

# Condition dependence and the nature of genetic variation for male sex comb bristle number in *Drosophila melanogaster*

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Received: 5 August 2010 / Accepted: 23 March 2011 / Published online: 5 April 2011  
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**Abstract** Genetic architecture of variation underlying male sex comb bristle number, a rapidly evolving secondary sexual character of *Drosophila*, was examined. First, in order to test for condition dependence, diet was manipulated in a set of ten *Drosophila melanogaster* full-sib families. We confirmed heightened condition dependent expression of sex comb bristle number and its female homologue (distal transverse row bristles) as compared to non-sex sternopleural bristles. Significant genotype by environment effects were detected for the sex traits indicating a genetic basis for condition dependence. Next we measured sex comb bristle number and sternopleural bristle number, as well as residual mass, a commonly used condition index, in a set of thirty half-sib families. Sire effect was not significant for sex comb and sternopleural bristle number, and we detected a strong dominance and/or maternal effect or X chromosome effect for both traits. A strong sire effect was detected for condition and its heritability was the highest as compared to sex comb and sternopleural bristles. We discuss our results in light of the rapid response to divergent artificial selection for sex comb

bristle number reported previously. The nature of genetic variation for male sex traits continues to be an important unresolved issue in evolutionary biology.

**Keywords** Condition dependence · *Drosophila melanogaster* · Sex comb · Condition · Genetic variation

## Introduction

The sex comb of *Drosophila*, an array of specialized bristles on the foreleg of males, is an emerging model system for studying the evolution of male secondary sexual characters. Sex combs are thought to be used to grasp a female's abdomen or spread her wings during mating, and have been shown to be important for mating to occur (Spieth 1952; Ng and Kopp 2008). The morphology of sex combs varies dramatically between closely related species indicating that their evolution is driven by directional sexual selection (Kopp and True 2002). Indeed, sex comb bristle number exhibits high intra- and inter-specific variation (Coyne 1985), and has been shown to affect mating success in opposite directions in natural *Drosophila simulans* (Markow et al. 1996) and *D. bipectinata* (Polak et al. 2004) populations. Previously we reported high intra-specific variation for sex comb bristle number within *D. melanogaster* and a rapid response to divergent artificial selection (Ahuja and Singh 2008). We also noted that within the low sex comb bristle number line males with higher bristle number had higher mating success. Here we further examine patterns of phenotypic and genotypic variation underlying *D. melanogaster* sex comb bristle number.

Condition dependence is an important source of intra-specific variation in male sex traits and a key element of

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10709-011-9572-2) contains supplementary material, which is available to authorized users.

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good genes models of sexual selection (Andersson 1994). The handicap hypothesis predicts that costly male sex traits exhibit heightened condition dependence as compared to non-sex traits (Cotton et al. 2004), where condition is defined as the pool of resources available for allocation to competing life history traits (Bonduriansky and Rowe 2005). Males in good condition are assumed to signal their quality through greater sexual trait size while males in poor condition are unable to do so because of the viability costs associated with such extravagance (Iwasa and Pomiankowski 1994). Furthermore, if exaggerated sexual ornaments signal male genetic quality then condition dependence is expected to be heritable. In the first experiment we investigated condition dependence by assessing the effect of diet manipulation on the sex comb, its female homologue the distal transverse row bristles, and non-sex sternopleural bristles.

Genetic variation is expected to be depleted in traits subject to directional selection (Falconer and Mackay 1996). However, empirical evidence has shown that additive genetic variance for male sex traits is not depleted, but that they actually harbor higher levels than comparable non-sex traits (Pomiankowski and Møller 1995). In the second experiment we used a nested full-sib, half-sib mating design to test for and estimate heritability of sex comb and sternopleural bristle number. In addition we also assessed residual mass, a commonly used condition index. Theoretically one expects high genetic variance for condition since it is a complex summary trait and any allele that affects the ability of an individual to acquire or utilize resources will also affect condition (Andersson 1982).

Taken together these quantitative genetic analyses provide important insight into the genetic architecture of sex comb bristle number variation in *D. melanogaster*. We discuss our results in light of the response to divergent artificial selection for sex comb bristle number reported previously, and raise important issues regarding our understanding of the nature of genetic variation underlying male sex traits.

## Materials and methods

### Experiment 1: full-sib analysis

An out-bred base population was established by crossing six geographical *D. melanogaster* populations that were highly divergent for sex comb bristle number (for details see Ahuja and Singh 2008). A single virgin male and female from this population were crossed to initiate ten such full sibling families. The lines were then maintained on standard laboratory cornmeal-molasses-agar diet (15.6 g yeast, 7.8 g agar, 54.6 ml molasses, 78 g cornmeal per liter of water) for

three generations before the condition experiment was started. The rich conditioning diet contained 70 g yeast, 8 g agar, 60 ml molasses and 80 g cornmeal per liter of water while the poor condition diet comprised 13 g yeast, 8 g agar, 15 ml molasses and 20 g cornmeal per liter. These diets have been modified from Imasheva et al. (1999) who reported a significant decrease in larval viability under poor feeding conditions. Hence, larval density cannot be strictly controlled and vials with rich diet were transferred every 48 h while poor diet vials were transferred every 96 h. In this manner, poor diet vials had low quality as well as less quantity of food per individual. Five virgin male–female pairs from each full sibling family were introduced into two rich and poor diet vials each. The resulting progeny were aged on the respective diet for 5 days and were stored at 20°C for subsequent morphological measurements. Thirty male and thirty female progeny from each full sibling family were scored for a total of 300 individuals of each sex in each diet.

Sex comb and sternopleural bristle number in males and distal transverse row bristle number in females were counted under a light microscope. The length of the first tarsus in males and females was used as an index of body size, and along with male sex comb length were measured using an ocular micrometer in arbitrary micrometer units (72 units = 1,000 µm). All characters were measured on both, left and right sides of the fly and absolute trait sizes were calculated as the average of the left and right side score. Since measurements of continuous traits were performed twice, we calculated the average of the two measurements made on each side to obtain the score for that side. Repeatability values, calculated using the method of Bland and Altman (1996), were 1.09, 2.75 and 3.5 ocular units for male sex comb length, male tarsus length and female tarsus length, respectively.

Data analysis was conducted using the Statistix1 add on package in Microsoft Excel. The data were not normally distributed (Kolmogorov–Smirnov one-sample tests;  $P < 0.01$ ), and we were unable to transform to approximate a normal distribution. However, given the large sample sizes, under the central limit theorem, parameter estimates are expected to be close to normally distributed and parametric tests were used. We also confirmed the validity of results obtained using non-parametric tests as indicated. Absolute trait sizes between treatments were compared using a *t* test and repeated with a Mann–Whitney *U*-test. We measured strength of condition dependence by estimating magnitude of treatment effect. Standardized mean effect size was calculated for each trait using Hedge's *g* statistic, where  $g = t \sqrt{(n_1 + n_2/n_1n_2)}$  and  $n_1$  and  $n_2$  represent sample size of each group (Nakagawa and Cuthill 2007). To correct for body size, a mixed model ANCOVA was conducted with tarsus length as covariate and family

(genotype), treatment (environment) and their interaction (genotype by environment) as factors. We confirmed these results with a second, albeit less reliable, method to correct for body size: Relative trait sizes (absolute trait size divided by tarsus length) between treatments were compared using a Mann–Whitney *U* test.

#### Experiment 2: half-sib analysis

Thirty virgin males and 90 virgin females were randomly taken from the base population. They were aged for 5 days and each male was then crossed to three females in a standard diet vial supplemented with live yeast (*ad libitum*). Males were discarded after 48 h and females were separated and transferred to petri dishes with standard medium for egg laying for 48 h. Twenty larvae obtained from each female were transferred to individual vials. Flies collected from each individual vial were aged for 4 days and frozen for morphological measurements. Five male progeny of each dam from each half-sibling family were scored in this manner for a total of 450 individuals scored.

Condition is defined as the resources available for utilization, and we used body mass corrected for body size as a surrogate measure since residual mass is expected to be positively correlated with reserves such as fat or sugar (Kotiaho 1999). Body size was estimated from wing length, measured as the length of the longitudinal vein L3 from the intersection with the anterior cross vein to the tip of the wing. Length of each wing was measured twice, and repeatability value for this trait was 1.3 ocular units. Average of the left and right side wing measurement was calculated as the score for each individual. Flies were individually weighed to the nearest 0.001  $\mu\text{g}$  on a Mettler UMT2 microbalance. Residuals from log transformation of mass and wing length were used to correct for the fact that a unit of reserves may be more useful for larger individuals than smaller ones (Kotiaho 1999). The use of residual mass as a condition index carries several assumptions and caveats (Green 2001), and while we did not directly test these in our system overall this surrogate has been shown to provide a reasonable estimate of condition in other taxa (Schulte-Hostedde et al. 2005; Birkhead et al. 2006). Sex comb and sternopleural bristle numbers were scored as described previously. Data were analyzed with nested ANOVA with sire and dams (sires) as factors. Standard procedures were used for the calculations of heritability (Lynch and Walsh 1998). Coefficients of genetic variation were calculated as  $CV_A = 100\sqrt{V_A}/x$ , where  $V_A$  is the additive genetic variance and  $x$  is the mean of a trait (Houle 1992). Since residuals have a mean of zero, these were standardized by adding mean body mass for calculating  $CV_A$  of condition (Kotiaho et al. 2001; Birkhead et al. 2006).

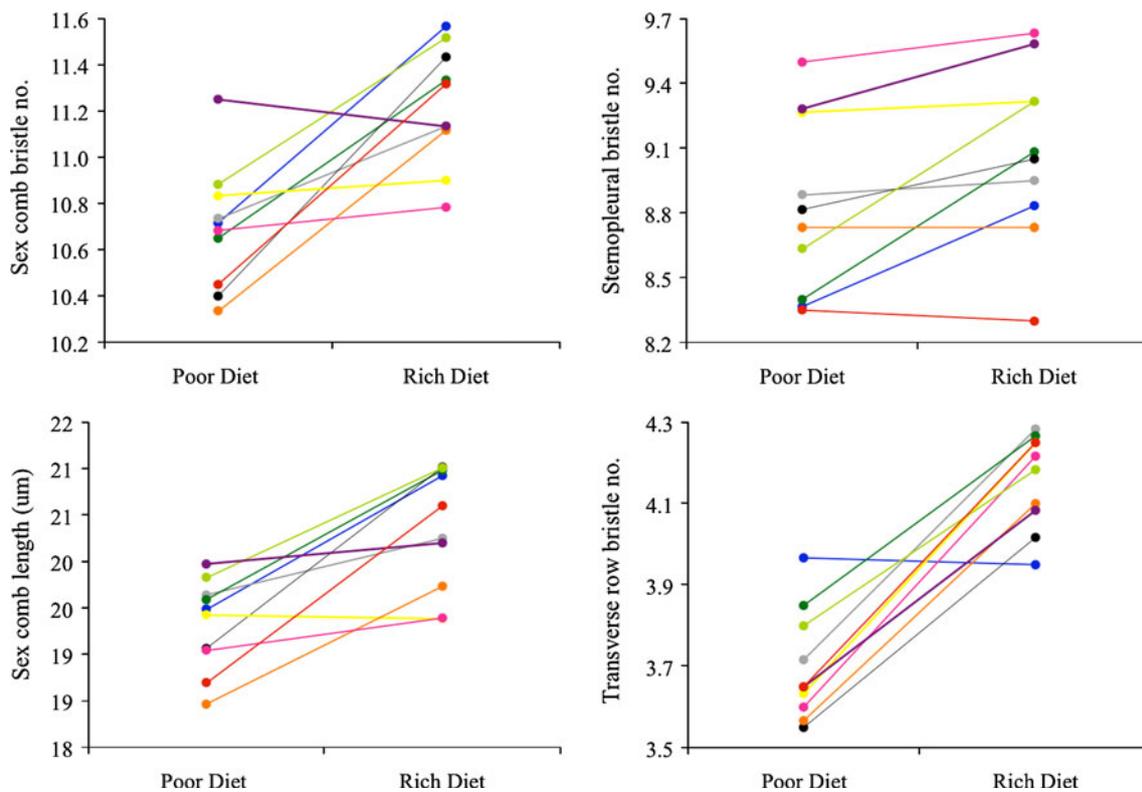
## Results

Thirty flies from each full-sib family from each diet regimen were scored for the bristle traits under study (Fig. 1). For each trait, flies reared on the rich diet exhibited larger absolute trait sizes as compared to flies reared on the poor diet (Table 1; Fig. 1). Not surprisingly, we detected strong correlations between sex comb bristle number and sex comb length on each diet (Supplementary Figure 1). As seen by the magnitude of effect size of diet treatment (Table 1), female transverse bristles exhibited the strongest condition dependence, followed by sex comb length and bristle number and finally male sternopleural bristles. Partitioning of variance due to effects from various sources revealed that for all four traits effect of diet treatment persisted even after controlling for body size (Table 2). In addition, the presence of genetic variation was suggested by a significant family effect. Most interesting were the effects due to interaction between family and diet: Significant genotype  $\times$  environment interaction was detected for male sex comb bristle number and length, as well as for female transverse bristles, but not for the non-sex male sternopleural bristle numbers. Results obtained using non-parametric tests (Supplementary Table 1) were qualitatively similar, confirming our findings.

Half-sib analysis revealed a marginally non-significant effect of sire on sex comb bristle number ( $P = 0.07$ ). Sire effect was not significant for sternopleural bristle number variation ( $P = 0.11$ ). A strong sire effect on condition ( $P = 0.005$ ) was detected with little residual variance and no significant dam effect (Table 3). On the other hand, the effect of dam was highly significant for both, sex comb and sternopleural bristles ( $P < 0.01$ ) (Table 3). Given the large dam component of variance, we cannot rule out dominance or maternal effects, and only the sire component was used to estimate heritability. Measurements of mean, heritability, and coefficient of additive genetic variation for each trait are presented in Fig. 2. Estimates of condition heritability ( $0.45 \pm 0.17$ ) were the highest as compared to sex comb ( $0.21 \pm 0.13$ ) and sternopleural bristle number ( $0.2 \pm 0.14$ ).  $CV_A$  for condition (4.4) derived from log transformed residuals was comparable to sternopleural bristle number (4.49) and greater than sex comb bristle number (3.4).

## Discussion

In addition to the sex comb we examined condition dependence of two related mechanosensory bristle traits: The most distal transverse row of females rotates and thickens to form the sex comb in males (Tokunaga 1962; Held et al. 2004). It exhibited a strong, positive correlated



**Fig. 1** Mean trait size of full-sibs reared on poor and rich diets. Each filled circle represents mean score of thirty flies, with each line representing a full-sib family

**Table 1** Comparison of mean ( $\pm$  SD) ( $n = 300$ ) absolute size of bristle traits in flies reared under poor and rich diet regimens

Trait	Poor diet	Rich diet	Comparison ( $t$ )	Effect size ( $g$ ) (95% CI)
Sex comb bristle no. ( $\delta$ )	10.69 (0.77)	11.22 (0.79)	7.9***	6.44 (8.08–4.8)
Sex comb length (um) ( $\delta$ )	19.321 (1.31)	20.34 (1.42)	9.2***	7.5 (9.16–5.85)
Sternopleural bristle no. ( $\delta$ )	8.82 (0.86)	9.08 (0.96)	3.7***	3.01 (4.62–1.41)
Transverse bristle row no. ( $\varphi$ )	3.7 (0.42)	4.16 (0.46)	13.2***	10.77 (12.48–9.05)

Effect size of diet treatment represents standardized difference between the two means for each trait

**Table 2** Analysis of covariance examining the effect of diet, line, and their interaction on four bristle traits in 10 isofemale lines

Factor	$df$	Sex comb bristle no.		Sex comb length		Sternopleural bristle no.		Transverse row bristle no.	
		MS	$F$	MS	$F$	MS	$F$	MS	$F$
Tarsus length	1	25.089	47.15***	168.948	127.54***	4.283	6.12*	0.874	6.14*
Diet treatment	1	21.734	40.84***	64.782	48.9***	5.597	8.00**	27.293	191.85***
Family	9	1.435	2.69**	7.689	5.8***	8.702	12.44***	0.396	2.78**
Treatment $\times$ family	9	1.587	2.98**	3.901	2.94**	1.093	1.563	0.483	3.39***
Error	579	0.532		1.320		0.699		0.142	

Tarsus length was used as the covariate to control for body size variation

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

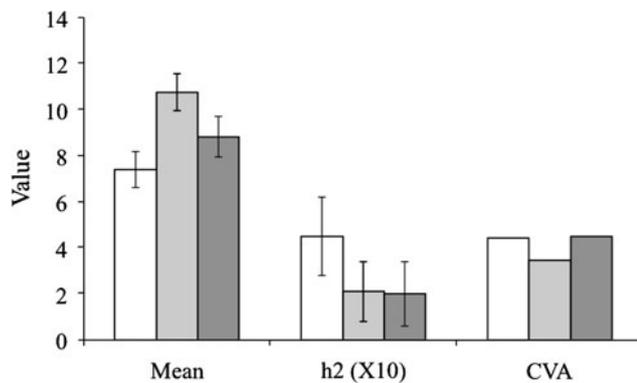
response to artificial selection for sex comb bristle number (Ahuja and Singh 2008) though the nature and direction of selection acting on this trait is unclear. Given this correlation with the male sex comb, female transverse bristles

are also expected to exhibit condition dependent expression (Bonduriansky and Rowe 2005; Punzalan et al. 2008). Sternopleural bristles on the other hand did not show a consistent correlated response to selection for sex comb

**Table 3** Nested analysis of variance of three traits in thirty *D. melanogaster* half sib families

Factor	df	Condition		Sex comb bristle no.		Sternopleural bristle no.	
		MS	F	MS	F	MS	F
Sire	29	0.067	2.63**	1.417	1.57	1.921	1.44
Dam (Sire)	60	0.025	1.24	0.9	1.66**	1.33	2.23***
Sibs (Dam)	360	0.02		0.54		0.59	
Total	449						

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$



**Fig. 2** Mean ( $\pm$  SD), heritability ( $\times 10$ ) ( $\pm$  SE) and coefficient of additive genetic variation for condition (white), sex comb bristle number (light gray) and sternopleural bristle number (dark gray)

bristle number and are thought to be evolving under weak stabilizing selection (Mackay 1985). They were used as a non-sex control and are theoretically predicted to exhibit weak condition dependence as compared to the male sex comb.

Size of both components of the male sex comb, bristle number and length, exhibited positive condition dependence and we also detected a significant genotype by environment effect for each trait. This suggests that different genotypes allocate resources to this trait at different rates and that condition dependence is heritable, although we cannot rule out the possibility that genotypes may differ in their overall genetic quality. We also detected significant condition dependence in the homologous female transverse bristle row and a significant genotype X environment effect. This suggests that loci involved in condition dependence are being expressed in both sexes and there is intersexual genetic correlation for condition dependence. Surprisingly, magnitude of effect of condition manipulation was stronger than that for the male sex comb, contrary to expectations if the evolution of condition dependence is driven primarily by sexual selection on males (Bonduriansky and Rowe 2005). Since the sex comb is a modification of the transverse rows and represents a later, sex-specific stage of development, this raises the possibility that condition dependence may be stronger in the early

stages of development as compared to later stages. Finally, while absolute male sternopleural bristle number also exhibited significant condition dependence, magnitude of effect of condition manipulation was weaker than the male sex comb and female bristles and the effect of genotype X environment was not significant. Moreover, it appears that changes in sternopleural bristle number due to condition manipulation are largely accounted for by changes in body size. Our results demonstrate heritable condition dependence of the sex comb, and contribute to the growing body of evidence that male sex traits exhibit heightened condition dependence as compared to non-sex traits (Cotton et al. 2004; Boughman 2007; Judge et al. 2008).

Half-sib analysis revealed a non-significant sire component for both bristle traits indicating that there may be little additive genetic variance underlying these traits. However, further inspection of variance components reveals a very strong dam effect which is rendering the sire component less significant. The strong dam effect indicates a maternal and/or dominance or X chromosome effect. Several studies suggest that heritability estimates based on sires may have a significant downward bias if the X chromosome contributes strongly to male fitness variance (Cowley and Atchley 1988; Long and Rice 2007; Connallon 2010) and given the large X of *Drosophila* this is a strong possibility in our study. Furthermore it is important to note the rapid response to divergent artificial selection for sex comb bristle number (Ahuja and Singh 2008) as well as significant genotype effects detected in the full-sib analysis, which indicate genetic variation for this trait. Estimates of heritability of sex comb and sternopleural bristle number were comparable. Overall there is somewhat conflicting evidence regarding the magnitude of additive genetic variance for sex comb bristle number. On the other hand, presence of a strong sire effect with little residual variance and no significant dam effects and high heritability confirms the presence of additive genetic variation for condition.

Under the genic capture model if the expression of male sex traits is condition dependent and if condition harbors high additive genetic variation, then male sex traits are expected to capture and express the high levels of genetic

variance in condition (Rowe and Houle 1996). In the present study we report condition dependence of sex comb bristle number and genetic variance for condition, but further investigation is required to dissociate X-linked and maternal effects to estimate genetic variation for the sex comb. The nature of genetic variation underlying male sex traits continues to be a controversial issue in evolutionary biology. While Pomiankowski and Møller (1995) reported significant heritability in a number of male sex traits, more recent studies report a lack of additive genetic variance in certain male traits (Griffith et al. 1999; Hine et al. 2004), even in the presence of condition dependence (Van-Homrigh et al. 2007). Much remains to be learned about the genetic architecture of male sex trait variation, and the sex comb is an important model system for further work in this direction.

**Acknowledgments** We thank Wilfried Haerty for help in planning this study and statistical analysis, and Safiah Mai for help with formulating and preparation of experimental diets. This research was funded by an OGS scholarship to AA and an NSERC (Canada) grant to RSS.

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