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Cell size is a factor in body size variation among Hawaiian and nonHawaiian species of *Drosophila*.

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Adult body sizes in the genus *Drosophila* are remarkably diverse, especially among the species endemic to Hawaii, which hosts the giants and the dwarfs of the *Drosophila* world. Body lengths among these species vary over a greater than five-fold range, encompassing flies that are much smaller than *D. melanogaster*, and others that are more than four times as large. This size variation raises questions as to the adaptive significance of these astounding differences within one evolutionary lineage, and the developmental factors that underlie this phenotypic variation. Size in multicellular organisms depends on both cell number and cell volume; but, are larger body sizes in related species due solely or primarily to increases in the number of cells, or do cell size differences contribute to this variation? To address this question, we compared cell sizes in six Hawaiian and two nonHawaiian species, selected to represent a range of adult body sizes. We used flies from stock cultures rather than field-collected individuals in order to minimize effects on body size due to variations in temperature or nutrition (Robertson, 1959; Partridge *et al.*, 1994).

The Hawaiian species included one member of the modified-mouthparts group, *D. mimica* (strain K85P1), and five picture-winged flies: *D. silvestris* (strain U34B4) and *D. heteroneura* (strain W48B6) of the *planitibia* species group, and *D. grimshawi* (strain G1), *D. disjuncta* (strain U59G44), and *D. hawaiiensis* (strain Y17P5) of the *grimshawi* species group. All were reared by standard methods for Hawaiian *Drosophila* on yeastless Wheeler-Clayton medium (Wheeler and Clayton, 1965) at 17°C, 75% humidity, and a 12:12 light/dark cycle. The continental species were *D. melanogaster* and *D. paulistorum* (Orinocan), kindly provided by Dr. Lee Ehrman. These species were maintained at room temperature on Carolina Instant Drosophila medium, supplemented with yeast. Using a stereomicroscope fitted with an ocular micrometer, four indices of adult body size – body length, thorax length, wing length, and tibia length of the right prothoracic leg – were measured for twelve individuals of each sex (see Kacmarczyk, 1999, for details of measurement points). Mean lengths and standard errors were calculated for males and females of each species. Body mass (average wet weight per fly) was estimated by weighing groups of sexed flies sedated by chilling. To estimate cell sizes, we used computerized image analysis of mounted right wings of five individuals of each sex and species, to make trichome counts in a 0.01 mm^2 area of the posterior cell of the wing. The selected region, just slightly anterior to the intersection of the posterior cross vein and the fifth

Table 1. Interspecific and intersexual differences in adult body size traits, body mass and cell size.

Species	Body length (mm)		Wing length (mm)		Thorax length (mm)		Fore-tibia Length (mm)		Body Mass (g)		Wing Cell Area x 10^{-4} mm^2	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
D. silvestris	5.87± 0.13	6.54± 0.12	5.50± 0.15	5.80± 0.07	2.61± 0.05	2.67± 0.04	1.35± 0.04	1.31± 0.02	0.012± 0.0004	0.017± 0.0009	2.57± 0.12	2.79± 0.11
D. heteroneura	5.63± 0.08	6.07± 0.07	5.30± 0.10	5.30±0.06	2.45±0.04	2.42± 0.03	1.98± 0.86	1.08± 0.01	0.010± 0.0006	0.011± 0.001	2.67± 0.02	3.21± 0.05
D. disjuncta	4.86± 0.14	4.97± 0.09	4.02± 0.07	4.10± 0.06	2.13± 0.04	2.22± 0.04	0.90± 0.01	0.88± 0.02	0.005± 0.0002	0.006± 0.0003	1.36± 0.06	1.56± 0.08
D. grimshawi	4.76± 0.09	4.71± 0.13	4.09± 0.11	3.92± 0.07	1.98± 0.06	1.88± 0.04	0.93± 0.04	0.79± 0.01	0.006± 0.0003	0.008± 0.0001	1.56± 0.04	1.52± 0.08
D. hawaiiensis	4.42± 0.06	4.68± 0.09	4.26± 0.04	4.42± 0.05	1.97± 0.02	2.09± 0.03	0.82± 0.01	0.87± 0.01	0.006± 0.0002	0.008± 0.0003	2.13± 0.05	2.18± 0.09
D. mimica	3.67± 0.05	3.98± 0.10	3.30± 0.03	3.60± 0.07	1.62± 0.02	1.80± 0.04	0.73± 0.01	0.75± 0.02	0.003± 0.0001	0.004 ±0.0007	2.13 ±0.05	2.21 ±0.11
D. melanogaster	1.95± 0.03	2.25± 0.03	1.91± 0.01	2.15± 0.02	0.89± 0.01	0.99± 0.01	0.46± 0.00	0.48± 0.00	0.0007± 0.000	0.001± 0.000	1.49± 0.03	1.81± 0.02
D. paulistorum	1.86± 0.03	2.17± 0.04	1.69± 0.05	1.85± 0.02	0.70± 0.01	0.80± 0.02	0.42± 0.01	0.44± 0.01	0.0004	0.0006	1.08± 0.00	1.18± 0.03

longitudinal vein, corresponds to region three of Dobzhansky (1929). Hairs on both the dorsal and ventral wing surfaces were included in the count. Average areas of the wing epithelial cells were estimated for each individual by dividing the counting area (0.01 mm^2) by half the total number of hairs counted, since the wing is two cells thick. Given the extreme compression of the two wing cell layers, the wing cells can be viewed as flattened roughly circular disks, rather than as spheres. Thus, we estimated wing cell diameters from the cell area estimates, using the standard geometric relationship $D = 2\sqrt{(A/\pi)}$.

Table 1 presents mean values ($\pm$ S.E.s) of the four body size indices, body masses, and wing cell areas for males and females of the eight species. There is a three-fold range in body, thorax and wing lengths among these sampled species, and an approximately 30-fold range in body weights between the largest and smallest species. The several body size traits show the expected pattern of allometric scaling both within species and also across *Drosophila* species. The scatter plots of log-transformed data in Figure 1 present the linear regressions of male and female species means of four traits – body length, wing length, fore-tibia length, and wet weight – on thorax length, the standard index of *Drosophila* body size. All four size traits scale positively with thorax length, and are highly correlated. The correlation across species between fore-tibia length and thorax length is lower than for the other three comparisons, due in part to the unexpectedly long tibial segment of the prothoracic leg of males of *D. heteroneura* (Table 1, Figure 1C).

The null hypothesis that all members of the genus *Drosophila* have cells of roughly the same size is not supported. We found substantial interspecific variation in wing cell areas, with *D. heteroneura*, one of the largest species, having the largest cells for both males and females, and the smallest species, the South American *D. paulistorum*, having the smallest cells in both sexes. Wing cell areas in these *Drosophila* species vary over a 2.5-fold range for males, and a 2.7-fold range for females, overall spanning from $1.08 - 3.21 \times 10^{-4}$ mm^2.

The relationship between cell size and body size (Figure 2) was evaluated by comparing species wing lengths with estimates of wing cell diameters (in order to maintain the same dimensions). Although Catchpole (1994) has cautioned that wing length is not always the best predictor of body size, our interspecific data show that it correlates well with thorax length (Figure 1B) and other body size traits. Moreover, given that cell size was estimated from adult wing cells, it is logical to use a wing metric as the body size index. Spearman's rank correlation coefficient (r_s) between the two traits was found to be 0.801, which indicates a highly significant association between cell size

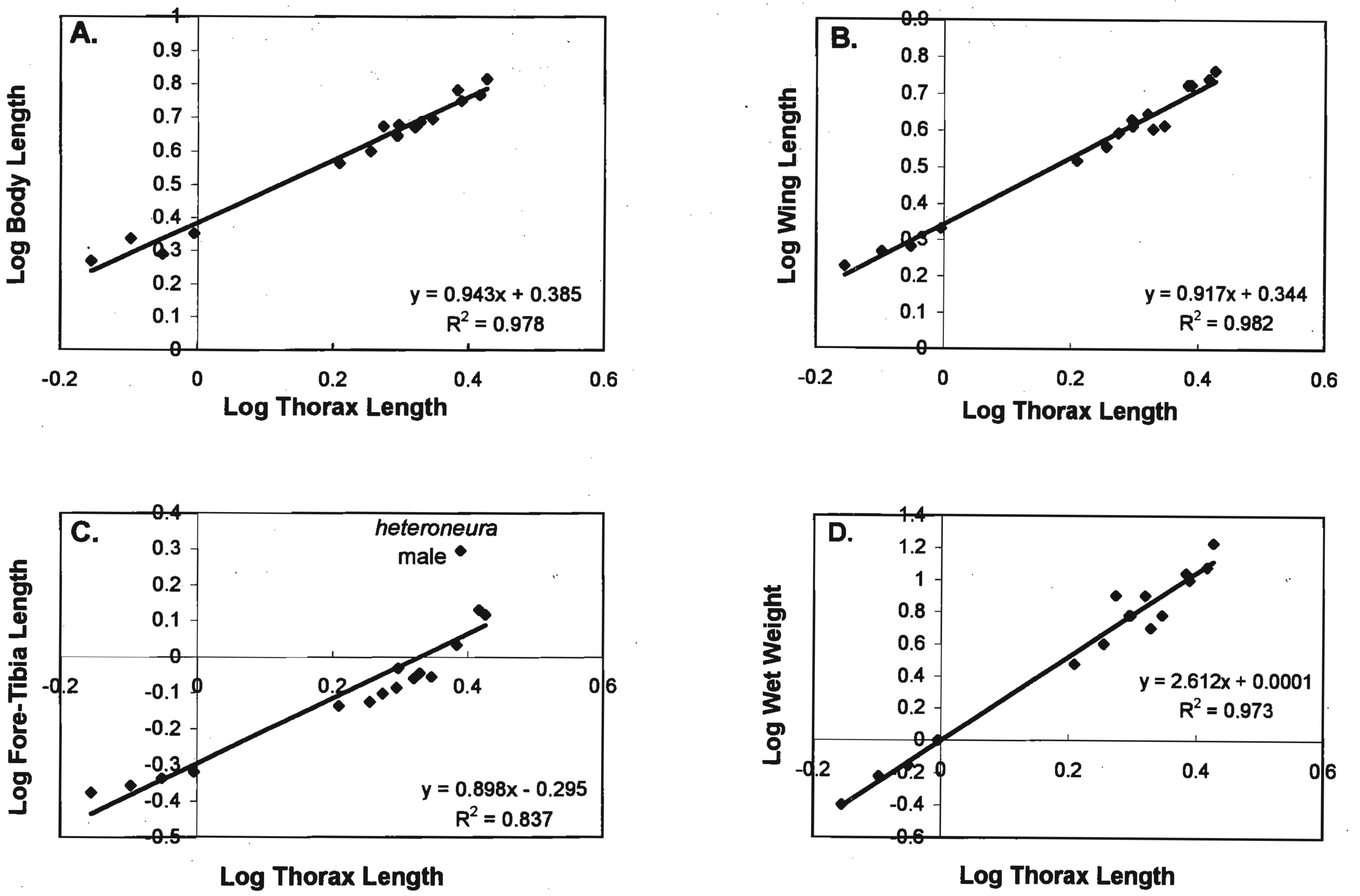

Figure 1. Scatter plots showing the allometric scaling of mean body length (A), wing length (B), fore-tibia length (C) and body mass (D) on thorax length across eight species of *Drosophila*. For each species, two mean values per trait were plotted, representing adult males and females, respectively. The equations for the standard linear regression lines are shown for the pairs of traits, along with the correlation coefficients.

and adult body size ($t_s = 5.00$, 14 d.f., $P < 0.01$) across species. Nonetheless, the relationship is complex, as our data contain some apparently anomalous cases of species of much the same size but rather different cell sizes (*e.g., D. disjuncta* and *D. hawaiiensis*), and other cases of species with almost equivalent cell sizes but quite different body sizes (*e.g., D. disjuncta* and *D. melanogaster*; see Table 1 and Figure 2). These apparent discrepancies bear further investigation and emphasize that differences in patterns of cell proliferation among species cannot be overlooked.

As well as the interspecific variation in cell size and body size traits, there is also substantial variation between the sexes. In general, females have larger body sizes, greater wet weights, and larger cell sizes than their conspecific males. To evaluate intraspecific differences between the sexes statistically, we used the one-tailed t-test. Of the 48 comparisons (6 traits in 8 species), thirty showed significance, confirming the considerable female-male size differences. Unexpectedly, the opposite pattern was observed in *D. grimshawi*, where males appeared to be larger than females with respect to their body, wing and thorax lengths, but these differences were not significant. Only fore-tibia length in *D. grimshawi* was significantly greater in males than females, a difference that was also apparent in three other large Hawaiian species, *D. silvestris*, *D. heteroneura*, and *D. disjuncta*. Surprisingly, although fore-tibia length in *D. heteroneura* scales differently with thorax length in males vs. conspecific females (Figure 1C), the sex difference is this trait was not statistically significant in this species. Nonetheless, the data suggest that fore-tibia length in the four picture-winged species mentioned above is a sexually selected trait. All have elaborate courtships, in which the male fore-tibia are vibrated against the female abdomen during the HUW (head-under-wings) stage prior to copulation (Spieth, 1982). Due in part to their egg mass, females were consistently heavier than males, although the weight difference was not statistically significant in two species, *D. heteroneura* and *D. paulistorum*, probably because the individuals sampled were not reproductively mature.

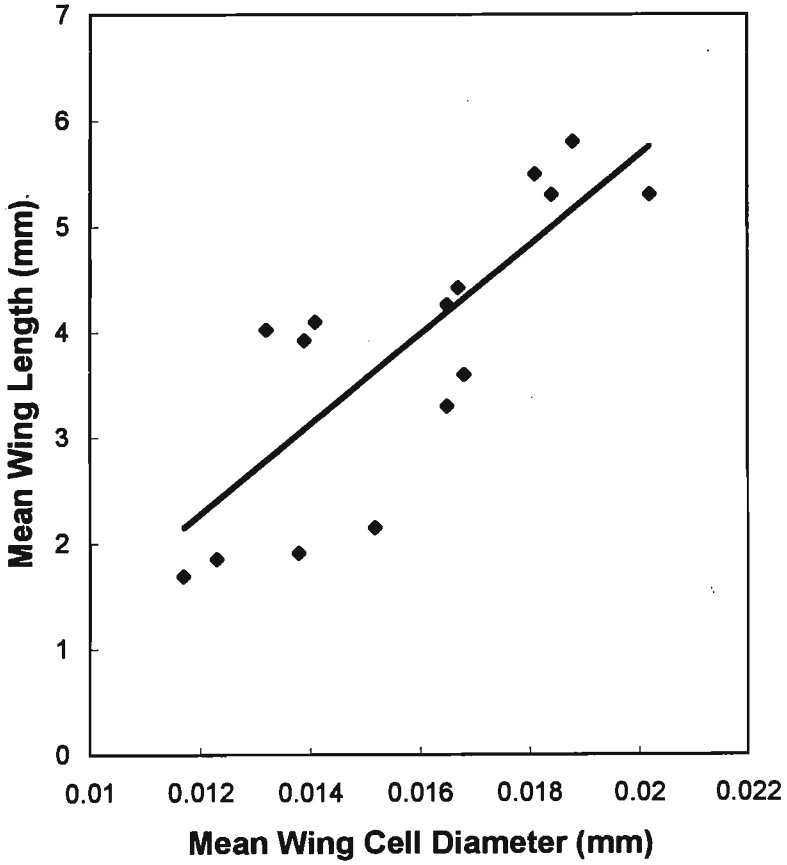

Figure 2. Cell size – body size relationship across *Drosophila* species evaluated by a comparison of species wing cell diameters with wing length.

With respect to cell size differences between the sexes, females were found to have larger wing cell areas than males in seven of the species, following the pattern of body size differences. In four of the species, *D. heteroneura, D. disjuncta, D. melanogaster,* and *D. paulistorum*, these differences were highly significant. Only in *D. grimshawi*, did males have larger cells than females (again, tracking the body size differences), but like the morphometric traits, the cell size differences between the sexes of this species were not significant. Intraspecific sex differences in cell size were not noted by Stevenson *et al.* (1995) in their survey of cell and organ sizes in Hawaiian *Drosophila* species, apparently because the sexes of individuals were not recorded.

Just why adult cell sizes should differ so markedly between the sexes and among *Drosophila* species is not immediately obvious. Larger body sizes could be achieved, if favored by selection, simply by more cell proliferation during an extended developmental period. Various rates of growth and development are found among the ecologically diverse Hawaiian *Drosophila* species (Kambysellis and Craddock, 1997), and these varied life histories undoubtedly play some role in the observed interspecific body size differences. Regulation of cell size and body size is complex and subject to both molecular and ecological constraints. For this reason, our lab is using the extraordinary array of Hawaiian *Drosophila* species to explore some of the genetic and developmental factors that have contributed to evolution of species differences in cell size and thus body size; further data from these investigations will be presented elsewhere.

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Immune response in the *tu-pb* melanotic tumor strain of *Drosophila melanogaster.*

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Introduction

Drosophila, like other insects, has developed an efficient host defense against microbial infection that induces both humoral and cellular reactions (Boman, 1995; Hoffman *et al.*, 1996; Hoffman and Reichart, 1997). The humoral reactions consist of proteolytic cascades, namely coagulation and phenoloxidase cascades, which lead to localized melanization and blood coagulation; they also consist of the rapid and transient synthesis of a battery of antimicrobial peptides. It has been reported that some genes controlling embryonic development [*dorsal (dl), Toll* and *cactus*] are expressed in larval and adult fat bodies, where their RNA expression is enhanced upon injury (Lemaitre *et al.*, 1995). Upon bacterial challenge, the Dorsal protein (Dl), normally localized in the cytoplasm of the fat body, is rapidly imported into the nucleus; this nuclear uptake of Dl occurs spontaneously in mutants exhibiting melanotic tumors. The cellular immune system, hemocytes and