<0.0001
Line Category	1	8.492	129.890	<0.0001
Temperature	1	7.412	0.822	0.393
Line Category × Temperature	1	7.433	0.0727	0.795
Line (Category) (var [SE] = −0.0423 [0.120]; 95% CI: −0.278, 0.194)				
Line (Category) × Temperature (var [SE] = 0.129 [0.195]; 95% CI: −0.254, 0.512)				
TOTC1				
Thorax Length	1	325.5	21.952	<0.0001
Line Category	1	8.192	8.706	0.0179
Temperature	1	6.870	0.334	0.576
Line Category × Temperature	1	6.895	0.204	0.665
Line (Category) (var [SE] = 0.177 [0.123]; 95% CI: −0.0642, 0.419)				
Line (Category) × Temperature (var [SE] = 0.0169 [0.0643]; 95% CI: −0.109, 0.143)				
TOTC2				
Thorax Length	1	61.77	6.839	0.0112
Line Category	1	5.425	192.505	<0.0001
Temperature	1	8.251	0.531	0.486
Line Category × Temperature	1	8.273	0.609	0.457
Line (Category) (var [SE] = −0.0442 [0.0491]; 95% CI: −0.1401, 0.0521)				
Line (Category) × Temperature (var [SE] = 0.0592 [0.0846]; 95% CI: −0.107, 0.225)				
Thorax Length				
Line Category	1	8.114	0.432	0.529
Temperature	1	8.194	0.0970	0.763
Line Category × Temperature	1	8.194	0.990	0.348
Line (Category) (var [SE] = 2.302×10^{-4} [1.246×10^{-4}]; 95% CI: -1.4×10^{-5}, 4.745×10^{-4})				
Line (Category) × Temperature (var [SE] = -7.122×10^{-6} [1.803×10^{-5}]; 95% CI: -4.246×10^{-5}, 2.821×10^{-5})				

Line is nested within Line Category and treated as a random effect. CI, confidence interval; df, degrees of freedom.

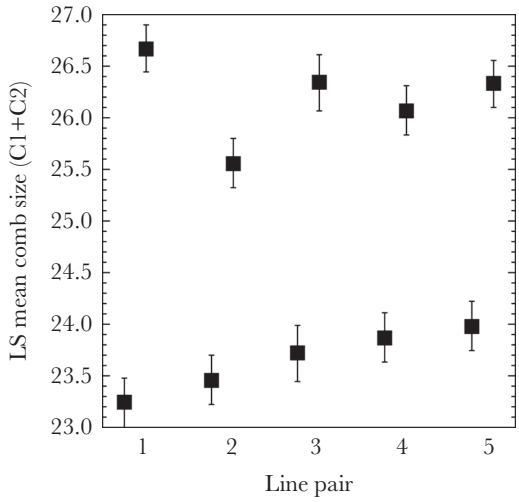

Figure 3

Least-squares (LS) mean (±1 SE) comb size of the 5 pairs of lines subjected to temperature treatments and fitness assays. LS means are from the model on TOTC1,2 described in Table 2. All pairs of means are statistically different ($P < 0.05$) from each other by the Tukey Honestly Significant Difference method.

were also not significant (Table 4). As shown in the previous paragraph, there was a strong effect of TOTC2 on mating probability, but not of TOTC1. In Model 2, we removed both comb size covariates and determined that the outcome of Model 1 did not change: Temperature ($F_{1,8} = 1.78$, $P = 0.219$), Line Category ($F_{1,8} = 0.63$, $P = 0.451$), and the Line Category × Temperature interaction ($F_{1,8} = 1.15$, $P = 0.314$) did not predict mating probability.

Table 3

Results of multiple logistic regression on mating success

Predictor	Numerator df	Denominator df	*F*	*P*
Thorax Length	1	323	6.56	0.0109
Temperature (31/25 °C)	1	323	090	0.342
Line	9	323	2.08	0.0307
Temperature × Line	9	323	1.40	0.187
TOTC1	1	323	0.27	0.602
TOTC2	1	323	12.35	0.0005

The model tests the fixed effects of Line (genotype) and the Line × Temperature (G × E) interaction without reference to which Line Category (i.e., high vs. low sex comb) the lines belong. df, degrees of freedom.

DISCUSSION

Indirect benefits of sexual selection are often broadly divided into sire effects that enhance offspring viability or mating success (attractiveness) of sons (Kirkpatrick and Ryan 1991; Møller and Alatalo 1999; Kokko et al. 2002; Qvarnström et al. 2006). Here, we tested for the effects of variation in a secondary sexual trait (the male sex comb in *D. bipectinata*) on traits falling into both these classes of indirect benefits. To do this, we used genetic (isofemale) lines extracted from a natural population, which were grouped into high (large-combed) or low line (small-combed) categories. We then contrasted high and low categories of lines in terms of pupal viability and male mating success. Our design permitted us to separate Line Category effects from among-line variation and in the case of mating success, to also separate Line Category effects from variation in comb size at the level of individual males.

Table 4

Results of multiple logistic regression on mating success, with Line Category in the model

Predictor	Numerator df	Denominator df	*F*	*P*
Thorax Length	1	331	5.55	0.0190
Temperature (31/25 °C)	1	8	1.21	0.304
Line Category (L/H)	1	8	0.71	0.423
Line Category × Temperature	1	8	1.60	0.241
TOTC1	1	331	0.33	0.566
TOTC2	1	331	11.93	0.0006

Line, treated as a random effect and nested within Line Category, is nonsignificant ($P = 0.274$). df, degrees of freedom.

First, we found that high sex comb lines produced offspring that were significantly more viable at the pupal stage than low lines, revealed as significant effects of Line Category on pupal survivorship. We also found that this effect held across 2 developmental temperatures, 25 and 31 °C. Because these temperatures overlap field conditions (Polak and Starmer 2005), we may expect that these indirect benefits as enhanced offspring viability operate in nature.

We did not, however, detect effects of Line Category on adult male mating success or body size, suggesting that relative sex comb size does not reflect genotypic properties that in turn influence mating success, at least under the conditions of our study and the measures of mating success that we used. It is, for example, possible that we would have detected an effect of Line Category had we used another metric of sexual performance, such as time elapsed to the onset of mating. Nevertheless, we find the lack of evidence for genotypic effects on mating success surprising, as mating success, body size, and associated activities, such as courtship, are complex phenotypes that should reflect adult body condition (Cordts and Partridge 1996; Droney 1998; Kotiaho et al. 2001). Because sex comb size is itself condition dependent and heritable (Polak and Starmer 2005; Polak and Taylor 2007), we expected that genotypes developing the largest combs collectively would express higher mating success. Not finding this effect is therefore of considerable interest, because it suggests that secondary sexual trait size in this system does not reveal the kind of overall genetic quality predicted by indicator models of sexual selection (Andersson 1982; Kodrick-Brown and Brown 1984; Iwasa and Pomiankowski 1999; Tomkins et al. 2004). Instead, sex comb size appears to reveal genotypic properties that elevate offspring fitness in a trait-specific manner, which, again, was restricted in our study to offspring viability. Genome-wide molecular profiling (Braendle et al. 2011; Hannum et al. 2013) could be used to define the genetic/epigenetic architecture of this intriguing effect.

Although there was no significant effect of Line Category, or of Line Category × Temperature interaction, on mating success, we did find significant effects of *individual-level* variation of both body size and comb size on this variable. Body size had a negative relationship with mating success, perhaps because smaller males are faster and more agile; smaller males may be able to find receptive females more quickly than larger males, as is often the case in scramble competition mating systems (Thornhill and Alcock 1983). In contrast to body size, there was a *positive* effect of individual variation in comb size on mating success. This effect was restricted to comb segment 2 (TOTC2), mirroring the pattern of sexual selection observed in the field, where TOTC2 (but not TOTC1) has been shown to predict male mating success (Polak et al. 2004). Thus, indirect benefits should still operate through a "sexy sons" effect in this system (Fisher 1930; Weatherhead and Robertson 1979; Kirkpatrick and Ryan 1991) because comb size is heritable (Polak et al. 2004; Polak and Taylor 2007).

It is puzzling that despite detecting this significant effect of individual variation in sex comb size on mating success, we found no such effect of Line Category. One possible explanation for these contrasting findings involves trade-offs, such that alleles contributing to large sex combs may negatively influence other physiological/behavioral properties of the organism via pleiotropy or epistatic effects, thereby attenuating Line Category effects on mating success. An additional but related possibility relates to our use of isofemale lines. Variation among isofemale lines captures both additive and nonadditive (dominance and epistasis) genetic effects (Falconer and Mackay 1996), so that possibly nonadditive genetic activity affected the expression of mating success in unpredictable ways across the lines, thus attenuating Line Category effects on mating probability. Indeed, mild inbreeding that undoubtedly occurred to some extent within our lines may have "released" nonadditive effects (David et al. 2005), potentially contributing epistatic variation to mating success, thus blurring Line Category effects on this variable. But why Line Category effects on pupal viability were not also eliminated remains an open question and underscores the possibility that distinct (or at least partially nonoverlapping) genotypic effects tie ornament size to the different fitness-related traits we measured.

Hence, we conclude from the above that indirect benefits of sexual selection occur as both enhanced offspring viability and mating success but that different genetic/developmental mechanisms underlie these effects. The results justify maintaining conceptual separation between these classes of genetic benefits (Andersson and Simmons 2006) and underscore our limited knowledge of the proximate mechanisms that underpin indirect benefits of sexual selection.

We did detect significant Line effects on mating success when the Line Category term was excluded from consideration (Table 3), indicating genotypic variation for mating success in the population apparently independent of sex comb size. This finding is not unexpected, however, as heritable traits other than morphological secondary sexual traits can of course influence sexual selection (e.g., Byers and Waits 2006). Traits such as male song, courtship vigor and cuticular hydrocarbon profiles, may have influenced male mating success in our experimental cages, all of which have been shown to have a genetic basis in *Drosophila* (Ritchie and Kyriacou 1996; Cooperman et al. 2007; Etges et al. 2010). In *D. montana*, genetic variation for a courtship song characteristic (carrier frequency) has been found, and interestingly, females preferring males with high carrier frequency receive indirect benefits from mate choice as increased offspring egg to adult survivorship (Hoikkala et al. 1998).

The effect on survivorship we observed occurred at 2 developmental temperatures at which rates of pupal mortality were similar, circumventing the potentially confounding effects of environmentally induced developmental selection (Møller 1997; Polak and Tomkins 2013). The extent to which the fitness traits of offspring might be influenced by more extreme thermal variation and by genotype × environment interaction is an interesting question to be addressed in future work. Indeed, the degree to which genetic benefits of sexual selection vary with environmental and maternal effects is a question of growing interest (Simmons 2005; Kokko and Heubel 2008; Ingleby et al. 2010, 2013; Hunt and Hosken 2014) and should also be examined in our system across a wider range

of environmental factors such as nutrient abundance and quality (Reed et al. 2010). Data on genetic and environmental modulation of good genes effects will go some way toward elucidating temporal and spatial variation in the strength of sexual selection and its evolutionary consequences.

In summary, the present study found positive genotypic effects of secondary sexual trait size on pupal offspring survivorship, but no such effects were detected for adult male mating success. A potential reason for these contrasting findings is that the proximate mechanisms linking sex comb size to offspring survivorship and mating success are distinct, and may involve the possibility, for example, that distinct "resource pools" (sensu Tomkins et al. 2004) or genetic architectures underlie covariation between ornament size and different fitness-related traits in offspring. Ornament size in the present system may thus not reveal the kind of broad-based genetic quality envisioned by indicator models of sexual selection.

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