Tipping the scales: Evolution of the allometric slope independent of average trait size

R. Craig Stillwell,[1,2,*] Alexander W. Shingleton,[3,4,*] Ian Dworkin,[4,5] and W. Anthony Frankino[1,6]

[1]*Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204-5001*

[2]*Department of Ecology and Evolution, University of Lausanne, Lausanne 1015, Switzerland*

[3]*Department of Biology, Lake Forest College, Lake Forest, Illinois 60045*

[4]*Department of Integrative Biology, Michigan State University, East Lansing, Michigan 48824*

[5]*Department of Biology, McMaster University, Hamilton, Ontario L9H 6X9, Canada*

[6]*E-mail: frankino@uh.edu*

The scaling of body parts is central to the expression of morphology across body sizes and to the generation of morphological diversity within and among species. Although patterns of scaling-relationship evolution have been well documented for over one hundred years, little is known regarding how selection acts to generate these patterns. In part, this is because it is unclear the extent to which the elements of log–linear scaling relationships—the intercept or mean trait size and the slope—can evolve independently. Here, using the wing–body size scaling relationship in *Drosophila melanogaster* as an empirical model, we use artificial selection to demonstrate that the slope of a morphological scaling relationship between an organ (the wing) and body size can evolve independently of mean organ or body size. We discuss our findings in the context of how selection likely operates on morphological scaling relationships in nature, the developmental basis for evolved changes in scaling, and the general approach of using individual-based selection experiments to study the expression and evolution of morphological scaling.

KEY WORDS: Allometry, constraint, morphological evolution, scaling, selection-artificial.

The scaling of body parts is a quintessential feature of animal morphology (Huxley 1932; Bonduriansky and Day 2003). In general, the size of most morphological traits scales nearly proportionally with the size of the body such that individuals have remarkably similar shapes across the range of body sizes within species. However, some body parts scale disproportionally with the body, producing shape variation across the range of adult size that can be subtle (e.g., genital size in *Drosophila* [Shingleton et al. 2009]) or striking (e.g., fiddler crab claws [Rosenberg 2002], beetle horns [Kawano 1997; Emlen and Nijhout 2000]). The scaling of morphological traits with body size can vary dra-

matically between even closely related species (e.g., among the stalk-eyed flies [Burkhardt and Motte 1985] or horned beetles [Emlen et al. 2005]). Indeed, such intra- and interspecific variation among scaling relationships is perhaps the most important source of morphological diversity (Thompson 1942; Newell 1949; Gould 1966; Frankino et al. 2009). Regardless of the pattern of scaling exhibited by a morphological trait, however, individuals of one sex typically deviate little from that scaling relationship across that range of body sizes displayed by the species. Although these patterns have been well established by over a century of research, it remains unclear how selection acts to produce them (Shingleton and Frankino 2013).

Scaling relationships are traditionally modeled mathematically using the allometric equation (Huxley 1924; Huxley and Tessier 1936; Gayon 2000), $y = bx^a$, where x is the size of one

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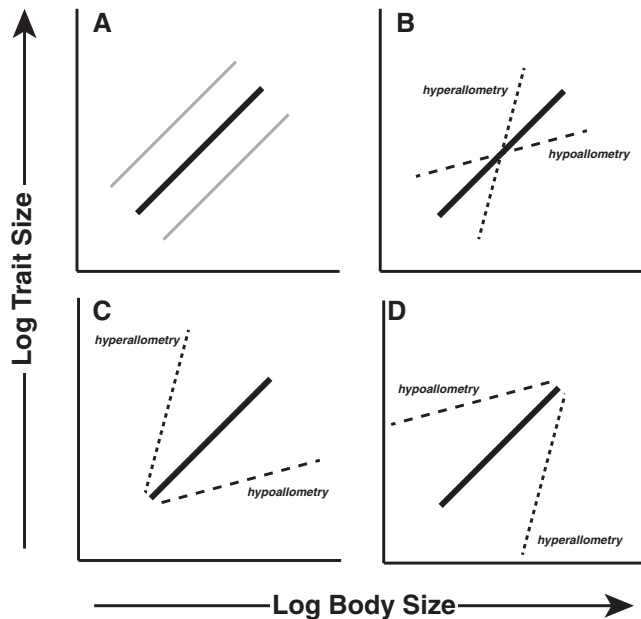
Figure 1. Variation among scaling relationships. Ancestral isometry (bold lines) can change by (A) altering average trait size, body size, or both (gray lines) while the slope remains unchanged. Alternatively, the slope can change to become hypo- (wide dash) or hyperallometric (narrow dash) by rotating about (B) some point internal to the distribution of observations or (C and D) some point outside the range of observed values. Slope evolution that occurs by rotating the relationship within the range of observed values may or may not include a change in mean trait size, whereas slope evolution resulting from rotation outside the range of observed values necessitates a change in mean trait size.

trait (usually body size) and *y* is the size of another (e.g., leg size). When the equation is applied to a population of individuals at the same developmental stage, the relationship is called a static allometry. Typically, the allometric equation is log-transformed to yield the linear $\log(y) = \log(b) + a\log(x)$, with an intercept, $\log(b)$, and a slope, *a*, also called the allometric coefficient. When $a = 1$, a condition called *isometry*, overall shape is maintained across body sizes within the population. In contrast, when $a < 1$ (*hypoallometry*) or $a > 1$ (*hyperallometry*), *y* changes disproportionally to *x*, and thus shape changes with size (Fig. 1). In this article, we consider static allometries where traits scale linearly on a log–log scale.

The parameters of the allometric equation summarize trait and body size covariation and can be used to compare morphologies among populations or other groups. The allometric coefficient (*a*) provides a common metric for comparing slopes among groups as it is scale-independent for traits measured in the same dimension (Kerkhoff et al. 2009; Knell 2009). In contrast, the allometric intercept ($\log(b)$) is scale-dependent and thus not similarly comparable among groups. Moreover, altering the slope

will almost always affect the intercept (Fig. 1B–D), and thus, the meaning of intercept variation among groups can be unclear when slopes also differ. This becomes particularly problematic when determining the extent to which morphological evolution can be attributed to relative changes in slope and intercept. One solution to this problem is to use an alternative, biologically meaningful, parameter to compare relative trait size among groups that differ in slope, such as the bivariate mean (Egset et al. 2012).

Isometry or slight hypoallometry is ostensibly the norm for morphological scaling relationships: within populations, smaller individuals are typically more-or-less the same shape as larger individuals (Voje 2016). The observations that changes in relative trait size underlie much morphological diversification and that most traits scale nearly isometrically with one another indicate that morphological evolution primarily involves changes in the bivariate mean rather than the slope of allometries (Gould 1966; Egset et al. 2012; Voje et al. 2013). There are a number of possible, nonexclusive, causes for these patterns. First, ecological function may favor approximately the same relative trait size across body sizes within populations, whereas different relative trait sizes may be favored among populations. This will select for (near) isometry within populations and produce greater interpopulational variation in the bivariate means than in the slopes. Support for this hypothesis comes from the observation that artificial selection on relative trait size can directly alter the bivariate mean without apparently changing the slope on a natural (e.g., Frankino et al. 2005, 2007) or log scale (Egset et al. 2012), whereas departures from the wild-type bivariate mean are selected against for functionally important traits (e.g., Frankino et al. 2005, 2007).

Alternatively, the developmental mechanisms that regulate trait and body size may act such that the pattern of covariation between them is difficult to change (Frankino et al. 2009; Shingleton and Frankino 2013), constraining evolution of the slope relative to that of the bivariate mean. At first glance, this appears to not be the case. There are several examples of artificial selection apparently altering the slope of a trait's scaling relationship with body size, albeit through selection on absolute or relative trait size rather than on the slope directly (e.g., Robertson 1962; Weber 1990; Wilkinson 1993). Moreover, direct genetic manipulation of the mechanisms controlling trait allometry suggest that, at a molecular–genetic level at least, the slope of morphological scaling relationships can be changed easily. For example, altering the expression of insulin-signaling genes in the developing wing of *Drosophila melanogaster* affects the allometric relationship of the wing to the body, causing *a* to range from 0.6 to 1.15 (Shingleton and Tang 2012). Thus, there would seem to be little developmental constraint limiting slope evolution in the short term. However, in both artificial selection experiments and developmental manipulations, these changes in slope are accompanied by changes in mean trait size (e.g., Fig. 1C, D).

Collectively, these data suggest that selection can change the bivariate mean of a scaling relationship without altering the slope, but that it may be difficult to change the slope without affecting the bivariate mean. Determining the evolutionary independence of mean trait size and parameters of the allometric equation is important as it impacts both our interpretation of the natural patterns in scaling described above, and how the evolution of scaling relationships should be modeled (e.g., Bonduriansky and Day 2003). One way to establish the evolutionary independence of average trait size and the allometric slope is to use artificial selection to isolate and alter the slope in such a manner that mean trait and body size remain unchanged.

Hitherto, only two experiments have attempted this, and they produced variable results. In the first experiment, conducted in guppies (*Poecilia reticulate*), Egset et al. (2012) found no response when they selected to rotate the slope of the caudal fin–body size allometry around the elevation, a measure closely related to the bivariate mean. However, their results should be interpreted with caution for three reasons. First, their selection regime was applied over only three generations, and was accidentally reversed in the second generation in the hypoallometry-selected treatment. Second, the behavior of their unreplicated control lineage complicated analysis of the response to selection. Third, Egset et al. (2012) may have selected primarily on the slope of the genetic rather than the environmental static allometry. This final reason warrants further explanation.

As is true of all phenotypic variation, the causes of size variation that generate scaling relationships can be partitioned into genetic and environmental sources. Consequently, static allometries can be partitioned into genetic static allometries—where size variation is due to allelic variation among individuals reared in the same environment—and environmental static allometries—where size variation reflects phenotypic plasticity among genetically identical individuals reared in different environments (Shingleton et al. 2007). Although this distinction is often overlooked, it is critical because the slope of an environmental static allometry need not be the same as the slope of a genetic static allometry for the same trait pair (Shingleton et al. 2009; Dreyer and Shingleton 2011). This indicates that the developmental mechanisms that generate size covariation among traits may differ for genetic and environmental static allometries, and each may contribute differently to the response to selection. Because Egset et al. did not manipulate diet, size variation in their laboratory-reared population of guppies may have been largely genetic—in which case they would have selected primarily on the slope of the genetic static allometry. It is possible that the slope of environmental static allometries is more responsive to selection than the slope of genetic static allometries. Indeed, the molecular–genetic changes that alter the slope of the wing–body allometry in *Drosophila* do so by changing the environmental (nutritional)

static allometry, where size variation is generated through variation in developmental nutrition (Tang et al. 2011; Shingleton and Tang 2012).

In the second experiment that sought to alter the slope of a morphological scaling relationship independent of average trait size, Bolstad et al. (2015) selected for 26 generations on the wing vein–wing size allometry in *D. melanogaster*. In this elegant experiment, they applied selection to populations fed an unrestricted diet and to other populations where the range of body size was increased by manipulating larval diet, ostensibly targeting the genetic and environmental static allometries, respectively. Intriguingly, they observed a response to selection in the well-fed populations, but not in the populations subjected to diet manipulation. These data suggest that the slope of the genetic static allometry may be more responsive to selection than the slope of the environmental static allometry.

Collectively, therefore, it is unclear how easily selection can independently target the slopes of morphological scaling relationships in populations where trait- and body-size variation is dominated by environmental variation. Given that environmental factors may account for well over 50% of size variation in many natural populations (Visscher et al. 2008), the evolvability of environmental static allometries is an important aspect of the evolution of morphology.

Here, we describe an experiment where we apply artificial selection for 17 generations to alter the allometric slope of the wing–body size scaling relationship in *D. melanogaster* while preserving mean trait size. Specifically, we used correlational selection to rotate the slope of the relationship around the bivariate mean to create populations with evolutionarily derived hypo- or hyperallometric scaling (Fig. 1B). To select primarily for a change in the environmental static allometry, we expand size variation in our experimental populations by rearing individuals from each cohort under two nutritional conditions (Stillwell et al. 2011). We find that selection can rotate the slope of a morphological scaling relationship without changing the bivariate mean, and thus demonstrate genetic variation for the environmental static allometry resulting from nutritionally induced size plasticity. However, the response to selection is perhaps not as strong as expected, suggesting that the slopes of allometric relationships may be somewhat difficult to evolve independently of mean trait size.

Materials and Methods
LINE FITTING AND ALLOMETRY
Allometries are linear on a log–log scale, and so their slope and intercept can be estimated using linear regression. There has been much debate over which type of regression to use in studies of allometric relationships (e.g., Smith 1980; Harvey 1982; Warton

et al. 2006; see discussion in Egset et al. 2012), and much of this debate focuses on the nature of the variation in the focal traits. For example, several authors reject the use of ordinary least-square (OLS) regression because it assumes no measurement error in the predictor (*x*-axis) trait (Rayner 2009) and is asymmetrical with respect to the *X* and *Y* variable (Smith 2009). Others reject the major-axis (MA) and reduced (standardized) major-axis (RMA) methods because they yield unreasonable estimations of the slope when there are biological deviations from the line of allometry (Hansen and Bartoszek 2012). We, however, favor the latter methods because, unlike OLSs, these do not assume a causative relationship between traits. For example, application of OLS to a trait–body allometry with trait size on the *y*-axis and body size on the *x*-axis explicitly assumes that trait size is a consequence of body size. Functionally, this is evident as asymmetry in slope when trait size is regressed on body size versus when body size is regressed on trait size. There are two reasons why a causal relationship between body size and trait size is unlikely. First, there is no clear biological definition of what body size is, and various authors use body length, area, volume, or mass, all of which capture potentially different aspects of an animal's structure and physiology (Chown and Gaston 2010; Stillwell et al., 2010; see discussion in Moczek 2006; Tomkins et al. 2006). Second, which of these aspects, if any, actually regulate trait size at a developmental level is unclear (Mirth and Shingleton 2012). Indeed, it is possible that trait size regulates body size developmentally, that trait and body size interact to determine final size, or both are regulated by other, unmeasured factors. We suggest that, in the absence of evidence of a causal relationship of size determination between traits, the application of MA or RMA regression, neither of which makes assumptions regarding the nature of the relationship between *x* and *y*, is more biologically sound than OLS.

STUDY SPECIES

As with nearly all animals, *Drosophila* spp. exhibit strong plasticity in body and trait size in response to variation in access to nutrition during ontogeny (e.g., Atkinson 1979; Thomas 1993); nutritional limitation produces smaller adults with reduced body and morphological trait size (Mirth and Shingleton 2012). However, the degree of size plasticity among morphological structures relative to the size of the body can differ among traits, producing variation in both the intercepts and slopes among morphological scaling relationships (Shingleton et al. 2009). Moreover, comparisons of isogenic lineages reveal variation among genotypes for slopes and bivariate means for the same morphological scaling relationships (e.g., see figures in Karan et al. 1998; Karan et al. 1999). In sum, these patterns indicate standing variation in the slopes and bivariate means of the wing size–body size scaling relationship in *Drosophila*.

STOCK POPULATION AND REARING PROTOCOLS

Establishment and care of the stock population prior to the start of the experiment is detailed in the Supporting Information. In brief, the experiment was initiated five generations after collection of the stock population from Fenn Valley Winery, Fenn Valley, MI (42.5ºN, 86.1ºW) in the fall of 2010. During each of these generations, we reared tens of thousands of flies, divided into up to 20 subcultures, with variation among subcultures in larval density, larval and adult holding temperature, food quantity and quality, oviposition substrate, and adult age during egg collection. Adults from all subcultures were mixed each generation, to maintain a single, free-mating super-colony. The purpose of this protocol is to prevent adaptation to a single laboratory environment and maintain genetic variation within the stock population. Trait measurement, rearing and selection procedures are described below and visualized in Figure S1.

MEASUREMENT OF TRAIT SIZE

Estimation of wing and body size is described in detail elsewhere (Stillwell et al. 2011). Briefly, individuals were collected as pupae and the area of the pupal case imaged (dorsal view) and measured as a proxy of body size. After imaging, pupae were placed individually in 1.5 or 2 mL Eppendorf tubes that had been punctured with an 18-gauge needle to enable gas exchange and that contained about 0.25–0.50 mL fly food. After eclosion of adults, the wings of live, intact flies were gently flattened between two pieces of glass attached to a "wing grabber" (Weber 1990; Houle et al. 2003) imaged and measured, before the flies were returned to their uniquely numbered vial until selection. Software used to estimate pupal and wing size consists of custom modules that run within ImagePro, and are available upon request.

ARTIFICIAL SELECTION PROCEDURE

Egg collection, larval rearing, and adult maintenance were all performed at 22ºC. To establish the starting population for the experiment, several thousand eggs were collected from the colony over 18 h and again 24 and 48 h later, creating three age cohorts. Eggs were transferred in lots of 50 into 40 mL vials containing 7 mL fly food. When about 20% of the oldest cohort reached the wandering stage, we removed all larvae in all cohorts from the food matrix and placed them by cohort into vials containing only compressed, wet cotton plugs. Thus, all larvae were subject to one of three types of feeding treatments: *late-starved larvae*, which were removed from their food at or just prior to the wandering stage; *mid-starved* larvae, which were removed from their food toward the middle of their final growth instar; and *early-starved larvae*, which were removed from their food about 48 h before wandering (Stillwell et al. 2011). Not all of the early-starved larvae survived the treatment, but those that did were presumably near but above the minimal size for adult eclosion. In combination, our

feeding treatments generated populations of adults possessing a wide range of wing and body sizes, where size variation among groups is due primarily to variation in access to nutrition during ontogeny. Because the mid-starvation treatment produced larvae that were intermediate in size (and thus not selected to rotate the scaling relationship slope), this treatment was dropped after generation three.

In two complementary artificial selection treatments, we sought to increase or decrease the slope of the wing–body allometry, referred to as hyperallometry- and hypoallometry-selection lineages, respectively, without affecting mean wing or body size. We established three independent hyperallometry selection lineages, three hypoallometry selection lineages, and three control lineages from the stock population. Individuals were identified for selection as follows. First, we pooled same-sex individuals from the feeding treatments within each lineage and fit a single scaling relationship to the log-body size (*x*-axis) and log-wing size (*y*-axis) data using RMA regression. We then imposed a saddle-shaped fitness function where individuals with extreme body and wing sizes along hyper- or hypoallometric scaling relationships had higher fitness (as described in Bolstad et al. 2015). To do this, flies in the largest and smallest quartiles for body size were ranked according to the signed perpendicular distance of their wing size from the regression line. For hyperallometry-selected lineages, we selected individuals in the smallest quartile for body size that possessed the greatest negative perpendicular distance (i.e., the largest deviation below the regression line; Fig. S1) and individuals from the largest quartile for body size possessing the greatest positive perpendicular distance (i.e., the largest deviation above the regression line). For the hypoallometry-selected lineages, we selected individuals in the smallest quartile for body size that had the greatest positive perpendicular distance, and individuals from the largest quartile for body size with greatest negative perpendicular distance. The wing–body size allometry of the hyper- and hypoallometry-selected individuals was therefore rotated counter-clockwise or clockwise around the bivariate mean, relative to the wing–body allometry of the whole population.

For nearly all generations, 18 individuals per sex, per body-size quartile (36 individuals/sex total) were selected from each lineage, although in a few generations this number was reduced to 16 individuals per sex per body-size quartile when fly mortality was high. If a selected fly was found dead in its chamber before the crosses were set, we replaced it with the fly with the next most extreme phenotype. Control lineage flies were treated identically to selection lineage flies, however individuals were selected at random and thus without regard to their position along the allometry.

We used a body-size based crossing design among selected individuals within lineages to control for potential effects of body size on reproductive success, in the following manner. Within each lineage, the selected individuals of each sex at each end of the body-size distribution were divided haphazardly into two groups, generating four groups for each sex. These groups were then crossed in a full factorial crossing scheme: large bodied males × large bodied females, large bodied males × small bodied females, small bodied males × large bodied females, small bodied males × small bodied females. We collected an equal number of eggs from each cross and used these to generate the next generation, which typically comprised about 300 individuals of each sex. In this manner, we aimed to minimize the potentially large effects of body size on mate choice, fecundity, or ability to compete for mates (Partridge and Farquhar 1983; Partridge et al. 1987; Pitnick 1991; Lefranc and Bundgaard 2000; Pitnick and Gracia-Gonzalez 2002; Turiegano et al. 2013) from favoring the contribution of genotypes that had achieved the largest body size. Because eggs were collected from each cross type and split equally between feeding treatments, the progeny of all cross types were represented equally at each end of the size distribution of the descendent population subject to the diet treatment and selection (Fig. S1). The experiment was designed in this fashion to maximize the likelihood of selecting on the allometric slope while holding the bivariate mean constant, although the bivariate mean was nevertheless still free to evolve.

Statistical Analysis
ESTIMATION OF THE SELECTION DIFFERENTIAL AND EVOLUTIONARY CHANGES IN SLOPE

We calculated the slope (RMA) of the wing–body allometry (on a log–log scale) for each generation in each lineage by sex using the *smatr* package in *R*. To control for changes in slope due to environmental variation or evolutionary changes unrelated to our selection regime, we normalized the slope of each lineage in each generation by subtracting from it the mean slope of the three control lines in that same generation. We calculated the selection differential as the signed difference between the RMA fit to the all individuals of one sex from a population before selection and an RMA fit to only the selected individuals of that sex. We tested for evolution of the slope by regressing the corrected slopes against their cumulative selection differential for each lineage, calculated as the cumulative sums of the absolute value of the corrected selection differentials for each sex within each replicate population.

We tested for evolution in the allometric slope by fitting the normalized slopes against generation or cumulative selection differential by OLS, with a zero intercept. We did this for each lineage by sex, using the *lm()* function in the *base* package in R, and for all lineages selected in the same direction (hyper- or
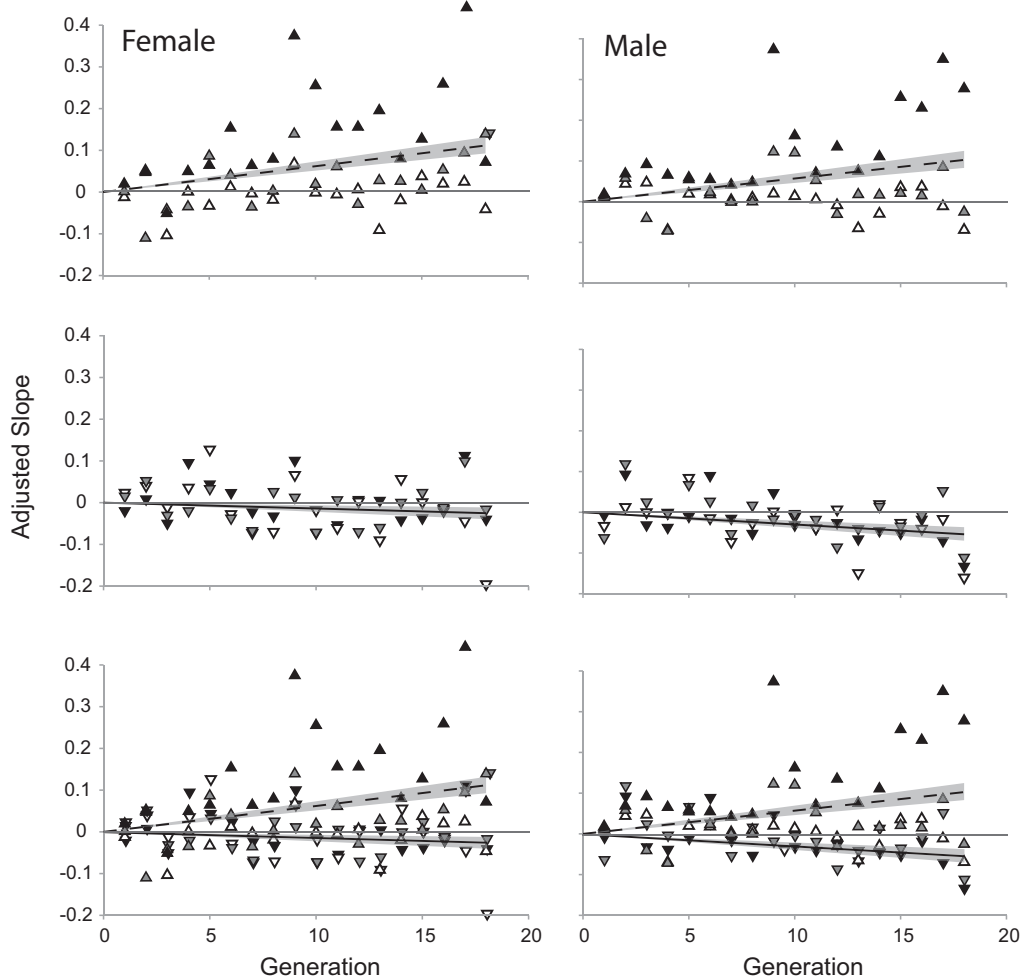
Figure 2. Response of the allometric slope to selection across 17 generations by sex. Upward pointing triangles represent the slopes each generation in populations subject to selection for hyperallometry (top row) and downward pointing triangles show those selected for hypoallometry (middle row); shades identify one of three replicates in each direction. All slopes are shown relative to the average of three control lineage slopes (horizontal gray lines). Trends for the response to selection are shown as regressions bordered by 95% CI (shaded areas), calculated as described in the Methods. Bottom row shows both selected directions combined. All slopes are distinguishable from zero, indicating a response to selection (see Results).

hypoallometry) nesting lineage within selection direction, using the *lmer()* function in the *lme4* package in R. We re-sampled the entire dataset with replacement and repeated each analysis 10,000 times to generate 95% confidence intervals for the slope of the relationship between the normalized allometric slope and generation/cumulative selection differential. If the 95% confidence interval did not contain zero, we concluded that there was a significant change in slope.

TEST FOR EVOLUTIONARILY INDEPENDENT CHANGE IN THE BIVARIATE MEAN

To test whether our selection regime rotated the wing–body allometry around the bivariate mean, we determined the extent to which the bivariate mean changed from generation to generation in each lineage. Any change in the bivariate mean along the axis of the allometry may have been a consequence of unintended selection on body or trait size, or due to stochastic environmental effects on size. Consequently, for each generation we partitioned change in the bivariate mean into two vectors: one that was along the axis of the allometry in the previous generation, and one orthogonal to it (Fig. S2). We normalized each of these vectors by subtracting from it the mean of the corresponding vector from the three control lines. We then tested whether, across generations within a lineage, the loadings of these vectors differed significantly from zero. Any consistent non-zero loadings of the orthogonal vector were interpreted as a change in the bivariate mean away from the ancestral axis of allometry, and hence a change in mean wing size independent of mean body size (Fig. S2).
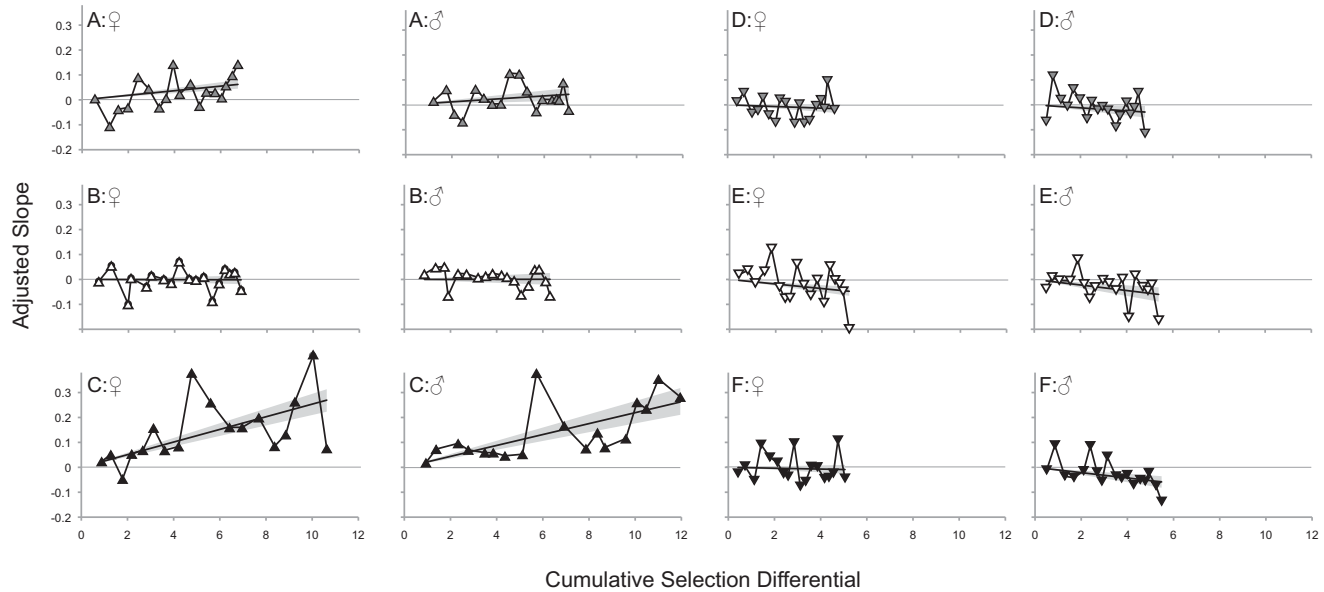
VARIATION PARTITIONING

To determine the fraction of trait variation due to the dietary manipulation, we fitted two linear mixed effect models to the data, with and without feeding treatment as an explanatory variable:

$$Y_{isgk} = u + S_s + L_I + G_{g(i)} + e_{isgk}, \qquad (1.1)$$

$$Y_{isgk} = u + S_s + L_I + G_{g(i)} + E_k + e_{isgk}, \qquad (1.2)$$

where Y is the untransformed trait size for each individual, u is mean trait size, S is sex, E is feeding treatment, L is lineage (random), and G is generation nested within lineage. The increase in R^2 with inclusion of E gives the fraction of variation that is due to the dietary manipulation (Legendre and Legendre 2012). The value of $1 - R^2$ for the complete model (1.2) is the amount of variation that is unexplained by the model, that is, variation within starvation treatments controlling for sex, lineage and generation, and is an estimate of genetic variation. Note that this likely overestimates genetic variation substantially, because there is nutritionally induced size variation within feeding treatments. This is because larvae within feeding treatments were from eggs laid across an 18-h period, and so larvae starved at the same time were not necessarily the same age or developmental stage within the third instar, generating additional variation in final adult size.

All data files and R scripts used for the analyses are available from the Dryad data repository.

Results

In 17 generations, our selection regimes significantly changed the slope of the wing–body scaling relationship in the hyper- and hypoallometry lineages relative to controls in both males and females (Fig. 2; Table 1). This demonstrates that there is segregating genetic variation for the allometric slope mediated by trait-specific nutritional sensitivities. Nevertheless, despite strong selection, the response was erratic and varied substantially within lineages over time, between sexes within a lineage, and among lineages subjected to the same selection regime (Figs. 3, S3; Tables 1, 2).

Our selection regime did not significantly affect the bivariate mean of the wing–body allometry. There were no statistically consistent changes in mean wing or body size in any treatment lineage relative to controls, either along the slope of the allometry or orthogonal to it, in males or females (t-test, $P > 0.2$ for all; Fig. S2). Consequently, evolution of the allometric slope occurred via rotation about the bivariate mean and was not accompanied by change in mean wing size or mean body size.

Variance partitioning revealed that the majority of the variation in wing and body size in our experiment was attributable to the nutritional manipulation rather than genetic variation, when

Table 1. Evolution of allometric slopes in response to selection.

Selection Direction		Line	Females Heritability	Low CI	High CI	Mean Heritability	Low CI	High CI	Males Heritability	Low CI	High CI	Mean Heritability	Low CI	High CI
Hyper-allomet		A	*0.0039	0.0029	0.0052				*0.0026	0.0010	0.0040			
		B	0.0000	−0.0012	0.0010				−0.0001	−0.0014	0.0013			
		C	*0.0148	0.0117	0.0178	*0.0062	0.0051	0.0073	*0.0148	0.0120	0.0175	*0.0057	0.0046	0.0069
Hypo-allomet		D	−0.0008	−0.0018	0.0004				*−0.0018	−0.0030	−0.0006			
		E	*−0.0030	−0.0043	−0.0019				*−0.0036	−0.0050	−0.0021			
		F	−0.0005	−0.0016	0.0006	*−0.0014	−0.0022	−0.0006	*−0.0036	−0.0047	−0.0025	*−0.0030	−0.0039	−0.0020

Units are change in slope per generation relative to control lineages, with 95% confidence intervals (CI). A significant response to selection is indicated when the CI does not include zero and is marked with an asterisk. The mean changes in slope are shown in Figure 2. See Methods for details.

Figure 3. Evolutionary response of the individual lineages as a function of the cumulative selection differential. Slopes for each replicate (triangles) are shown relative to the average of the three control lineages (gray horizontal line) and fit with a regression forced through a *y*-intercept of zero that reveals the response to selection. Upward pointing triangles indicate lineages selected for hyperallometric (steep) slopes, whereas downward pointing triangles indicate lineages selected for hypoallometric (shallow) slopes. Symbol shading matches replicate identity in Figure 2. Regressions are bound by 95% CI (shaded region) and indicate the realized heritability of each lineage to selection.

controlling for sex, lineage, and generation. The proportion of wing and pupal size variation that was due to dietary manipulation was 41% and 54%, respectively. In contrast, the proportion of size variation that could be attributed to genetic variation was, at most, 39% for both traits. This likely substantially overestimates genetic variation for size because variation in the time of oviposition during the egg collection period meant that larvae were unavoidably subject to manipulation at slightly different developmental stages within starvation treatments.

Discussion

Morphological scaling is central to the expression and evolution of form. It ensures that correct body proportion is maintained in the face of genetic and ecological variations that influence trait growth. Moreover, morphological diversification occurs largely through changes in body proportion (Huxley 1932; Bonduriansky and Day 2003) as reflected in the patterns of variation in the intercepts and slopes of scaling relationships. Although a wealth of knowledge exists regarding patterns of scaling relationship evolution, we know little about the proximate or ultimate processes that generate them (Frankino et al. 2009; Shingleton and Frankino 2013). Our study addresses a fundamental topic regarding scaling relationship evolution, namely the degree to which the slope can evolve independently from mean trait size. Our data indicate that the allometric relationship between wing and body size in

D. melanogaster can evolve both hypo- and hyperallometrically by rotating around an unchanging bivariate mean (i.e., Fig. 1B). Although ours is not the first study to attempt to alter the slope of a morphological scaling relationship without changing mean trait size (Egset et al. 2012; Bolstad et al. 2015), ours is the first to observe a response in populations expressing the full, natural range of body size generated largely through environmental variation.

Although we altered the slope of a static allometry through artificial selection, the response may be viewed as relatively, and perhaps surprisingly, weak. In particular, the final slopes were not as extreme as those observed in populations subject to developmental manipulation, where the slope of the wing–body allometry can range from ∼0.6 to 1.2 (75% to 150% of the wild-type slope; Tang et al. 2011; Shingleton and Tang 2012). Nor were our observed changes in slope as substantial as those in other artificial selection experiments where the slope was targeted directly (Bolstad et al. 2015), or where the slope changed as a consequence of selection on mean trait size (data from Wilkinson 1993; analyses not shown). Our response to selection was also erratic, both within and between lineages, as has been the case for other attempts to alter the allometric slope without affecting mean trait size (e.g., Bolstad et al. 2015). This is in contrast to responses in selection experiments that target the intercept of allometric relationships, which appear to respond smoothly and rapidly to selection (e.g., Bolstad et al. 2015). If our observed response to

Table 2. Realized heritabilities of the allometric slope.

Selection Direction	Line	Females Heritability	Low CI	High CI	Mean Heritability	Low CI	High CI	Males Heritability	Low CI	High CI	Mean Heritability	Low CI	High CI
Hyper-allomet	A	*0.0092	0.0061	0.0123				*0.0060	0.0026	0.0096			
	B	−0.0002	−0.0031	0.0030				0.0001	−0.0038	0.0043			
	C	*0.0253	0.0197	0.0307	*0.0146	0.0116	0.0174	*0.0219	0.0177	0.0263	*0.0138	0.0109	0.0166
Hypo-allomet	D	−0.0033	−0.0075	0.0014				*−0.0060	−0.0114	−0.0009			
	E	*−0.0092	−0.0132	−0.0046				*−0.0111	−0.0164	−0.0057			
	F	−0.0018	−0.0060	0.0024	*−0.0049	−0.0080	−0.0019	*−0.0107	−0.0148	−0.0064	*−0.0095	−0.0127	−0.0063

Heritabilities for each lineage were calculated from the regression coefficient of the change in allometric slope (relative to the control) against the cumulative selection intensities, with 95% confidence intervals (CI). A significant response to selection is indicated when the CI does not include zero and is marked with an asterisk. Values correspond to those shown in Figure 3. See Methods for details.

selection is weaker or less consistent than expected, we should seek to determine why this is so.

It is possible that artifacts of our feeding manipulation may have reduced the response to selection. Our feeding treatment imposed abrupt, developmentally timed starvation, which could differ in effect from chronic diet restriction *Drosophila* larvae may experience in nature. As a result, these two forms of nutritional deprivation may generate different morphological scaling relationships. We tested this hypothesis and found no difference in wing:body size scaling in flies that were cut off from nutrition entirely as compared to those experiencing chronic malnourishment (Fig. S4). Consequently, the scaling relationships that we subjected to selection appear to not differ from those generated by variation in access to nutrition in natural populations.

Alternatively, quantitative genetic constraints could have impeded the response to selection; low genetic variation for either trait or high genetic covariation between them would restrain the response to selection (Falconer 1981). However, the evolutionary impact of genetic correlations or covariances is determined ultimately by the proximate developmental mechanisms underlying them (e.g., Rice 1998; Wolf et al. 2001, 2004; Rice 2002, 2004). Thus, taking a developmental perspective on the expression and evolutionary independence of mean trait size and scaling relationship slope could prove useful.

The slope of a nutritional static allometry is controlled by the relative sensitivity of the trait and the body to variation in nutrition during development, that is, their relative nutritional plasticity (Shingleton et al. 2007). In most animals, including *Drosophila*, nutritionally induced plasticity in size is regulated by the response of growing tissues to levels of circulating insulin-like peptides, which are released in a nutrition-dependent manner (Mirth and Shingleton 2012). Traits that are very sensitive to changes in insulin signaling have a growth rate that is highly attuned to variation in nutrition during development, and thus such traits exhibit high nutritional plasticity (Emlen et al. 2012). Conversely, traits that are insensitive to changes in insulin signaling are nutritionally implastic (Shingleton et al. 2009; Dreyer and Shingleton 2011; Tang et al. 2011; Shingleton and Tang 2012). It follows that changing the insulin sensitivity of a trait should affect the slope of its nutritional static allometry with the body. Functionally, insulin sensitivity is changed by altering the expression of key insulin-signaling genes within the trait (Tang et al. 2011; Shingleton and Tang 2012). However, such manipulations also affect mean trait size (e.g., Fig. 1C, D; Tang et al. 2011; Shingleton and Tang 2012). Consequently, it is plausible that the mechanisms that regulate the slope of a nutritional static allometry may be the same as, or intimately tied to, those that regulate mean trait size (Emlen et al. 2012; Shingleton and Tang 2012; Shingleton and Frankino 2013).

Such pleiotropy between slope and mean size may explain why our selection regime resulted in weak and erratic response

in allometric slope. By selecting equal numbers of individuals from the extremes of the size distribution, we attempted to keep mean wing and body size the same while altering their allometric slope. However, if the alleles that influence trait and body size influence the allometric slope pleiotropically, then selection to maintain mean wing and body size may oppose selection to alter the allometric slope, weakening the slope's response to selection. If true, this hypothesis predicts that selection to change the slope of the wing–body scaling relationship without concern for mean trait size (e.g., Fig. 1C, D) will produce a more rapid, consistent, and extreme response than observed in our experiment.

More generally, our results may have been affected by an assumption common to all selection experiments on scaling relationships. To date, every artificial selection experiment aimed at changing relative trait size or the parameters of the allometric equation have used individual-based selection procedures (e.g., Robertson 1962; Weber 1990; Wilkinson 1993; Emlen 1996; Monteiro et al. 1997; Beldade et al. 2002; Frankino et al. 2005, 2007; Egset et al. 2012; Bolstad et al., 2015). Researchers select subpopulations of individuals based on their relative position in morphospace such that, when taken as a group, the selected subpopulation yields the desired scaling relationship (Fig. S1). The underlying assumption in these studies is that an individual's position in morphospace is heritable to some degree, and that over time artificial selection will produce an evolutionarily derived population with novel intercepts or slopes. When the goal is to alter the intercept or bivariate mean (Fig. 1A), researchers implicitly assume that the position of an individual above or below the population allometry, that is, how far off the regression line selected individuals fall, would be the same at other body sizes for that individual. In other words, it is assumed that trait size relative to body size is constant for an individual across all possible body sizes. Moreover, when the goal of the experiment is to alter the slope of the scaling relationship (Fig. 1B–D), it is assumed that crossing selected individuals from the different portions of morphospace (Fig. S1) will produce a progeny population with a novel slope similar to that fit to the selected subpopulation. These assumptions may not be valid; no study has estimated intraspecific variation in morphological static allometries, let alone partitioned the genetic and environmental components of this variation. Consequently, the evolutionary relationship between individual genotypic and phenotypic variation and the population-level parameters of allometric intercepts or slopes is not known. One method to clarify this relationship may be to explicitly build the individual variation underlying the developmental mechanisms that regulate allometric scaling relationships into our understanding of the parameters of the allometric equation as expressed by a population.

Our results undermine the idea of an absolute constraint limiting the independent evolution of the allometric slope and mean

trait size (e.g., Egset et al. 2012). However, the pattern of responses observed here and elsewhere (Egset et al. 2012; Bolstad et al. 2015) suggests that slope evolution by rotation around the bivariate mean (Fig. 1B) may be difficult, particularly when the environment contributes significantly to trait and body size variation, as is common in nature. Determining why this is so requires integrating our understanding of how variation in the mechanisms that integrate trait growth leads to intraspecific variation in scaling relationship parameters, and how this interacts with patterns of selection to affect the evolution of morphological scaling. Elucidating these issues may explain why, in the laboratory and in nature, the most common route to morphological diversification seems to involve changes in absolute trait size, relative trait size, and the slope of the scaling relationship, but rarely the slope alone.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Artificial selection, crossing and rearing scheme.
Figure S2. Two types of change in bivariate mean.
Figure S3. Visualization of the change in slopes over time.
Figure S4. Wing:body scaling in flies subject to acute versus chronic malnourishment.