Sex combs, allometry, and asymmetry in *Drosophila*

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There has been recent debate about the expected allometry of sexually-selected traits. Although sexually-selected traits exhibit a diversity of allometric patterns, signalling characters are frequently positively allometric. By contrast, insect genitalia tend to be negatively allometric, although the allometry of nongenital sexually-selected characters in insects is largely unknown (with some notable exceptions). It has also been suggested that there should be a negative association between the asymmetry and size of bilaterally-paired, sexually-selected traits, although this claim is controversial. We assessed the allometry and asymmetry (fluctuating asymmetry, FA) of a nongenital contact–courtship structure, the sex comb, in replicate populations of three species of *Drosophila* (we also measured wing FA). Sex combs are sexually-selected characters used to grasp the female's abdomen and genitalia and to spread her wings prior to and during copulation. Although species differed in the size of the sex combs, all combs were positively allometric, and comb allometry did not generally differ significantly between species or populations. Comb and wing asymmetry did vary across species, although not across populations of the same species. However, FA was trait specific and was never negatively associated with trait size.   © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **103**, 923–934.

ADDITIONAL KEYWORDS: developmental stability – Diptera – *D. melanogaster* – *D. pseudoobscura* – *D. simulans* – fluctuating asymmetry – scaling.

INTRODUCTION

The study of allometry (i.e. changes in trait dimensions relative to changes in overall organismal size) has a long history (Huxley, 1932; Huxley & Teissier, 1936; Huxley, Needham & Lerner, 1941; Gould, 1966). The scaling relationship among individuals of the same species between one trait and total body size, or between two traits at a single developmental stage, is called static allometry (Cock, 1966; Gould, 1966). Most traits tend to display negative static allometry (Eberhard, 2002; Cuervo & Møller, 2009) and perfect isometry appears to be rare (Gould, 1966). By contrast to general scaling patterns, many sexually-selected traits show positive allometry (Petrie, 1988; Green, 1992; Simmons & Tomkins, 1996; Kodric-Brown, Sibly & Brown, 2006), leading to the suggestions that positive allometry is indicative of (directional) sexual

selection (Petrie, 1988; Green, 1992; Kodric-Brown *et al.*, 2006). However, positive allometry can be generated without directional selection (Bonduriansky & Day, 2003). Additionally, despite claims that sexually-selected traits always show positive allometry (Kodric-Brown *et al.*, 2006), a recent review found that many sexually-selected characters do not scale in this way (Bonduriansky, 2007), although signalling characters and weapons were generally positively allometric (Bonduriansky, 2007). What is also apparent from the review by Bonduriansky (2007) is that the allometry of structures under sexual selection often evolves rapidly and divergently in closely-related species (Baker & Wilkinson, 2001; Emlen, Hunt & Simmons, 2005; Shingleton *et al.*, 2007) and geographically isolated populations of the same species can also differ in trait allometry (Moczek & Nijhout, 2003).

In contrast to characters used in sexual signalling, the male genitalia of insects and spiders, which are also sexually selected (Eberhard, 1985; Hosken &

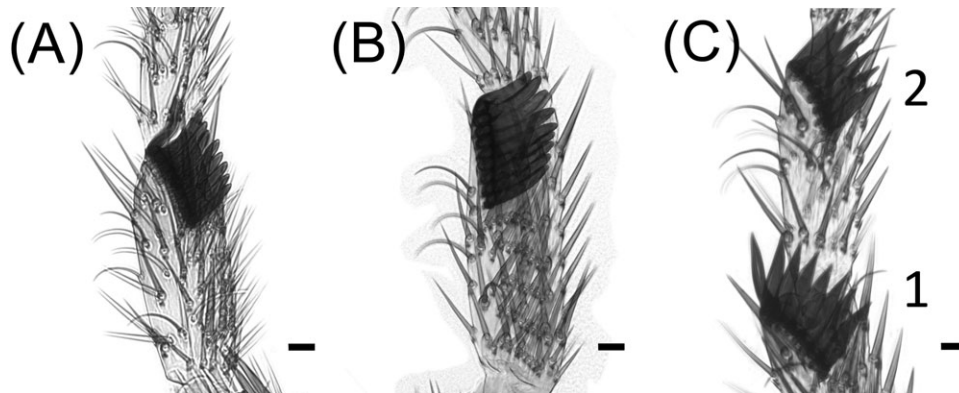*Corresponding author. E-mail: d.j.hosken@exeter.ac.uk

Figure 1. The sex combs of *Drosophila simulans* (A), *D. melanogaster* (B), and *Drosophila pseudoobscura* (C). Measurements were taken along the base of the sex combs. Combs are oriented with distal end of leg facing upwards and scale bars represent 20 μm. *Drosophila pseudoobscura* has two sex combs, one each on the first and second tarsus (annotated as 1 and 2), whereas *D. simulans* and *D. melanogaster* have a single sex comb on the first tarsus.

Stockley, 2004), tend to show negative allometry (Eberhard *et al.*, 1998; Hosken, Minder & Ward, 2005). The low allometric slopes of arthropod genitalia can be explained by mechanical and stimulatory versions of the one-size-fits-all hypothesis (Eberhard, 2009) but, although the allometry of insect genitalia has been extensively investigated, fewer studies have looked at the allometry of nongenital characters (e.g. forelegs and sex combs) in insects (although exaggerated traits such as horns are notable exceptions; Kodric-Brown *et al.*, 2006) (Bonduriansky, 2007). This may partly be because it is not always clear whether particular nongenital characters are sexually selected in insects or not.

In addition to debates about the scaling of sexually-selected characters, there have also been disputes about the relationship between trait symmetry and sexual selection (Møller & Swaddle, 1997; Tomkins & Simmons, 2003). Fluctuating asymmetry (FA), small random deviations from perfect symmetry in bilateral traits (Van Valen, 1962), which is a measure of developmental instability, has been suggested to reflect an individual's genetic quality (Møller & Swaddle, 1997). Higher quality individuals are predicted to have lower FA and, at the same time, also bear larger sexual traits. Essentially, larger sexually-selected traits should be more symmetrical because only high quality individuals can pay the costs of the larger traits and maintain developmental stability (Møller & Swaddle, 1997). Furthermore, FA levels should also be correlated across characters because FA reflects general quality (Møller & Swaddle, 1997). However, although FA has been linked to sexual selection and fitness in some taxa, this does not appear to be the case in many species (Tomkins & Simmons, 1995; David *et al.*, 1998; Hunt & Simmons, 1998; Martin & Hosken, 2002; Cuervo &

Møller, 2009; for a review, see Tomkins & Simmons, 2003).

The sex comb(s) of *Drosophila* are male-specific secondary sexual characters. They consist of a row of stout, modified mechanosensory bristles (comb teeth) on the fore-leg tarsus (Fig. 1) and are found in all species groups in *Sophophora*, with the exception of the Neotropical *saltans* and *willistoni* groups (Lakovaara & Saura, 1982). They are sexually selected, being involved in male–female tactile interactions during courtship and mating (Cook, 1977), although selection on the combs varies across species. For example, in free-living *Drosophila simulans*, mating success is negatively associated with tooth number and comb size (Markow, Bustoz & Pitnick, 1996), whereas, in *Drosophila bipectinata*, males with larger and more symmetrical sex combs have greater mating success (Polak, Starmer & Wolf, 2004). By contrast, sex comb size in *Drosophila pseudoobscura* does not appear to significantly affect mating success (Markow *et al.*, 1996). Little is known about the allometry of sex combs, however, prompting calls for investigations of how they scale with body size (Bonduriansky, 2007). In the present study, we investigated the allometry and asymmetry of sex combs for two distinct geographical populations of each of three closely-related *Drosophila* species: *Drosophila melanogaster*, *D. simulans*, and *D. pseudoobscura*.

MATERIAL AND METHODS

We used three *Drosophila* species, with each represented by two populations from distinct geographical areas. *Drosophila simulans* populations came from Tuncurry (T) and Walpole (W$_s$) in Eastern and Western Australia, respectively. The *D. melanogaster* Canton-S (C) population came from the *Drosophila*

Table 1. Details of sex comb tooth number in different populations of three species of *Drosophila* (numbers indicate mode and range)

| Species | Population | First tarsomere teeth number (range) | | Second tarsomere teeth number (range) | |
		Right comb	Left comb	Right comb	Left comb
Drosophila	T	9 (7–11)	9 (7–11)	0	0
simulans	W_s	9 (6–11)	9 (7–11)	0	0
Drosophila	C	10 (8–13)	10 (8–12)	0	0
melanogaster	W_m	10 (7–12)	10 (8–12)	0	0
Drosophila	S	7 (5–9)	7 (5–8)	6 (4–6)	5 (5–7)
pseudoobscura	L	6 (4–8)	6 (5–8)	5 (4–6)	5 (4–7)

T, Tuncurry; W_s, Walpole; C, from the *Drosophila* stock centre; W_m, Walpole; S, Sholow, AZ; L, Lewiston, MT.

stock centre and the other was from Walpole (W_m) in Western Australia. *Drosophila pseudoobscura* populations were collected from Sholow, AZ (S) and Lewiston, MT (L) in the USA. Details of sex comb tooth arrangement in all populations are listed in Table 1. All fly stocks were laboratory adapted for being housed in population cages for > 50 generations and reared on *Drosophila* quick mix medium (supplied by Blades Biological) at 25 °C (±0.1 °C) under a 12 : 12 light/dark cycle. Flies were sexed under light anaesthesia with CO_2 within 8 h of eclosion. Virgin males were then collected and housed in 45-mL vials at a density of no more than ten males per vial. After 3 days, males were collected under light CO_2 anaesthesia and preserved in 70% ethanol at –80 °C before measurement. All flies were treated with 10% KOH solution at 75 °C for 45 minutes, and transferred to 80% glycerol–ethanol solution after being passed through graded washes (*sensu* Atallah *et al.*, 2009). The right and left wings of males along with both pro-thoracic legs were removed from each fly and then mounted on slides using Hoyer's medium, for measurement of sex comb, tarsus, and wing dimensions. Measurements were made on at least 100 males of each species, with a minimum of 50 males measured per population. Empirical evidence in Diptera suggests that wing length tends to scale negatively, whereas hind-tibia length (HTL) scales isometrically with body size (Eberhard, 2002). A preliminary analysis was conducted using both measures and results (not shown) suggested higher positive slopes when we use HTL instead of wing length. Therefore, we conservatively used wing length as a measure of body size (Gilchrist & Partridge, 1999) instead of HTL.

Wing (×50) and foreleg images (×300) were acquired using a Leica inverted microscope connected to a digital image acquisition system on a personal computer and later analyzed manually using NIH Image J. Wing length (WL) was measured as the length of the first posterior cell, from anterior cross-vein (the
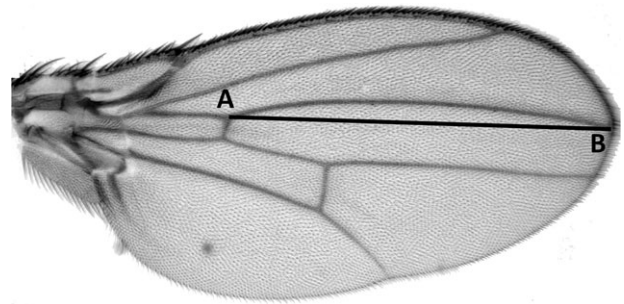
Figure 2. Wing length measured between points A and B.

junction of the longitudinal vein III) to the distal tip (border of the wing) of vein III (Fig. 2). Both wings of each individual were measured and an average value calculated. Comb length was measured as the greatest length subtended by the sex comb (CL) along its base, and sex comb tooth number (TN) was also counted. All traits were measured twice on different days without reference to the previous measurement to allow analysis and partitioning of measurement error (ME), which is essential for subsequent FA analysis (Palmer, 1994). Regression of WL, CL, and TN measure one on measure two showed that they were significantly associated (WL: $r^2 = 0.999$, $F_{1,318} = 1.4^{e+8}$, $P < 0.001$; CL: $r^2 = 0.998$, $F_{1,318} = 2.04^{e+6}$, $P < 0.001$; TN: $r^2 = 0.998$, $F_{1,318} = 1.6^{e+6}$, $P < 0.001$) and repeatability estimates (Lessells & Boag, 1987) were accordingly high ($r = 0.99$ for all traits measured). We also assessed the impact of our mounting technique on measurement error and, again, regression of measure 1 on measure 2 (after remounting specimens) revealed that our techniques were highly repeatable (CL: $r^2 = 0.92$, $\beta = 0.96$, $F_{1,40} = 4.25^{e+2}$, $P < 0.001$; TN: $r^2 = 1$, $\beta = 1$, $F_{1,160} = 1.57^{e+32}$, $P < 0.001$). Before performing any analyses, data were checked for potential outliers *sensu* Palmer (1994) and Palmer & Strobeck (2003). Grubb's test revealed seven extreme data points, which were

removed (Palmer & Strobeck, 2003). The filtered dataset was used for all subsequent analyses.

Comb allometry was then quantified by regressing $\log_{10}$-transformed comb lengths against $\log_{10}$ wing lengths and obtaining the regression slope. Several regression techniques were used (Table 2), although model 2 regression or structural models are recommended for allometric analyses (Harvey & Pagel, 1991); model 1 regression [ordinary least squares (OLS) regression] assumes that there is no measurement error in the predictor variable. Model 2 regression, on the other hand, assumes errors in both x and y directions, whereas structural models assume errors are uncorrelated and that the value of λ (ratio of the two error variances) is known (Harvey & Pagel, 1991). Although we present regression slopes calculated using all methods [OLS, major axis (MA), standardized major axis (SMA = reduced major axis), ranged major axis regressions (RaMA), along with structural relationship (STR/ML) regression (maximum likelihood regression)], we base subsequent analyses on MA slopes and intercepts because MA regression is a preferred method for calculating allometry (Harvey & Pagel, 1991). Regression slopes for OLS, MA, SMA, and RaMA methods were computed in 'R' using the lmodel2 package (Legendre, 2008), and their statistical significances (> 0) were assessed in JMP, version 8.0 (SAS Institute Inc.), and SPSS, version 15 (SPSS Inc.). SMATR (http://web.maths.unsw.edu.au/~dwarton/programs.html) implemented on the R software platform (R Development Core Team) was used to test MA slopes for isometry ($\beta = 1$) using one-sample *t*-tests. We were interested to determine whether sex comb allometry differed across populations and species. Accordingly, we tested for a common slope within species and then across species, using Barlett corrected maximum likelihood tests (Warton *et al.*, 2006; 2007).

The asymmetry of each trait was measured as the signed (R–L) difference. FA1 is the absolute value of this measure. A two-way analysis of variance was used to assess whether asymmetry could be distinguished from measurement error (ME) (Palmer, 1994; Palmer & Strobeck, 2003). This is essential because ME can confound FA interpretations. Further analyses were performed using a worksheet provided by A.R. Palmer (http://www.biology.ualberta.ca/palmer/DataFiles/FA_Calc.xls). We calculated FA4a, FA10a, ME1, ME3, ME5, and ME1 as a percentage of FA4a (Table 3) *sensu* Palmer (1994) and Palmer & Strobeck (2003); however, subsequent analyses are based on FA1 values. *Drosophila simulans* (W_s) and *D. melanogaster* (W_m) populations showed significant directional asymmetry in comb length. As a result and to be conservative, comb length FA was excluded from all further FA analyses. All remaining populations displayed nonsignificant levels of skew and kurtosis after Bonferroni correction (data not shown). Although all of these analyses were conducted at the population level, species level analyses also indicated that FA was discernable from ME (data not shown).

RESULTS

We first used multivariate analysis of variance (MANOVA) to see how species and populations differed in the average size (mean of left and right characters) of the traits we measured. In this analysis, we were only interested in the sex combs on the first tarsal segment because this trait was shared by all species; only *D. pseudoobscura* has multiple combs. Species and population nested within species were our predictor variables, and wing length, sex-comb length, and comb tooth number were the dependent variables. This analysis revealed a significant effect of species (Wilks' lambda: $F_{6,624} = 570$, $P < 0.001$) and population (within species) (Wilks' lambda: $F_{9,759.5} = 9.11$, $P < 0.001$) on the multivariate combination of these traits. Univariate post-hoc tests of the species effect revealed that comb length, tooth number, and wing length all varied across species ($F > 169$, $P < 0.001$) (CL: *D. simulans* = *D. pseudoobscura* < *D. melanogaster*. TN: *D. pseudoobscura* < *D. simulans* < *D. melanogaster*. WL: *D. simulans* < *D. melanogaster* < *D. pseudoobscura*). Within species, wing length was found to be significantly different between populations of *D. simulans* ($F_{1,105} = 42.5$, $P < 0.001$: $W_s > T$) and *D. pseudoobscura* ($F_{1,103} = 18.2$, $P < 0.001$: $S > L$), although populations of *D. melanogaster* did not differ significantly in wing length ($F_{1,106} = 2.27, P = 0.14$). For comb length and comb tooth number, only *D. pseudoobscura* showed a significant difference between populations (CL: $F_{1,103} = 8.42$, $P = 0.004$; TN: $F_{1,103} = 4.36$, $P = 0.039$: in both cases, $S > L$); these traits did not differ significantly across population in the other two species ($F < 1.67$, $P > 0.199$).

All the Type II regression slopes and the slope generated from the structural model suggest positive allometry for comb length (Table 2), and it is these models that are recommended for allometric slope estimation (Harvey & Pagel, 1991). We also fitted quadratic equations to the allometry data, although polynomial equations were not significant, even before Bonferroni correction (data not shown). All MA slopes were significantly greater than 1 for all populations (T: $F_{1,47} = 12.9$, $P < 0.001$; W_s: $F_{1,56} = 59.2$, $P < 0.001$; C: $F_{1,48} = 59.9$, $P < 0.001$; W_m: $F_{1,56} = 36.0$, $P < 0.001$; S: $F_{1,47} = 63.7$, $P < 0.001$; L: $F_{1,54} = 34.8$, $P < 0.001$; Fig. 3). We also calculated the allometry of the second sex comb (on the second tarsus) of *D. pseudoobscura* and found this displayed positive allometry in all models (excluding OLS) (Table 2) and,

Table 2. Details of allometry of (log) comb length (regressed against (log) wing length) as calculated by various methods

Allometric relationship of CL (first tarsus) to WL

Species/population	N	Method			Intercept	Slope (± SE)	F (d.f.)	P
Drosophila simulans								
T	49	OLS	r^2	0.230	−0.522	0.749 (0.199)	14.03 (47)	0.000
		MA			−5.359	2.346 (0.626)		
		SMA			−2.983	1.561 (0.199)	NA	
		RaMA			−7.040	2.901 (NA)		
		ML			−4.257	1.982 (NA)		
W_s	58	OLS	r^2	0.137	−0.902	0.867 (1.265)	8.92 (56)	0.004
		MA			−14.550	5.338 (1.787)		
		SMA			−5.391	2.338 (1.265)	NA	
		RaMA			−16.469	5.967 (NA)		
		ML			−11.150	4.224 (NA)		
Drosophila melanogaster								
C	50	OLS	r^2	0.100	−0.650	0.797 (0.344)	5.36 (48)	0.025
		MA			−19.335	6.833 (2.952)		
		SMA			−5.971	2.516 (0.344)	NA	
		RaMA			−21.447	7.515 (NA)		
		ML			−14.752	5.353 (NA)		
W_m	58	OLS	r^2	0.127	−0.395	0.712 (0.250)	8.13 (56)	0.006
		MA			−11.928	4.433 (1.554)		
		SMA			−4.384	1.999 (0.250)	NA	
		RaMA			−14.240	5.179 (NA)		
		ML			−8.836	3.435 (NA)		
Drosophila pseudoobscura								
L	56	OLS	r^2	0.357	−1.745	1.095 (0.199)	30.00 (54)	0.000
		MA			−6.355	2.546 (0.465)		
		SMA			−4.088	1.833 (0.199)	NA	
		RaMA			−7.435	2.886 (NA)		
		ML			−5.375	2.237 (NA)		
S	49	OLS	r^2	0.174	−1.596	1.050 (0.333)	9.93 (47)	0.002
		MA			−15.029	5.258 (1.669)		
		SMA			−6.270	2.514 (0.333)	NA	
		RaMA			−16.679	5.775 (NA)		
		ML			−11.931	4.287 (NA)		
Allometric relationship of CL (second tarsus) to WL:								
Drosophila pseudoobscura								
L	56	OLS	r^2	0.073	0.012	0.517 (0.249)	4.28 (54)	0.043
		MA			−15.178	5.298 (2.561)		
		SMA			−4.408	1.908 (0.249)	NA	
		RaMA			−19.105	6.534 (NA)		
		ML			−10.744	3.902 (NA)		
S	49	OLS	r^2	0.088	−0.796	0.771 (0.363)	4.51 (47)	0.039
		MA			−22.726	7.641 (3.598)		
		SMA			−6.653	2.606 (0.363)	NA	
		RaMA			−25.352	8.464 (NA)		
		ML			−17.412	5.977 (NA)		

P-values are from significance test of ordinary least squares (OLS), major axis (MA), and ranged major axis (RaMA) slopes against zero. The SE of the standardized major axis (SMA) slope is equal to the standard error of the slope calculated for an OLS model and the SE of the MA and maximum likelihood (ML) slopes are equal.

T, Tuncurry; W_s, Walpole; C, from the *Drosophila* stock centre; W_m, Walpole; S, Sholow, AZ; L, Lewiston, MT; CL, comb length; WL, wing length; NA, not applicable.

Table 3. Descriptors of fluctuating asymmetry and measurement error derived from the results of a mixed model analysis of variance of sex-comb length, sex-comb tooth number, and wing length, for different populations

	Drosophila simulans T			Drosophila melanogaster C			Drosophila pseudoobscura L				
	CL	TN	WL	CL	TN	WL	CL − T1	CL − T2	TN − T1	TN − T2	WL
(a) FA1 mean (in μm for CL) +- SE	1.5596 / 0.8882	0.2653 / 0.1816	−2.1976 / 1.3669	1.0217 / 0.8826	0.18 / 0.1731	1.3568 / 1.8236	0.8805 / 0.6383	0.1706 / 0.6476	0.0714 / 0.0982	−0.0536 / 0.0898	−3.1551 / 1.6808
(b) $FA4a = 0.798\sqrt{MS_{SI}}$ (in μm for CL)	4.9719	1.0045	7.6556	4.9808	0.9765	10.291	3.8526	3.9062	0.5365	0.5318	10.0481
(c) FA10a (in μm for CL)	19.3346 / d.f. = 48	0.7845 / d.f. = 47	45.9479 / d.f. = 48	0.3257 / d.f. = 48	19.3926 / d.f. = 49	83.0781 / d.f. = 49	11.5763 / d.f. = 54	11.9042 / d.f. = 54	0.2126 / d.f. = 49	0.2176 / d.f. = 53	79.1937 / d.f. = 55
(d) $ME1 = 0.798\sqrt{MS_{err}}$ (in μm for CL)	0.3093	0.0987	0.2986	0.3321	0	0.3091	0.3149	0.3121	0.1306	0.0754	0.3191
(e) ME1 as % FA4a	6.2203	9.83	3.9002	6.6677	0	3.0033	8.1732	7.9908	24.3468	14.1793	3.1759
(f) ME3 = MS_{err} as % MS_{SI}	0.3869	0.9663	0.15	0	0.4446	0.09	0.668	0.6385	5.9268	2.0102	0.1
(g) repeatability (ME5)	0.7243	0.5112	0.8701	0.6913	1	0.9172	0.5704	0.5815	0.1241	0.3032	0.8984

	Drosophila simulans W_s			Drosophila melanogaster W_m			Drosophila pseudoobscura S				
	CL	TN	WL	CL	TN	WL	CL − T1	CL − T2	TN − T1	TN − T2	WL
(a) FA1 mean (in μm for CL) +- SE	2.5001 / 0.6237	0.069 / 0.141	−0.8191 / 1.3216	2.8489 / 0.6982	0.2241 / 0.1477	0.2971 / 1.8544	1.8867 / 0.6874	0.9095 / 0.6457	0.1224 / 0.1153	0 / 0.0922	1.0431 / 1.8387
(b) $FA4a = 0.798\sqrt{MS_{SI}}$ (in μm for CL)	3.8271	0.8394	8.0453	4.2507	0.8787	11.2606	3.8537	3.6641	0.644	0.5084	10.2666
(c) FA10a (in μm for CL)	11.4221 / d.f. = 56	0.5489 / d.f. = 56	50.7418 / d.f. = 57	14.1013 / d.f. = 56	0.602 / d.f. = 56	99.4654 / d.f. = 57	11.5834 / d.f. = 47	10.4559 / d.f. = 47	0.7488 / d.f. = 49	0.1978 / d.f. = 46	82.6739 / d.f. = 48
(d) $ME1 = 0.798\sqrt{MS_{err}}$ (in μm for CL)	0.3154	0.0741	0.3192	0.3305	0.0741	0.3478	0.3137	0.3306	0	0.0806	0.329
(e) ME1 as % FA4a	8.2409	8.8264	3.9675	7.7745	8.4313	3.089	8.1393	9.0217	0	15.8538	3.2048
(f) ME3 = MS_{err} as % MS_{SI}	0.6791	0.7791	0.16	0.6044	0.7108	0.1	0.6625	0.8139	0	2.5144	0.1
(g) repeatability (ME5)	0.5577	0.5233	0.8454	0.5864	0.5463	0.9003	0.6048	0.5543	1	0.2836	0.9085

FA1, FA4a, and FA10a: descriptors of fluctuating asymmetry (FA). ME1 reports measurement error in the original units, and ME3 represents the mean difference between replicate measurements as a proportion of mean difference between sides (mean squares of the sides × individual interaction). ME5 expresses FA variation as a percent of the total between-sides variation (including ME), and provides a standardized measure of FA repeatability (Palmer & Strobeck, 2003). CL, comb length; TN, sex comb tooth number; WL, wing length.
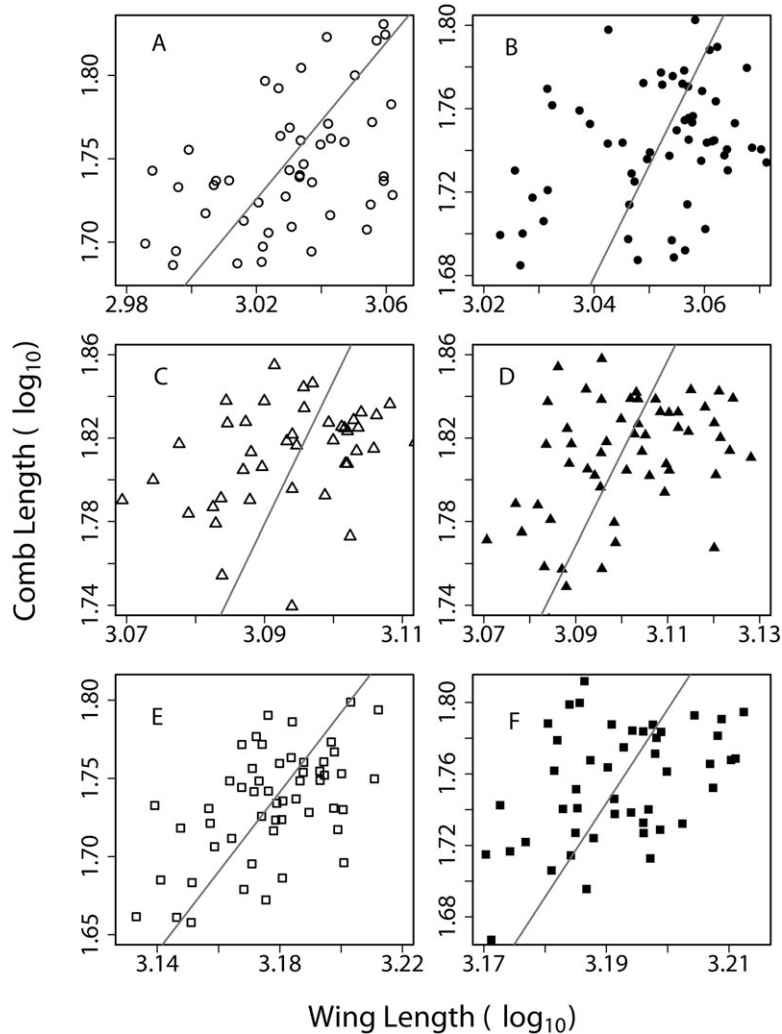
Figure 3. Major axis regression plots showing scaling association between comb length and body size (wing length) for six *Drosophila* populations. A, *Drosophila simulans* – Tuncurry. B, *Drosophila simulans* – Walpole. C, *Drosophila melanogaster* – Canton-S. D, *Drosophila melanogaster* – Walpole. E, *Drosophila pseudoobscura* – Sholow. F, *Drosophila pseudoobscura* – Lewiston. Wing length and comb length are represented as $\log_{10}$ values on the *x*- and *y*-axes. Lines of best fit are shown in each panel. All slopes are positively allometric (Type II regression; Table 2).

again, the MA slope was greater than 1 (Population S: $F_{1,47} = 63.6$, $P < 0.001$; Population L: $F_{1,54} = 27.9$, $P < 0.001$).

MA slopes within species were then tested for differences using Barlett–corrected likelihood ratios (lr), and a common MA slope (β_{com}) was determined when possible. We found that populations of *D. simulans* (lr = 3.46, $P = 0.06$, $\beta_{com} = 4.03$) and *D. melanogaster* (lr = 0.61, $P = 0.44$, $\beta_{com} = 5.62$) did not significantly differ in their comb length allometry. However, *D. pseudoobscura* populations had significantly different slopes (lr = 4.58, $P = 0.03$). We then compared allometric slopes across species (for the first tarsal comb). However, because a common slope could not be calculated for *D. pseudoobscura* populations, these

were compared separately. Comb allometry for *D. simulans* was similar to that of *D. melanogaster* (lr = 3.42, $P = 0.06$, $\beta_{com} = 4.43$) and *D. pseudoobscura* (L: lr = 0.034, $P = 0.85$, $\beta_{com} = 2.60$; S: lr = 2.70, $P = 0.10$, $\beta_{com} = 4.04$). Similarly, *D. pseudoobscura* S had slopes that did not differ from *D. melanogaster* (S: lr = 0.03, $P = 0.87$, $\beta_{com} = 5.47$), although slopes of *D. pseudoobscura* L did (lr = 5.93, $P = 0.02$). Because *D. pseudoobscura* has two sex combs, we also compared them within and across the two populations. Within each *D. pseudoobscura* population, the two combs had allometric slopes that did not significantly differ (L: lr = 2.91, $P = 0.09$, $\beta_{com} = 3.08$; S: lr = 0.47, $P = 0.49$, $\beta_{com} = 6.19$). Furthermore, although comb one slopes were significantly different across the two

populations (as noted above: lr = 4.57, $P = 0.03$), those of comb two were similar (lr = 0.27, $P = 0.60$, $\beta_{com} = 6.70$).

We again used MANOVA to compare FA levels in wing length (WLFA) and sex comb tooth number (TNFA) across species and populations (with population again nested within species). We included wing length as a covariate in this analysis because levels of FA may vary simply because larger traits may have larger FA, and trait size differed across populations and species. This analysis revealed that the multivariate combination of these traits was significantly influenced by species (Wilks' lambda: $F_{4,624} = 3.45$, $P = 0.01$) but not population (nested within species) (Wilks' lambda: $F_{6,624} = 0.689$, $P = 0.66$). Body size was not associated with the multivariate combination of FA in WL and TN (Wilks' lambda: $F_{2,312} = 1.26$, $P = 0.29$). Univariate post-hoc tests of the species effect revealed that both WLFA ($F_{2,317} = 4.93$, $P = 0.008$) and TNFA ($F_{2,317} = 13.2$, $P < 0.001$) differed significantly between species. Post-hoc Bonferroni tests revealed that WLFA of *D. simulans* was significantly lower than that of *D. melanogaster* ($P = 0.013$) and *D. pseudoobscura* ($P = 0.034$), although *D. melanogaster* and *D. pseudoobscura* did not differ in WLFA ($P = 1.00$) (*D. simulans* < *D. pseudoobscura* = *D. melanogaster*). Post-hoc Bonferroni tests revealed that TNFA of *D. pseudoobscura* was significantly lower than that of *D. simulans* ($P < 0.001$) and *D. melanogaster* ($P < 0.001$), whereas *D. simulans* and *D. melanogaster* did not differ ($P = 1.00$) (*D. pseudoobscura* < *D. simulans* = *D. melanogaster*).

Individuals were ranked for WLFA and TNFA and Spearman's rank correlations were then used to assess the congruence of within individual FA by regressing WLFA rank against TNFA rank; this was carried out within populations (i.e. individuals were ranked within each population and regression were performed for each population), then within species, and then across all individuals. None of these analyses revealed a significant association (all |Rho| < 0.26, $P > 0.2$), except that there was a weak positive association between the two rank scores in one *D. melanogaster* population (W_m: Rho = 0.262, $P = 0.047$), which would be nonsignificant with Bonferroni correction. We also looked at potential associations between mean trait size and trait FA by regressing WLFA versus WL, TNFA versus TN, and TNFA versus CL; this was carried out within populations, then within species, and then across all individuals. OLS estimates for all three relationships were close to zero ($\beta < 0.1$, SE<0.1, $P > 0.05$) in each analysis, except for TNFA versus TN, where we see a significant positive association when all individuals are pooled ($\beta = 0.05 \pm 0.01$, $F_{1,318} = 17.2$, $P = 0.001$).

DISCUSSION

The major finding of the present study was that the sex combs of all species examined display positive allometry. Additionally, comb allometry did not significantly differ across populations, except for the allometry of the first comb in *D. pseudoobscura*, and there were few statistically significant differences in allometry across species. There were differences between species and populations in trait sizes and asymmetries, although little evidence that FA of single traits reflected overall developmental stability, or that FA was generally associated with trait size. We discuss each of these findings in turn.

Sex combs were positively allometric for all species examined. Thus, sex combs scale more like signalling characters (Alatalo, Höglund & Lundberg, 1988; Petrie, 1988; Baker & Wilkinson, 2001) than insect genitalia (Eberhard *et al.*, 1998; Hosken *et al.*, 2005). This is perhaps unexpected because there is evidence in one of our study species that sexual selection favours smaller combs (Markow *et al.*, 1996), whereas exaggerated signalling traits are usually favoured by sexual selection (Andersson, 1994). Furthermore, similar to male genitalia, combs are frequently brought into direct contact with females during mating. Both *D. melanogaster* and *D. simulans* males use sex combs for 'precision grasping' of extruded female genitalia before mounting, whereas *D. pseudoobscura* males use the sex combs to spread the females' wings during copulation (Spieth, 1952; Cook, 1977). These functions are similar to those of some genital characters such as non-intromittent claspers (Hosken & Ward, 2000) but, although genital claspers scale with negative allometry in at least some Diptera (Hosken *et al.*, 2005), the sex combs do not. Positive allometry has also been reported for the fore-legs of another fly and these are also used to hold onto females' wings during copulation (Eberhard *et al.*, 1998). Why the scaling differences exist when functional differences apparently do not (i.e. claspers versus sex combs) is not clear, and further investigations are needed to explore selection acting on the sex combs of our experimental populations. However, if heavier males require better anchorage to lift and position themselves onto females, this could potentially explain the positive allometry we report. Recent work on another species finds that fertilization success during competitive mating is positively associated with sex comb size (Polak & Simmons, 2009) and, in one of our *D. simulans* populations (T), pre- and post-copulatory success are positively associated (Hosken *et al.*, 2008). Because negative associations between mating success and comb size have been reported in this species (Markow *et al.*, 1996), this tentatively suggests that comb size could also be

negatively associated with sperm competitiveness. Again, this remains to be established.

The allometric relationships of sexually-selected traits frequently diverge rapidly (Baker & Wilkinson, 2001; Shingleton *et al*., 2007), and geographically isolated populations of the same species can also differ substantially in trait allometry (Moczek & Nijhout, 2003). In the present study, however, we did not find significant differences in sex comb allometry for the most part. Although populations of *D. pseudoobscura* differ from each other in the scaling of the first comb, they do not differ from each other for the second comb, and one population did not significantly differ from *D. simulans* or *D. melanogaster* (for the first comb), which were also similar to each other. Furthermore, there were no significant differences in comb scaling across populations of *D. simulans* or *D. melanogaster*. These significance levels were assessed with likelihood ratio tests and, when assessed with *F*-tests, comb allometry differed significantly between all populations. However, because the numerator and denominator sums of squares are not independent, testing MA slopes using *F*-tests it is not recommended (Warton *et al*., 2006). So, although there appear to be differences in the scaling of the sex combs, using the appropriate tests these are not significant and hence we must conclude allometry has not diverged greatly across our samples. Interestingly, the only species with statistically different MA slopes across populations (one of which differed from the other species too) was *D. pseudoobscura*. This is the one species that we investigated where there is no direct evidence that sexual selection acts on the sex combs (Markow *et al*., 1996). However, because our sample size at the species level is small ($N = 3$), it is difficult to draw conclusions from this. Additionally, despite the general similarity in comb allometry, all species differed in the absolute size of the characters we measured and there were some differences between populations within species too, primarily in wing length. Because flies were all reared under identical environmental conditions, this indicates genetic differences between populations for some traits, and similar findings have been reported across populations of other flies (Demont *et al*., 2008).

Across *Drosophila*, there is considerable variation in the number of comb teeth per row, number of rows, and in the orientation and position of rows (Kopp & True, 2002). Consistent with this, the total sex comb tooth number and comb length for all three species we investigated were significantly different from each other (for apparent orientation differences, see Fig. 1). Sex combs are similar to male genitalia in this regard (Eberhard, 1985; Hosken & Stockley, 2004), and even species such as *D. simulans* and *D. melanogaster*, which are morphologically very similar, clearly differ in these characters. Although species differed in comb attributes, populations within species differed far less, a pattern also reported for other sexual traits (Karr & Pitnick, 1996; Civetta & Singh, 1998). There are many reasons for a lack of within species differentiation, although divergence of sexual trait morphology across species suggests the precise focus of (sexual) selection on the combs varies between species (Han *et al*., 2010). Consistent with this, sex combs are used for female stimulation in *D. simulans* and *D. melanogaster*, whereas, in *D. pseudoobscura*, they are used to spread the female wings during mounting (Spieth, 1952; Cook, 1977). Furthermore, mating *D. simulans* males have significantly fewer sex-comb teeth than noncopulating males (Markow *et al*., 1996), which may explain the lower number of teeth in the combs of this species compared to *D. melanogaster*, where males with a lower number of comb teeth have significantly lower fitness compared to those with a higher number of comb teeth (Ahuja & Singh, 2008).

We found no evidence that sex-comb FA was associated with sex-comb size unless we pooled all individuals, although even then the association was positive. Arguably, this is the association we could expect if sexual selection is for smaller comb size, as appears to be the case in *D. simulans* (Markow *et al*., 1996). However, this association was only apparent across all individuals and not within or across *D. simulans* populations and, in previous work, there were also no associations between comb FA and comb size (Markow *et al*., 1996). Therefore, the present study, together with previous work on sex combs (Markow *et al*., 1996; Polak & Taylor, 2007), provides no evidence to support the predicted negative relationship between FA and (sexually-selected) trait size. This may be because combs are not particularly costly to produce, although this appears highly unlikely given that there is some evidence of condition dependence of sex combs (Polak & Starmer, 2005). However, a lack of FA/sexual-trait size associations have been reported for a number of other insects (Tomkins & Simmons, 1995; David *et al*., 1998) and, overall, the evidence for this association is weak at best (Polak, 2008). Additionally, although there is some evidence that FA occasionally influences mating success in two of our species, the reported associations are not always consistent with theory. For example, a positive association between wing FA and mating success has been reported in *D. pseudoobscura* and a negative association in *D. simulans*; there was no association in a third species (Markow & Ricker, 1992). However, this previous study did not assess measurement error and subsequent work found no associations between FA and mating success in either *D. simulans* or *D. pseudoobscura* (Markow *et al*., 1996). Furthermore, there is no association between FA and fecundity in

D. melanogaster (Woods *et al*., 2002), which also suggests that FA genetic quality associations are at times weak (Martin & Hosken, 2002), a stance further supported by the lack of consistency of within-individual FA that we found in the present study.

For FA to be useful as an indicator of general individual quality, it should at least be consistent across different traits measured on the same individual, even if the correlations are weak (Whitlock, 1996). However, although we found that trait asymmetry differed between species (but not between population), there were no significant associations between FA in different traits. This supports claims that FA is trait rather than individual specific (Palmer & Strobeck, 1986; Clarke, 1998; Hosken, Blanckenhorn & Ward, 2000), as may be expected if different traits are developmentally buffered to different degrees (Lüpold, McElligott & Hosken, 2004). However, this lack of congruence may only generally be true when comparisons are across trait classes (e.g. sexual versus nonsexual) (Polak *et al*., 2003), as in the present study, and comparisons of differences in congruence across characters (and character classes) may reveal important information about trait developmental integration (Klingenberg, 2003). In any case, there is currently little consensus on how informative FA is from a sexual selection perspective (Palmer, 1999; Møller & Cuervo, 2003; Tomkins & Simmons, 2003; Uetz & Taylor, 2003). It is possible that comb–FA associations were present but undetectable in our populations, and that our null results reflect a lack in statistical power. Furthermore, much emphasis has been put on the problem of distinguishing FA from ME (Palmer & Strobeck, 1986; Palmer, 1994). However, we have followed the guidelines of Palmer (1999) when performing all our calculations of FA, can discern ME from FA, and our sample sizes exceeded those recommended by Palmer (1999). We did find significant differences in trait FA across species but not across populations, and differences were not associated with simple trait-size differences that were controlled for in the analyses. Because all flies were reared at the same temperature, which is the temperature they have been reared at since their capture, these FA differences reflect variation in developmental stability under a standard developmental regime. This variation across species is unlikely to simply be the result of differences in captivity duration because the two *D. simulans* populations have been in the laboratory for very different lengths of time and their FA did not differ. It is possible that variation in heterozygosity across the different species has affected FA (Mitton, 1997; Woolf & Markow, 2003), although it would be fortuitous if the different populations of the same species had similar heterozygosity but different species did not.

As a result, we are not sure of the mechanistic basis for the FA differences we see across species, although variation in FA across populations and taxa, including *Drosophila*, has been reported previously (Mitton, 1997; Civetta & Singh, 1998).

In summary, we find that sex combs are positively allometric in all the populations and species that we sampled. Thus, sex-combs appear to scale similar to sexually-selected signalling traits. Despite some variation in slope estimates, differences across populations and species were mostly nonsignificant. Finally, there were differences in trait FA across species, although we found no consistent evidence that FA was associated with trait size.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Static allometry in various taxa.

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