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# Many ways to be small: different environmental regulators of size generate distinct scaling relationships in *Drosophila melanogaster*

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Static allometries, the scaling relationship between body and trait size, describe the shape of animals in a population or species, and are generated in response to variation in genetic or environmental regulators of size. In principle, allometries may vary with the different size regulators that generate them, which can be problematic since allometric differences are also used to infer patterns of selection on morphology. We test this hypothesis by examining the patterns of scaling in *Drosophila melanogaster* subjected to variation in three environmental regulators of size: nutrition, temperature and rearing density. Our data indicate that different environmental regulators of size do indeed generate different patterns of scaling. Consequently, flies that are ostensibly the same size may have very different body proportions. These data indicate that trait size is not simply a read-out of body size, but that different environmental factors may regulate body and trait size, and the relationship between the two, through different developmental mechanisms. It may therefore be difficult to infer selective pressures that shape scaling relationships in a wild population without first elucidating the environmental and genetic factors that generate size variation among members of the population.

**Keywords:** allometry; scaling relationship; size regulation; plasticity; environmental variation; nutrition

## 1. INTRODUCTION

Within a species, variation in the size of the body is accompanied by variation in the size of its constituent body parts (traits), a relationship called static allometry. Static allometry essentially describes the shape of a species (Bonduriansky & Day 2003) and it is no exaggeration to say that the evolution of morphology is largely the evolution of allometry. Consequently, the last 100 years have seen an ever-increasing accumulation of data concerning the scaling relationship of myriad morphological traits, and upon which insights into the evolution of morphology have been based (Huxley 1924; Gould 1966; Brown *et al.* 2000). More recent efforts have concentrated on the genetic and developmental basis of scaling relationships, to better understand the proximate mechanisms upon which selection has acted to create morphological diversity (Emlen & Allen 2003; Emlen *et al.* 2006; Shingleton *et al.* 2007, 2008).

A fundamental but often overlooked aspect of this research, however, is an appreciation of the genetic and environmental factors that generate variation in body and trait size. In principle, these different factors could impinge on different aspects of development and so produce different scaling relationships. Observed static allometries might therefore reflect the effects of multiple factors acting on multiple developmental pathways.

This would have significant implications for the study of allometry, both for identifying the developmental pathways that regulate allometry, and for understanding the selective pressures that act on those pathways. There is, however, a paucity of data exploring whether static allometries vary with the environmental and genetic factors that create them. Such studies are essential if we are to better understand the evolutionary and developmental mechanisms that shape allometric, and hence morphological, diversity. Here we describe such a study on the fruit fly *Drosophila melanogaster*.

Allometry is typically modelled using the allometric equation  $y = ax^b$ , where  $y$  and  $x$  are measurements of morphological traits and  $b$  is the allometric coefficient (Huxley & Tessier 1936). Log-transforming the measurement data produces a linear relationship,  $\log(y) = \log(a) + b \cdot \log(x)$ , with a slope of  $b$  and an intercept of  $\log(a)$ . The allometric coefficient  $b$  is particularly important in studies of scaling relationships, since it controls how shape changes with size. When  $b = 1$ , the relationship between  $x$  and  $y$  is called isometry, with the relative size of each trait remaining constant irrespective of absolute size. When  $b < 1$  or  $b > 1$ , the relationship is hypo- or hyperallometric, respectively, with the relative size of  $y$  decreasing (hypoallometry) or increasing (hyperallometry) with an increase in absolute size.

Implicit to the concept of allometry is that there is variation in body size and organ size and covariation between them. Several factors are known to regulate body and organ size in *Drosophila*, including developmental nutrition (Robertson 1963), temperature (James *et al.* 1997), rearing density (Lefranc & Bundgaard 2000),

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oxygen level (Peck & Maddrell 2005) and genetic background. Isometry, hyper- and hypoallometry can therefore be viewed as description of how different traits respond to the factors that regulate size (Shingleton *et al.* 2007). Isometry results when both traits show the same response to a size regulator. Hyper- or hypoallometry occurs when the  $y$  trait responds relatively more (hyperallometry) or less (hypoallometry) than the  $x$  trait to a size regulator. When the size regulator is an environmental factor, allometry is essentially a description of the phenotypic plasticity of trait  $y$  relative to trait  $x$ .

When viewed from this perspective, we might expect traits to show varying degrees of plasticity in response to different environmental conditions. Consequently, different environmental variables may create distinct scaling relationships. Previous studies have indicated that the size response of developing *Drosophila* to changes in temperature may be different from their response to changes in developmental nutrition. *Drosophila* are smaller when reared at higher temperatures, and, for the epidermis at least, this size reduction is primarily through a reduction in cell size rather than cell number (Partridge *et al.* 1994; de Moed *et al.* 1997; Azevedo *et al.* 2002). In contrast, the reduction in size that accompanies reduced developmental nutrition is, for the wing and eye at least, through a reduction in both cell size and cell number (Robertson 1959; Shingleton *et al.* 2005). More germane work on *Drosophila buzatti* shows that the slope of the allometry between wing and body size may be steeper for thermal variation than for nutritional variation (Thomas 1993). Finally, work in the horned beetle *Onthophagus acuminatus* indicates that the scaling relationship between horn length and body size is different for populations reared on different diet qualities (Emlen 1997).

These data suggest that organ size is not simply a developmental consequence of body size: a reduction in body size may or may not be accompanied by a corresponding reduction in organ size, depending on the environmental factor that regulates size. Thus a thermal static allometry may be quite different from a nutritional static allometry. To better explore the relationship between the environment and static allometry, we subjected three isogenic *Drosophila* strains to three environmental factors known to regulate body and organ size: temperature, nutrition and rearing density. We tested the hypothesis that static allometric relationships reflect the specific contribution of the environmental and genetic factors that create variation in body and organ size.

## 2. MATERIAL AND METHODS

### (a) Fly stocks

We used three isogenic strains in our study. Oregon R is a common 'wild-type' laboratory strain. 157 and 187 (provided by P. Schmidt) were isolated from lines collected in Maine and differ only at their third chromosome via balancer-mediated chromosome substitution.

### (b) Environmental variable 1: nutrition

Flies from each line were allowed to oviposit on apple-juice plates for 4 hours. Eggs were then washed and transferred to food vials, 50 eggs per vial. Food vials contained either standard cornmeal/molasses medium, or medium diluted to 1, 2, 5, 10 and 50 per cent, in 2 per cent agar in water. We set

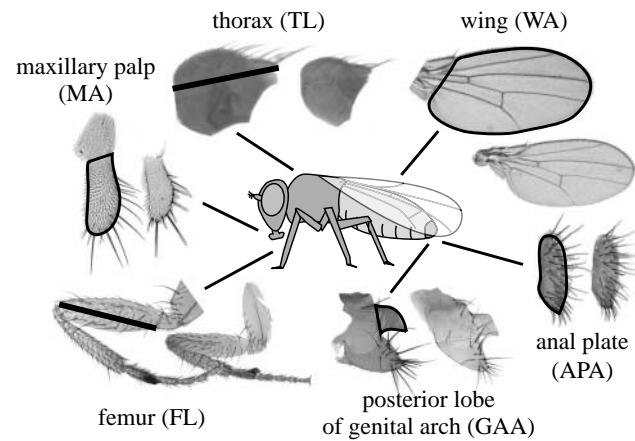


Figure 1. Illustration of *D. melanogaster* showing the range of body part sizes for OreR flies reared on different diets. For each body part, the organ on the left is from a fly reared on a 100% diet, while the organ on the right is from a fly reared on a 2% diet. Images are shown at the same scale within body parts but at different scales across body parts. Morphological measurements are outlined in black.

up at least 3–6 vials for each genotype–diet combination (survival of flies reared on low-quality diets was low, so additional vials for the 1, 2 and 5 per cent diets were set up). The larvae were left to hatch and develop at 25°C. Upon eclosion, adults were transferred to 70 per cent ethanol in water and stored at room temperature for measurement. The experiment was repeated for flies reared at 17°C.

### (c) Environmental variable 2: temperature

Eggs were collected and transferred to 100 per cent food vials as described above. The vials were then transferred to either 17, 19, 21, 25 (all genotypes), 23 or 24°C (OreR only). We set up at least three vials for each genotype–temperature combination. Larvae were left to develop, and the adults were collected and stored as described above.

### (d) Environmental variable 3: density

Flies from each line were allowed to oviposit on standard cornmeal/molasses medium plates for 4 hours. The plates were left for a further 24 hours at 25°C, until the larvae were in their first instar (L1). L1 larvae were then transferred to 110 per cent food vials, each vial receiving either 50, 100, 200 or 300 larvae. We set up three vials for each genotype–density combination. Larvae were left to develop, and the adults were collected and stored as described above.

### (e) Morphology

We dissected the wing, maxillary palp, genital arch, anal plate and the first leg from each fly. Because we could not reliably dissect the anal plate and genital arch from only one side of the body, all body parts were dissected without consideration for whether they came from the left or the right side. All body parts were mounted in dimethyl hydantoin formaldehyde. We measured the area of the wing (WA), maxillary palp (MPA), posterior lobe of the genital arch (GAA) and anal plate (APA) and the length of femur (FL; figure 1) using a Leica DM6000B compound microscope and Retiga 200R digital camera. We measured the length of the thorax (TL) from where the neck meets the pronotum to the posterior tip of the scutellum, using Leica MZ16FA dissecting microscope and a Leica DFC250 digital cameras. We measured no more than 10 flies from any one vial. Image processing was performed

using IMAGEPRO v. 6.1. Measurement error was quantified by re-measuring the body parts of 10 flies, three times. Percentage measurement error (%ME) was calculated using the methods of Bailey & Byrnes (1990) and is reported in the electronic supplementary material.

### (f) Analysis

Linear measurements were squared prior to analysis, to convert them to the same dimension as area measurements. All the data were then log transformed. We found no significant difference in size between vials of flies subjected to the same treatment ( $p > 0.05$  for all), so all data for each treatment were pooled. Finally, we grouped all individuals of each genotype which were subjected to the same environmental variable: temperature, nutrition (25°C), nutrition (17°C) and density (25°C). Variation in body and organ size among individuals within any group was therefore assumed to be a consequence of variation in the environmental factor. There were a total of 12 datasets comprising a combination of three genotypes and four environmental variables.

#### (i) Phenotypic plasticity

Plasticity, the change in a phenotype caused by a change in the environment, can be measured as:

$$\sigma_{\text{PL}}^2 = \sigma_{\text{E}}^2 + \sigma_{\text{E}\cdot\text{G}}^2,$$

where  $\sigma_{\text{PL}}^2$  is a trait's plastic variance,  $\sigma_{\text{E}}^2$  is its environmental variance and  $\sigma_{\text{E}\cdot\text{G}}^2$  is its genotype–environment interaction variance (Scheiner & Lyman 1989). For each environmental factor we fitted the data to the following model:

$$Y_{ijk} = u + E_i + G_j + E \cdot G_{ij} + e_{ijk},$$

where  $Y$  is the morphological measurement (WA, MPA, GAA, APA, FL, TL),  $E$  is the effect of the particular environmental factor (nutrition, density or temperature) and  $G$  is the effect of the particular genotype (OreR, 157, 187). Both  $E$  and  $G$  were treated as random factors. We used the `lmer()` function in the `lme4` package in R (R-Development-Core-Team 2007) to estimate the variance components for  $E$  and  $E\cdot G$  ( $\sigma_{\text{E}}^2$  and  $\sigma_{\text{E}\cdot\text{G}}^2$ , respectively) using maximum likelihood. These were then summed to calculate trait plasticity. Each dataset was sampled with replacement to generate 1000 bootstrap datasets, which were analysed and used to construct a 95 per cent confidence interval of each trait's plasticity.

#### (ii) Multivariate allometric coefficients

We used a multivariate approach to test whether different environmental factors produce different allometries in the different genotypes. For multivariate log-transformed data, the allometric coefficient is reflected by the loadings of the first eigenvector of the variance–covariance matrix, the ‘allometric vector’. Isometry occurs when all loadings of the first eigenvector equal  $1/\sqrt{n}$ , where  $n$  is the number of variables. The bivariate allometric coefficient for any two variables is the ratio of their loadings in the first eigenvector, while multiplying the loadings by  $\sqrt{n}$  gives the bivariate allometric coefficient for each trait against a measure of overall body size (Jolicoeur 1963; Klingenberg 1996).

We used the `pca()` function in the `labdsv` package in R, or the `eigen()` function in the `base` package of R, to extract the first eigenvector from the covariance matrix of log-

transformed data for each dataset. These vectors reflect the thermal, nutritional and density static allometries in the different genotypes. We used a random-variable bootstrap method to estimate the accuracy of each allometric vector (Tzeng & Yeh 2002). We sampled each dataset with a replacement to generate a bootstrap dataset of the same size as the original, which was then analysed. For each analysis we performed 10 000 bootstrap iterations. We used the distribution of the loadings of the first eigenvector for these bootstrap datasets to construct confidence intervals for the loadings from the observed dataset.

#### (iii) Comparisons of multivariate allometries

The angle between any two allometric (first principal component) vectors indicates the similarity of their multivariate allometries (Klingenberg 1996; Zelditch *et al.* 2004; Gerber *et al.* 2008). We computed this angle ( $\theta_0$ ) as the arc cosine of the inner product of the two first eigenvectors for pairs of treatments. The larger this angle, the more different the allometric coefficients. This is analogous to measuring the angle between the major axes of two bivariate allometric plots. We used a permutation test to generate a null distribution of  $\theta_0$ , which allowed us to examine whether the difference between two multivariate allometries was significant (Tzeng & Yeh 2002). First we pooled the data from the two environmental variables being compared. Next, we sampled this pooled dataset, without replacement, to create two new permuted datasets. We then calculated the angles between the two permuted datasets' allometric vectors ( $\theta_A$ ). This was repeated 10 000 times to generate a distribution of expected angles under the null hypothesis that the observed data share the same multivariate allometry. The position of the angle from the observed data ( $\theta_0$ ) was determined among the ordered angles ( $\theta_A$ ) from the permuted datasets. The proportion of  $\theta_A$  greater than or equal to  $\theta_0$  was used as a  $p$ -value under the null hypothesis that the two observed multivariate allometries were sampled from the same distribution.

To better visualize how the multivariate allometries differed among themselves, we used the angles between the multivariate allometric coefficients as a measure of distance, and used the resulting distance matrix to construct a distance tree. Angles were calculated in R, using the method described above, put into a distance matrix and turned into a distance tree using hierarchical clustering, in R. This process was repeated for 1000 bootstrap datasets. The resulting trees were converted into a standard New Hampshire tree format, and a majority consensus tree was calculated using the `CONSENSE` package in `PHYLIP`, along with bootstrap values for individual branches (Felsenstein 2005).

## 3. RESULTS

While it is well established that environmental factors such as temperature, nutrition and rearing density contribute to variation for body and organ size in *Drosophila*, it is unclear whether they do so in a similar manner. Specifically, does manipulation of these variables produce flies with similar patterns of allometry? To address this, we independently manipulated all three of these factors and examined the consequences on the patterns of multivariate allometry. Our results overwhelmingly indicate that different sources of environmental variation result in different multivariate allometries.

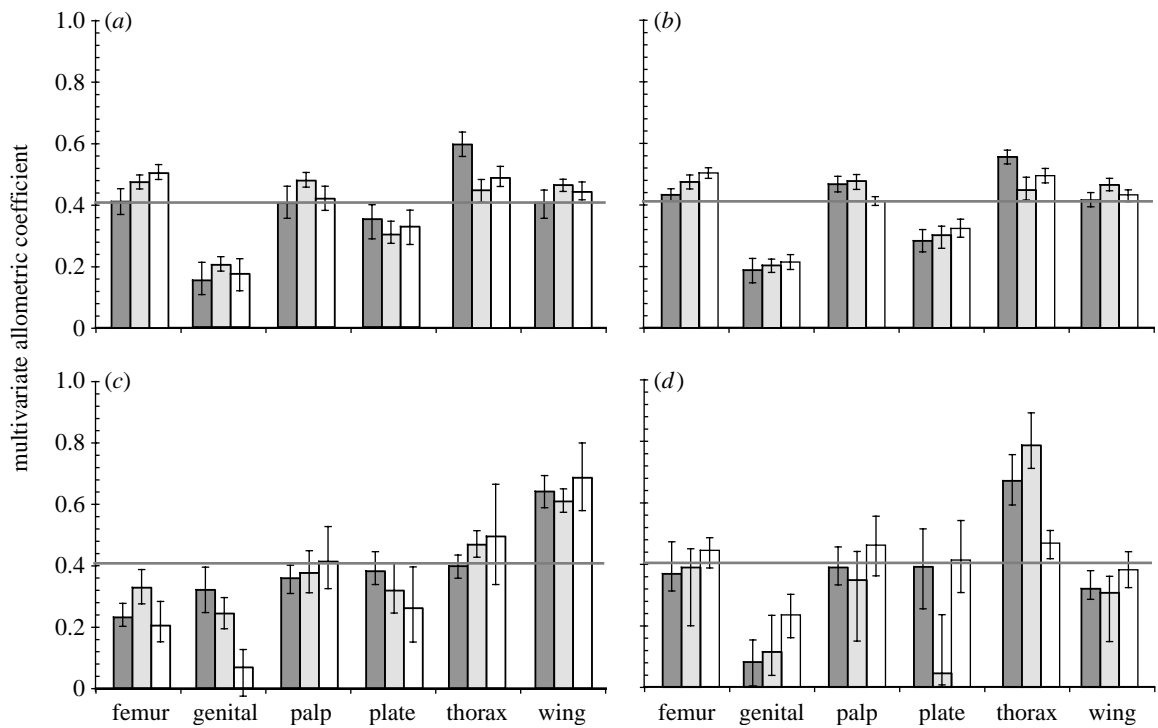


Figure 2. Loadings of the first eigenvector of multivariate allometries for different environmental variables and genotypes. Horizontal grey lines indicate expected loadings if trait is isometric to body size. Loadings above this line indicate hyperallometry, loadings below this line indicate hypoallometry. Error bars are 95% confidence intervals. (a) Density, (b) nutrition (25°C), (c) temperature and (d) nutrition (17°C). Dark grey bars, 157; light grey bars, 187; and white bars, OreR.

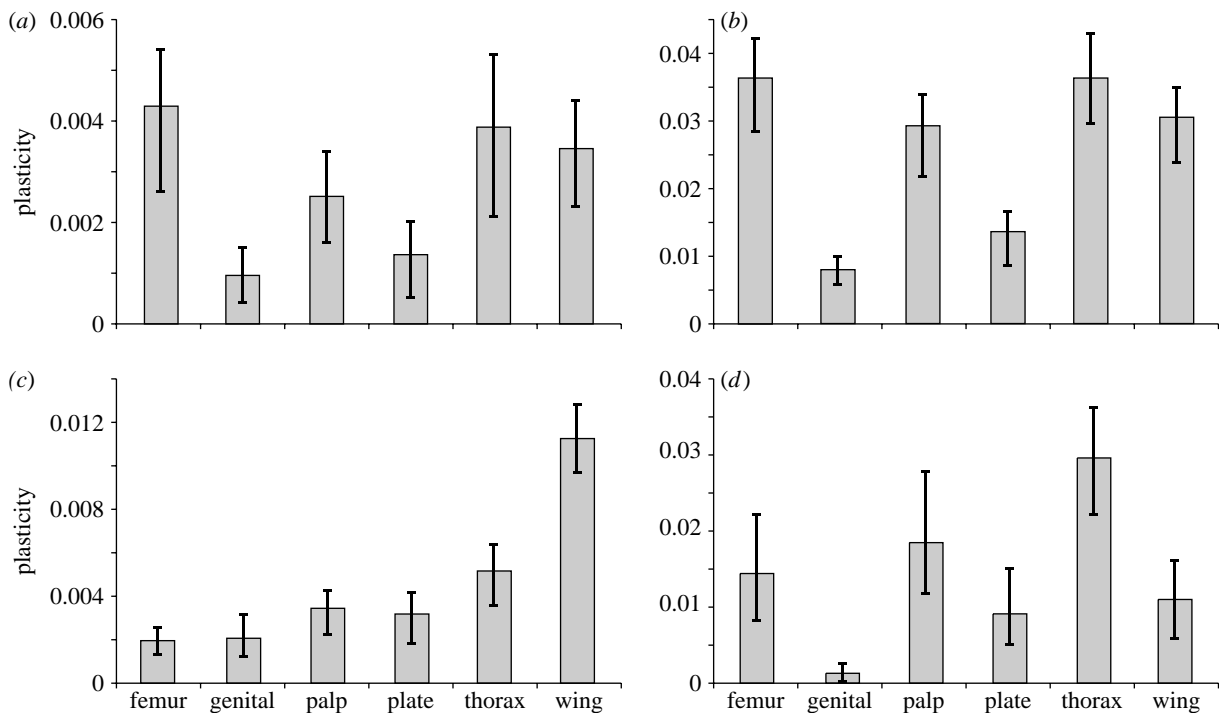


Figure 3. Trait plasticity in response to different environmental variables. Error bars are 95% confidence intervals. (a) Density, (b) nutrition (25°C), (c) temperature and (d) nutrition (17°C).

When subjected to variation in an environmental size regulator, different traits showed quantitatively distinct scaling relationships with one another and with overall body size. Figure 1 shows the range of trait sizes for different organs of OreR flies reared under different nutritional conditions at 25°C. Figure 2 shows the loadings of the first eigenvectors for each of the genotypes and for each environmental variable. Multiplying the loading by 2.45 ( $\sqrt{n}$ , where  $n=6$ ) gives the bivariate allometric

coefficient of each trait against overall body size. Thus, traits with a loading less than 0.408 ( $1/\sqrt{n}$ , where  $n=6$ ) are hypoallometric to body size, while traits with a loading greater than 0.408 are hyperallometric to body size.

The pattern of allometry varied from trait to trait and depended on the environmental variable and the genotype. The response to variation in nutrition and rearing density at 25°C produced very similar patterns of allometry across all three genotypes: the posterior lobe

Table 1. Angles (degrees) between multivariate allometric vectors under different environmental conditions for genotypes 157, 187 and OreR with corresponding *p* values (in parentheses). (\*Significant at Bonferroni corrected  $p < 0.0017$ .)

157	nutrition (25°C)	density (25°C)	temperature
density (25°C)	6.58 (0.326)		
temperature	22.74 (<0.001)*	23.90 (<0.001)*	
nutrition (17°C)	12.58 (<0.001)*	10.56 (0.004)	31.34 (0.002)
187	nutrition (25°C)	density	temperature
density (25°C)	6.54 (0.669)		
temperature	13.43 (<0.001)*	14.64 (<0.001)*	
nutrition (17°C)	31.89 (<0.001)*	28.16 (<0.001)*	34.44 (0.048)
OreR	nutrition (25°C)	density (25°C)	temperature
density (25°C)	2.41 (0.581)		
temperature	28.11 (<0.001)*	26.57 (<0.001)	
nutrition (17°C)	8.295 (0.004)	8.90 (0.038)	30.78 (0.001)*

of the genital arch and the anal plate were hypoallometric to body size, while all other body parts were either isometric or slightly hyperallometric to body size (figure 2*a,b*). The thermal static allometries were, however, quite different. Although the genitals were again hypoallometric to body size, the anal plate was closer to isometry with body size, and the wings were highly hyperallometric (figure 2*c*). There was also a greater difference among genotypes in their thermal static allometry than in their density or nutritional static allometries. However, where the genotypes differed most was in their nutritional static allometries at 17°C (figure 2*d*). Although the nutritional static allometry of OreR was similar at both 25 and 17°C, in both 157 and 187 the thorax was more hyperallometric and the wings more hypoallometric at 17 than at 25°C.

Patterns of allometry reflected trait plasticity. Figure 3 shows the plasticity of each trait under the different environmental conditions. Within each treatment, traits that were hypoallometric to body size showed relatively low levels of plasticity, while traits that were hyperallometric to body size showed relatively high levels of plasticity.

To more formally test whether multivariate allometries differed among environmental treatments and genotypes, we determined the angle between pairs of allometric vectors and tested whether the angle differed significantly from zero, using a permutation test. Table 1 shows pairwise comparisons of the multivariate allometry among environmental factors for each genotype. In all three genotypes, the nutritional and density static allometries at 25°C were not significantly different from one another. However, both the nutritional and density static allometries differed significantly from the thermal static allometry. There was also a trend for the nutritional static allometry at 17°C to differ from all other static allometries, although this was not significant for all comparisons when using a Bonferroni correction for multiple comparisons.

Table 2 shows pairwise comparisons of the multivariate allometry among genotypes for each environmental variable. There was a trend for different genotypes to have different static allometries for each environmental variable, although many of these were not significant after using a Bonferroni correction for multiple comparisons.

Table 2. Angles (degrees) between multivariate allometric vectors for genotypes 157, 187 and OreR under different environmental conditions with corresponding *p* values (in parentheses). (\*Significant at Bonferroni corrected  $p < 0.0017$ .)

nutrition (25°C)	157	187
187	7.36 (0.005)	
OreR	6.85 (0.079)	5.42 (0.254)
density (25°C)	157	187
187	8.49 (0.007)	
OreR	8.93 (0.002)	4.29 (0.37)
temperature	157	187
187	9.15 (0.042)	
OreR	24.52 (<0.001)*	20.27 (0.004)
nutrition (17°C)	157	187
187	17.60 (0.015)	
OreR	19.87 (<0.001)*	34.82 (<0.001)*

Figure 4 shows a consensus distance tree of the different multivariate allometries, using the angle between allometries as a measure of distance. Multivariate static allometries that are most similar to each other appear closest to each other on the tree. The bootstrap value for each branch is an indication of confidence in the position of that branch. Internal branches that have less than a 50 per cent bootstrap value are not well supported by the data, and so the groups of allometries they separate are probably not different from each other. The tree illustrates that the density and nutritional static allometries were the same among all three genotypes, with less than 50 per cent bootstrap support for any internal branches in this part of the tree. By contrast, there was much higher bootstrap support for internal branches separating the thermal static allometries and the 17°C nutritional static allometries from all other static allometries.

The finding that different environmental factors generated different allometries meant that flies that were ostensibly the same size had different body proportions.

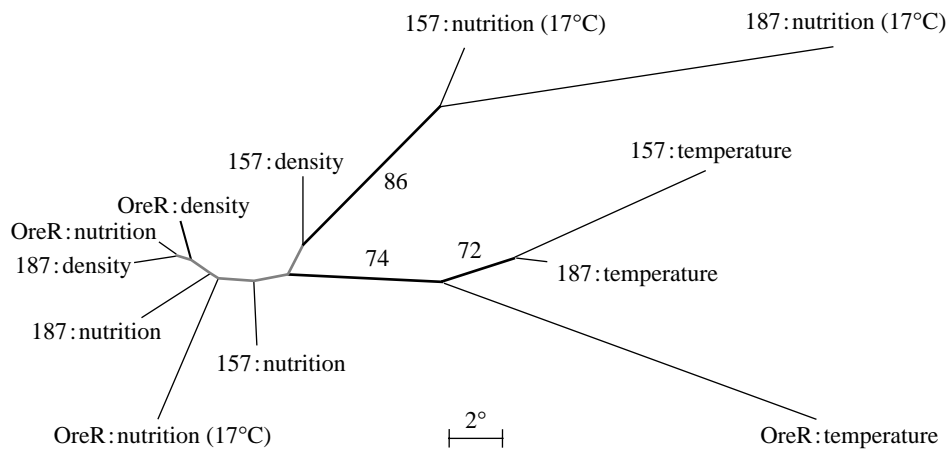


Figure 4. Distance tree summarizing similarity among multivariate allometries in different genotypes under different environmental conditions. Grey internal branches have less than 50% bootstrap support. Black internal branches have greater than 50% bootstrap support, with bootstrap values shown adjacent to branch. Branch lengths indicate angular difference (in degrees) between multivariate allometric vectors.

For example, even though flies reared at low nutrition at 17°C had slightly smaller thoraxes than well-fed flies reared at 25°C, they had much larger wings (figure 5). Indeed, our results (figure 2) indicate the thorax does not always scale isometrically to body size, and may not represent an ideal proxy for overall size.

#### 4. DISCUSSION

Change in allometry underlies much of the evolution of morphology. Despite decades of research elucidating the patterns of allometry within and among species, very little is known of the proximal developmental and physiological mechanisms that create scaling relationships. Consequently, we have only a rudimentary understanding of the genetic basis for allometric change. Central to this problem has been a lack of clarity concerning the environmental and genetic factors that underlie variation in body and trait size, and hence create allometries. Our results show that different environmental factors create different static allometries in *D. melanogaster*. These data indicate that studies of the developmental basis and evolution of allometries should take into account the sources of variation that create the allometry, in particular when such allometries are used for making inferences about condition dependence.

##### (a) *Individual organs respond differently to different environmental variables*

Our results indicate that trait allometry depends on the environmental factor that creates size variation, and differs between traits. For example, wing area is hyperallometric to body size under conditions of variable temperature, but isometric or hypoallometric under conditions of variable nutrition. The reverse is true for the femur. Thus variation in trait size is not simply a consequence of variation in overall body size. Further, different environmental factors interact in their regulation of static allometries. For example, the nutritional static allometry of the thorax was isometric at 25°C but became hyperallometric at 17°C. Research on the horned beetle *O. acuminatus* also hints that different regulators of size may interact in their influence on trait allometry (Emlen 1997): in this species, the relationship between body size and horn length varies with diet quality.

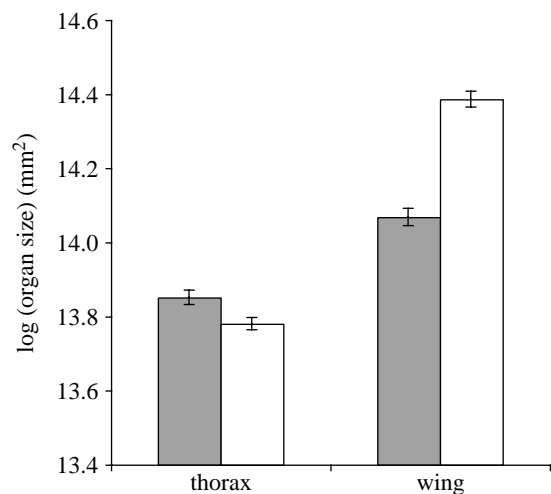


Figure 5. Genetically identical flies of the same 'size' had different body proportions. 157 flies reared on 100% food at 25°C (grey bars) had slightly larger thoraxes but considerably smaller wings than 157 flies reared on 20% food at 17°C (white bars). Error bars are standard errors. Thoraxes and wings are significantly different in size at  $p < 0.05$ .

Theoretical models of allometry (Bonduriansky & Day 2003; Kodric-Brown *et al.* 2006) have explained patterns of allometry in terms of resource allocation, with the total available resources (as indicated by body size) being allocated to individual growing tissues (as indicated by trait size). This model is supported by evidence that experimental removal of an organ results in an increase in the size of the remaining organs (Nijhout & Emlen 1998), presumably through the allocation of more resources. An unfortunate consequence of the term 'allocation' is that there is a tendency to see trait size as a 'read out' of body size (Kodric-Brown *et al.* 2006). However, body size variation need not be a consequence of variation in available resources, and organ size variation need not therefore be a consequence of the pattern of the allocation of these resources. Resource allocation models of allometry may accordingly only apply to nutritional static allometries, when resources are limiting. Instead the plasticity induced from other sources of variation may result from adaptation to other agents of selection.

**(b) Different organs respond differently to the same environmental variable**

The results from this study suggest that individual organs respond at least semi-autonomously to the environmental factors that regulate size. Plasticity of wing size is distinct from that of the thorax, and for both, plasticity varies between different environmental size regulators. This is all the more surprising given that both the wing and the dorsal thorax of *Drosophila* are derived from the same imaginal disc, the precursors of adult organs that grow exclusively during the larval stages of insect development. In order to explain the developmental basis of allometry, we must therefore not only elucidate those factors that coordinate growth across the body in response to an environmental or genetic variable, but also the basis of the autonomous responses of organs, and tissue within those organs, to those factors (Shingleton *et al.* 2008).

The factors that coordinate organ growth in response to nutrition include circulating insulin-like peptides and amino acids (Edgar 2006), which influence growth via the insulin- and target of rapamycin (TOR)-signalling pathways, respectively. The nutritional plasticity of individual organs appears to reflect their sensitivity to changes in signalling through these pathways. For example, mutation of the insulin receptor (*Inr*) reduces signalling through the insulin-signalling pathway, and has a greater effect on wing size than on genital size (Shingleton *et al.* 2005). The same is true for mutations that affect signalling through the TOR pathway (A.W. Shingleton 2008, unpublished data). The congruence of the response of individual organs to changes in nutrition with their response to changes in insulin- and TOR-signalling provides important indications of the proximate mechanisms that regulate trait plasticity and allometry in *Drosophila*.

The factors that coordinate growth in response to rearing in density have not yet been explored in *Drosophila*. Inter- and intraspecific competition often inhibits growth in animals and plants. In some cases, for example in lamprey (Rodríguez-Munoz *et al.* 2003) and anuran tadpoles (Petranka 1989), this can occur through pheromones or other chemicals released into the environment. Such a chemical competition potentially regulates body and trait size through a distinct signalling pathway, with the potential of producing unique allometries. Our finding that nutritional allometries did not differ from density allometries, however, suggests that rearing density affects size via nutritional signalling pathways in *Drosophila*, presumably through interference competition. Nevertheless, in *Caenorhabditis elegans*, density is in part sensed by a 'dauer pheromone' released from conspecifics, which in turn regulates the insulin-signalling pathway (Golden & Riddle 1984; Butcher *et al.* 2007). We cannot exclude the possibility that *Drosophila* also use a pheromone to signal density, which similarly regulates the insulin-signalling pathway of developing larvae.

The factors that coordinate organ growth in response to temperature are unknown (but see Davidowitz *et al.* 2004), as are the mechanisms that regulate how individual organs respond to these factors. Owing to the differences between thermal and nutritional static allometries, size variation in response to temperature appears not to be regulated solely through the insulin- and TOR-signalling pathways. Nevertheless, nutritional static allometries do vary with temperature (figure 1), indicating that the mechanisms

that regulate size with respect to temperature interact with those that regulate size with respect to nutrition.

One important question is why allometries are different for different environmental factors. For example, why should a fly that is small because of a high rearing temperature have proportionally smaller wings than a fly that is small because of low nutrition (figure 4)? One possibility is that the allometric relationship between organs is not directly shaped by selection but reflects pleiotropic consequences of selection on other aspects of development or physiology. However, it is difficult to believe that such a functionally important aspect of morphology such as wing loading is not a direct target of selection. A recent study has demonstrated that flies with lower wing loadings (wing area divided by body mass) have improved flight performance at lower temperatures (Frazier *et al.* 2008). Similarly, other selective pressures may account for the difference in nutritional and thermal static allometries in other traits. Further experimentation on the fitness consequences of relative organ size is necessary to address these questions.

**(c) Different genotypes respond differently to the same environmental variable**

The data revealed difference between genotypes in their multivariate allometries and reflect genetic differences in the relative plasticity of particular traits to particular environmental variables. For example, the thorax of genotype 187 was more plastic in response to changes in nutrition and density than the thorax of genotype 157, while the reverse was true for the femur. It is interesting to note that these differences in allometry lie on the third chromosome, since the two genotypes are otherwise genetically identical. The fact that we can observe genetic variation in allometry among only three genotypes suggests that we may be able to alter the multivariate allometry of a wild-type population of *Drosophila* using artificial selection. Subsequent mapping of the genes subjected to artificial selection will enable us to quickly identify the genes that regulate morphological scaling relationships, essential if we are to understand the genetic basis for morphological evolution.

**(d) Genital traits are hypoallometric to body size**

Hypoallometry of male genitalia is a general trend within the insects (Eberhard *et al.* 1998), and *Drosophila* is no exception. However, while myriad studies have examined the static allometry of male genitalia, this is one of only a few that have directly examined the relative condition dependence of genital versus somatic traits. Our data indicate that the posterior lobe of the genital arch is canalized relative to other traits with respect to all the environmental factors we tested, while the anal plate is also canalized, except under conditions of variable nutrition at 17°C. The anal plate is part of the analia rather than the genitalia proper. Nevertheless, both the genital arch and anal plate are derived from the genital imaginal disc and both contribute to the functional male apparatus.

The few other studies that have examined degree of plasticity of genital traits in insects revealed a similar pattern (although see Andrade *et al.* 2005). In the water strider *Aquarius remigis*, external genital morphology tends to be canalized with respect to rearing temperature



(Fairbairn 2005). In the water strider *Gerris incognitos* genital size, while varying with nutritional condition, is also less plastic relative to other traits (Arnqvist & Thornhill 1998). Similarly, the genitals of the dung beetle *Onthophagus taurus* are also largely unresponsive to variation in nutrition (House & Simmons 2003).

While the low plasticity of the genitals is consistent with their general static hypoallometry, the implications this has for the evolution of genital morphology are unclear. There are several competing hypotheses explaining genitalic evolution, some of which require that the genitals be relatively condition independent, and others require them to be condition dependent (Arnqvist *et al.* 1997). Importantly, theoretical studies suggest that it is difficult to infer the selective process that shape allometry from the pattern of the allometry alone (Bonduriansky & Day 2003). However, the finding that the size of the male genitals in *D. melanogaster* has low plasticity, the promise of elucidating the developmental mechanisms that regulate their plasticity, and the myriad molecular and genetic tools available to manipulate these developmental mechanisms, makes the fruit fly a very attractive model for understanding the selective pressure that shapes genital evolution. For example, it should be possible to alter the size, shape and plasticity of the male genitals and directly assay the effects on male mating success.

## 5. CONCLUSION

The data highlight an unexpected and largely overlooked aspect of allometry expression in animals: that the shape of an allometric relationship will depend on the environmental factors that create it. Consequently, intra- and interspecific variations in allometry need not reflect evolved differences between population and species, but rather the set of environmental conditions those populations and species are exposed to. These data therefore present a challenge to researchers of allometry. Allometric studies should make explicit the type of allometry being investigated, be it nutritional or thermal, environmental or genetic.

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