

# The Genetic Architecture of Morphological Scaling

Alexander Shingleton (✉ [ashingle@uic.edu](mailto:ashingle@uic.edu))

University Of Illinois Chicago <https://orcid.org/0000-0001-9862-9947>

Austin Wilcox

University Of Illinois Chicago

Isabelle Vea

University Of Illinois Chicago

Antony Frankino

University of Houston

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## Article

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# The Genetic Architecture of Morphological Scaling

Austin S. Wilcox<sup>1</sup>, Isabelle M. Veal<sup>1</sup>, W. Anthony Frankino<sup>2</sup>, Alexander W. Shingleton\*<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, University of Illinois Chicago, 840 W Taylor St, Chicago, IL

60607

<sup>2</sup> Department of Biology and Biochemistry, University of Houston, Houston, TX 77204

\* Author for Correspondence. [ashingle@uic.edu](mailto:ashingle@uic.edu)

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13

14 **Abstract**

15 Morphological scaling relationships between the sizes of individual traits and the body captures  
16 the characteristic shape of a species, and the evolution of scaling is the primary mechanism of  
17 morphological diversification. However, we have almost no knowledge of the genetic  
18 architecture of scaling, critical if we are to understand how scaling evolves. Here we explore the  
19 genetic architecture of *population-level* morphological scaling relationships – the scaling  
20 relationship fit to multiple genetically-distinct individuals in a population – by describing the  
21 distribution of *individual* scaling relationships – genotype-specific scaling relationships that are  
22 unseen or cryptic. These individual scaling relationships harbor the genetic variation that  
23 determines relative trait growth within individuals, and theoretical studies suggest that their  
24 distribution dictates how the population scaling relationship will respond to selection. Using  
25 variation in nutrition to generate size variation within 197 isogenic lineages of *Drosophila*  
26 *melanogaster*, we reveal extensive variation in the slopes of the wing-body and leg-body scaling  
27 relationships among individual genotypes. This genetic variation reflects variation in the  
28 nutritionally-induced size plasticity of the wing, leg and body. Surprisingly, we find that  
29 variation in the slope of individual scaling relationships primarily results from variation in  
30 nutritionally-induced plasticity of body size, not leg or wing size. These data allow us to predict  
31 how different selection regimes alter scaling in *Drosophila* and is the first step in identifying the  
32 genetic targets of such selection. More generally, our approach provides a framework for  
33 understanding the genetic architecture of scaling, an important prerequisite to explaining how  
34 selection changes scaling and morphology.

35

## 36 **Introduction**

37 Static morphological scaling relationships (commonly referred to as *static allometries*  
38 (Klingenberg and Zimmermann, 1992) describe the size relationships among morphological  
39 traits as they co-vary with body size among individuals at the same developmental stage in a  
40 population, species, or other biological group (Shingleton, 2010). In as much as the shape of an  
41 animal is determined by the relative size of its constituent body parts, differences in  
42 morphological scaling relationships capture variation in body shape within and among animal  
43 groups. Because morphological diversity is dominated by variation in animal shape, the study of  
44 morphological scaling relationships has been the focus of evolutionary biologists for well over a  
45 century (Huxley, 1924, 1932; Gould, 1966; Thompson and Bonner, 1992; Gayon, 2000).  
46 Nevertheless, until recently, almost nothing was known regarding the developmental-genetic  
47 mechanisms that regulate morphological scaling and that are the proximate targets of selection  
48 for morphological change (Tang *et al.*, 2011; Emlen *et al.*, 2012; Casasa *et al.*, 2017). Even less is  
49 known of the distributions of genetic variation in these mechanisms that should determine how  
50 scaling responds to selection. This is primarily because, unlike most other morphological traits,  
51 scaling is ostensibly a characteristic of a group rather than an individual. Because groups of  
52 animals are, typically, genetically heterogeneous, describing the genetic architecture of  
53 morphological scaling is therefore challenging.

54

55 Historically, the literature has been concerned mostly with *population* scaling relationships  
56 (Huxley and Tessier, 1936; Gould, 1973; Klingenberg and Zimmermann, 1992; Wilkinson, 1993;

57 Dreyer *et al.*, 2016). Here, size variation among individuals results from genetic and  
58 environmental variation, and the line fit to trait-body size data reveals how these covary in a  
59 that population and environment. (For clarity, here we restrict the term 'trait' to morphological  
60 characteristics other than body size). When trait and body size are plotted on a log-log scale,  
61 the slope of their relationship is referred to as the *allometric coefficient* (Huxley and Tessier,  
62 1936) which reflects the relative sensitivity of the trait and body to the myriad environmental  
63 and genetic factors that affect their size (Shingleton *et al.*, 2007).

64  
65 More recently, attention has turned to *individual* scaling relationships (Figure 1; Dreyer et al.  
66 2016). These result from co-variation in trait and body size due to variation in a single  
67 environmental or genetic factor, with all other size regulatory factors held constant (including  
68 genotype). Variation in slopes and intercepts among individual scaling relationships reflect  
69 genetically-based differences among individuals in how trait and body size respond to the  
70 varying size-regulatory factor. When the size-regulatory factor is environmental – yielding an  
71 individual environmental scaling relationship – variation among scaling relationships is a  
72 consequence of genotype-by-environment interactions. Importantly, different environmental  
73 factors will generate different scaling relationships for the same genotype. For example, in  
74 *Drosophila melanogaster*, the effects of nutrition during development on body size generate  
75 different morphological scaling relationships among traits than the effects of temperature  
76 during development (Shingleton *et al.*, 2009). Similarly, when the size-regulatory factor is  
77 genetic – yielding an individual genetic scaling relationship – variation among scaling  
78 relationships is a consequence of genotype-by-genotype interactions. This would be generated

79 by allelic variation at a single locus interacting epistatically with an otherwise constant genetic  
80 background. In reality each individual occupies only a single point on their individual scaling  
81 relationships, reflecting the particular combination of environmental and genetic effects that  
82 determine trait and body size in that individual. Fitting a line to a collection of these points from  
83 genetically heterogenous individuals in a population, each experiencing a unique combination  
84 of environmental factors, generates a population scaling relationship (Figure 1A).

85

86 Thus, underlying population scaling relationships are collections of unseen, or cryptic, individual  
87 scaling relationships. The distribution of individual scaling relationships in a population will  
88 place individuals at particular locations around the population scaling relationship, and  
89 selection on these individuals may alter the distribution of individual scaling relationships,  
90 which in turn may change the slope or intercept of the population-level scaling relationship  
91 (Dreyer *et al.*, 2016; O'Brien *et al.*, 2017; Houle *et al.*, 2019). Mathematical modeling suggests  
92 that the response of the population scaling relationship to selection is dependent on the  
93 distribution of the underlying individual scaling relationships; the same selective pressure  
94 applied to two ostensibly identical population scaling relationships can generate very different  
95 responses if the underlying distribution of individual scaling relationships differs (Dreyer *et al.*,  
96 2016). Consequently, if we are to understand the evolution of population scaling relationships,  
97 we need to understand the genetic architecture of the individual scaling relationships that  
98 underlie them.

99

100 While the concept of individual scaling relationships is straightforward, measuring them is not.  
101 Individual environmental scaling relationships can be generated by fitting a line to the trait-  
102 body size combinations expressed by genetically-identical individuals reared across an  
103 environmental gradient. Individual genetic scaling relationships can be generated by fitting a  
104 line to the trait-body size combinations expressed by individuals possessing allelic variation at  
105 only a single locus in an otherwise co-isogenic background and reared in a single environment.  
106 For many animals, such environmental and genetic control is impractical or impossible to  
107 impose. The measurement of individual scaling relationships is tractable in *Drosophila*,  
108 however, as the long-term maintenance of (near) isogenic populations is routine and genetic  
109 variation can be generated at a single gene or locus (Frankino *et al.*, 2019; Houle *et al.*, 2019).

110  
111 In this paper we focus on the genetic architecture of population scaling relationships by  
112 characterizing the distribution of individual scaling relationships, using isogenic lineages of *D.*  
113 *melanogaster* as a model. The individual scaling relationships for each genotype were  
114 generated by varying access to food during development; because trait and body size results  
115 from variation in developmental nutrition, we refer to these individual scaling relationships as  
116 *nutritional scaling relationships* (Dreyer *et al.*, 2016). We have previously used this simple diet  
117 manipulation to generate variation in wing and body size in *D. melanogaster* (Stillwell *et al.*,  
118 2011; Frankino *et al.*, 2019). Here we apply this approach to 197 isogenic lineages of *D.*  
119 *melanogaster*, to determine the distribution of individual nutritional wing- and leg-body scaling  
120 relationships in this population. Further, we assay the nutritionally-induced size plasticity of  
121 these traits and the body within each lineage. We use these data to explore the genetic

122 architecture of nutritional scaling within and among traits and the variation in relative trait  
123 plasticity that accounts for this architecture.

124

## 125 **Material and Methods**

### 126 *Fly Stocks*

127 All flies used in this study came from The *Drosophila* Genome Resource Panel (DGRP). The  
128 DGRP is a library of ~200 fully sequenced inbred isogenic *Drosophila* lineages that originated  
129 from a single outbred population (Mackay *et al.*, 2012) collected from Raleigh, NC, USA. Flies  
130 were maintained on standard cornmeal molasses medium (Frankino *et al.*, 2019) and  
131 maintained on a 12:12 light cycle at 22°C and 75% humidity.

132

### 133 *Starvation treatment*

134 *Drosophila* egg collection, rearing, and phenotyping followed our established protocols (Stillwell  
135 *et al.*, 2011, 2016; Frankino *et al.*, 2019). For each DGRP lineage, females oviposited for three  
136 days. At 24h, 48h and 72h, eggs were collected, divided into lots of 50 and placed into vials  
137 containing 10ml of standard cornmeal molasses medium. This generated three age cohorts of  
138 flies (D0, D1 and D2, respectively). When third instar larvae from D0 began to pupariate, larvae  
139 from each cohort were removed from the food and placed into empty food vials with a wet  
140 cotton plug to provide moisture. Pupae were removed from these vials and transferred to  
141 individual 1.5ml Eppendorf tubes, each with a small hole in the lid, to complete development to  
142 adulthood. Larvae in the D0 cohort were starved for between 0-24h before pupariation, larvae  
143 in the D1 cohort were starved for between 24-48h before pupariation, and larvae in the D3

144 cohort were starved for between 48-72h before pupariation. Because larvae stop feeding ~24h  
145 before pupariation (Testa *et al.*, 2013), D0 larvae were essentially allowed to feed *ad libitum*  
146 and more-or-less achieved full adult body size. In contrast, D1 and D2 larvae were starved  
147 before larval wandering, reducing adult size depending on their size at initiation of starvation.  
148 Across all three cohorts, our starvation treatment therefore generated nutritionally-induced  
149 variation in body size. Flies were collected in nine temporal blocks, with five lineages repeated  
150 across all blocks to serve as a control.

151

### 152 *Body and Trait Size Measurement*

153 Body and trait size were measured using established protocols (Shingleton *et al.*, 2009; Stillwell  
154 *et al.*, 2011). Briefly, *Drosophila* adults were dissected, and their right wing and right first leg  
155 mounted in dimethyl hydantoin formaldehyde (DMHF). Pupal area (a proxy for body size), wing  
156 area, and femur length (a proxy for leg length) were measured across the full range of body size  
157 for ~50 individuals per sex per lineage; Figure 2). All traits were measured via semi-automated  
158 custom software (Metamorph, Molecular Devices LLC) that captures images from a digital  
159 camera-equipped microscope (Leica DM6000B, Leica Microsystems Inc). Femur length was  
160 squared to put it in the same dimension as wing and pupal area, and all measurements were log  
161 transformed to ensure scale invariance across traits of different sizes.

162

### 163 *Statistical Analysis*

164 All data as well as the *R* scripts used to analyze them are provided on Dryad. We collected data  
165 from >12 flies per sex per lineage, with a mean of 65 flies per sex per lineage. Block effects

166 were removed by fitting the model  $T = K$  to the data, where  $T$  is body/trait size and  $K$  is block.  
167 We then used the residuals of the fit for each trait/body as a measure of trait/body size  
168 independent of block. Theoretical studies indicate that major axis (MA) model II regression best  
169 captures the developmental mechanisms that generate morphological scaling relationships  
170 (Shingleton, 2019), so where possible we used this method to fit the individual scaling  
171 relationships. However, for completeness, and when testing more sophisticated models (e.g.  
172 when lineage was treated as a random factor) we fit the relationship using Model I linear  
173 regression, using maximum likelihood (R package: lme4; Bates et al. 2014), and Bayesian  
174 methods (R package: MCMCglmm; Hadfield 2010).

175

176

## 177 **Results**

### 178 *Population and Individual Morphological Scaling Relationships*

179 Almost all published scaling relationships are population-level scaling relationships, where each  
180 point on a plot of body size against trait size is a genetically distinct individual. To estimate the  
181 population scaling relationship between wing or leg and body (pupal) size in our *Drosophila*  
182 population, we first sampled one individual of each sex from each lineage (genotype), pooled  
183 these observations to create a population, and then calculated the slope and intercept of the  
184 major axis (MA) Model II regression of trait size against body size. We repeated this 10,000  
185 times to generate a 95% confidence intervals for the slope and the intercept for the female and  
186 male wing-body and leg-body population morphological scaling relationships (Table 1, Figure 2).  
187 There were no differences between the sexes in the slope of either of these scaling

188 relationships (Table 1). In contrast, the intercept for the wing-body size population-level scaling  
189 relationship was higher in females than in males, while the intercept for the leg-body  
190 population scaling relationship was higher in males than in females (Table 1). This was  
191 supported by an MA regression of mean trait size against mean body size among lineages  
192 (Figure 2), which also detected no sex differences in the slope of either the wing-body or leg-  
193 body population scaling relationship (wing-body slope:  $P = 0.806$ ; leg-body slope:  $P = 0.315$ ,  $n =$   
194 194), but found a significant sex difference in intercept (wing-body intercept & leg-body  
195 intercept:  $P < 0.0001$  for both,  $n = 194$ ).

196

197 We next explored the individual scaling relationships across the 197 isogenic lineages. We  
198 estimated the slope and intercept of the wing-body and leg-body individual scaling  
199 relationships for each sex in each lineage using MA regression. Across lineages, the wing-body  
200 individual scaling relationships were steeper and had a higher intercept in females than in  
201 males (paired t-test,  $p < 0.0001$  for slope and intercept, Table 1), indicating that females had  
202 larger wings than males relative to body size, and that this difference in relative wing size  
203 increased disproportionately to overall body size within lineages. In contrast, the leg-body scaling  
204 relationship had lower intercept in males than females (paired t-test,  $p < 0.0001$ , Table 1), and  
205 tended to be shallower, although the difference in slope was not significant (paired t-test,  
206  $p = 0.0831$ , Table 1). These distributions were supported when fitting the individual scaling  
207 relationships using a linear-mixed model and treating lineage as a random factor  
208 (Supplementary Tables 2 and 3).

209

210 Within females and males, there was significant variation among genotypes in slope for both  
211 the wing-body and leg-body individual scaling relationships (Figure 2), when the relationships  
212 were fit using either an MA regression (treating lineage as a fixed factor; Supplementary Table  
213 4) or a linear mixed-model regression (treating lineage as a random factor; Supplementary  
214 Table 5). For females, the coefficient of variation (CV) for the wing-body and leg-body MA  
215 slopes was 17.2% and 24.2% respectively, while for males the CV for the wing-body and leg-  
216 body MA slopes was 15.4% and 18.6% respectively. An important caveat is that these estimates  
217 of genetic variation are among isogenic lineages and so may not reflect the additive genetic  
218 variation for slope in an outbred population (Houle *et al.*, 2019). Among lineages, there was a  
219 significant correlation between male and female slopes for both the wing-body scaling  
220 relationship ( $p = 0.30$ , 95% CI: 0.16- 0.42) and the leg-body scaling relationship ( $p = 0.40$ , 95%  
221 CI: 0.28- 0.52). Fitting an MA regression to this correlation revealed that, for both wing-body  
222 and leg-body scaling relationships, as the slope of the individual scaling relationship increased  
223 among lineages, the female slope increased more than the male slope (Supplementary Figure  
224 1).

225

### 226 *Distribution of Cryptic Individual Scaling Relationships*

227 Theoretical studies suggest that the distribution of individual scaling relationships in a  
228 population determines the response to selection on the scaling relationship slope (Dreyer *et al.*,  
229 2016). These distributions can be classified as either broomstick, seesaw, or speedometer;  
230 these names are derived from objects that move in a manner that looks like a plot of individual  
231 scaling relationships under each distribution. Classification is determined by where the

232 morphological scaling relationships, on average, intersect relative to the observed range of trait  
233 sizes (Figure 3). Previously, we used the mean point-of-intersection among all pairs of individual  
234 scaling relationships to classify their distribution (Frankino *et al.*, 2019). However, pairs of near-  
235 parallel individual scaling relationships can generate substantial outliers in the distribution of  
236 points-of-intersection, biasing the mean. To circumvent this problem, here we instead used the  
237 median point of intersection (MPI) to classify the distribution of the individual scaling  
238 relationships. More specifically, we compared where the MPI lies relative to the observed  
239 morphological scaling relationships (Figure 2). For a speedometer distribution, the MPI is closer  
240 to the origin than is the bivariate mean; for a broomstick distribution, the MPI is farther from  
241 the origin than is the bivariate mean; finally, for a seesaw distribution, the MPI lies  
242 approximately at the bivariate mean. For both the individual wing-body and the leg-body size  
243 scaling relationships, the MPI was close to the bivariate mean trait size in males and in females:  
244 that is, the distribution of individual scaling relationships appeared to be a seesaw in both  
245 sexes.

246

247 An artificial selection experiment to increase or decrease relative wing size (wing:thorax ratio)  
248 resulted in a corresponding increase or decrease in the slope of the wing-thorax scaling  
249 relationship, respectively (Robertson, 1962). This finding suggests a positive correlation  
250 between relative wing size and the slope of the wing-body scaling relationship among  
251 genotypes. This would occur if the distribution of individual scaling relationships were  
252 speedometer (Figure 1), which appears to contradict our observation that the distribution of  
253 wing-pupal individual scaling relationships is seesaw. Indeed, we found no correlation between

254 a lineage's relative wing size and the slope of its wing-body scaling relationship, in either males  
255 or females (OLS regression:  $R^2 < 0.006$ ,  $P > 0.31$  for both).

256

257 However, unlike our study, Robertson (1962) did not use diet manipulation to increase the  
258 range of body size among flies, and so likely selected only well-fed individuals. In our study,  
259 these are the largest flies that occupy the upper-right portion of their individual scaling  
260 relationships (black lines, Figure 4A). For a seesaw distribution (Figure 1B), large size-class flies  
261 will also show a positive correlation between their wing-body slope and relative wing size  
262 among lineages. This hypothesis was supported by our data. We found there was a significant  
263 positive relationship among lineages between mean relative wing size for the largest 25% of  
264 individuals in a lineage and the slope of the lineage's wing-pupal scaling relationship, in both  
265 males and females (Figure 4B' & C'). Conversely, for a seesaw distribution, the smallest flies  
266 should show a negative correlation between wing-body slope and relative wing size (grey lines,  
267 Figure 4A), which was also supported by our data (Figure 4B & C).

268

269 We also examined the relationship among lineages between the leg-body slope for a lineage  
270 and relative leg size in the largest and smallest individuals from that lineage. As was the case for  
271 the wing, there was a positive correlation between mean relative leg size for the largest 25% of  
272 individuals in a lineage and the slope of the lineage's leg-pupal scaling relationship, in both  
273 males and females (Figure 4E' & F'). We could not detect, however, any correlation between  
274 relative leg size and slope using data from the smallest 25% of individuals in each lineage

275 (Figure 4E & F). This suggests that the distribution of individual scaling relationships between  
276 the leg and the body is more of a speedometer than seesaw (Figure 1B).

277

### 278 *Morphological Scaling and Size Plasticity*

279 Individual scaling relationships reflect variation in body size and covariation in trait size; that is,  
280 size plasticity caused by a particular environmental or genetic factor. When size variation is due  
281 to an environmental factor, the slope of an Individual scaling relationship captures the  
282 genotype-specific size plasticity of the trait (on the y axis) relative to that of the body (on the x  
283 axis) (Shingleton *et al.*, 2007). When trait size is more plastic relative to body size, the slope of  
284 the scaling relationship is greater than one; when the trait exhibits less size plasticity than the  
285 body, the slope will be less than one. Variation in the slope of individual nutritional scaling  
286 relationships can therefore be due to variation in the plasticity of trait size, variation in the  
287 plasticity of body size, or some combination of both.

288

289 To explore the relationship between trait- and body-size plasticities and the slope of individual  
290 scaling relationships, we used the range in trait and body size between the largest and smallest  
291 10% of individuals within a lineage as a measure of size plasticity. We found significant  
292 correlations between the plasticity of trait pairs (wing v. leg, leg v. body, wing v. body) both  
293 within and between sexes (Table 4). Similarly, we also found significant correlations in the  
294 plasticity of the same trait between sexes (Table 4). We may *a priori* expect trait plasticities to  
295 be correlated due to the systemic effects of nutrition on overall body size (Shingleton *et al.*,  
296 2007). We therefore regressed wing- and leg- size plasticity against body-size plasticity using

297 OLS regression, and used the residual values as a measure of trait-size plasticity that was  
298 independent of body-size plasticity. This analysis revealed significant correlations in trait-size  
299 plasticity among appendages and between sexes, independent of body-size plasticity  
300 (Supplementary Table 6).

301

302 Finally, we investigated the extent to which trait- or body-size plasticity explains among-  
303 genotype variation in the slope of the wing-body and leg-body individual scaling relationships.  
304 To do this, we regressed the slope of the individual scaling relationships against their trait- and  
305 body-size plasticities, by sex across all lineages. The  $R^2$  of these linear regressions capture the  
306 proportion of slope variation that is due to plasticity in trait size relative to plasticity in body  
307 size (Supplementary Table 7). For both sexes, variation in the plasticity of body size explained  
308 more of the variation in the slope of the individual scaling relationships than did variation in the  
309 plasticity of either the wing or the leg (Figure 4). Indeed, there was no significant relationship  
310 between leg plasticity and the slope of the leg-body individual scaling relationship among  
311 lineages for either sex (Figure 4 C & D). Further, variation in body size plasticity explained more  
312 of the variation in the slope of individual scaling relationships in females than in males, for both  
313 wing-body and leg-body scaling. This suggests that variation in body size plasticity is greater in  
314 females than in males. Pairwise comparisons of the wing, leg and body size plasticity variances  
315 between females and males supported this hypothesis: Variance in body size plasticity was  
316 significantly greater among females than among males ( $F_{192,192} = 1.40, P=0.02$ ), which was not  
317 true for variance in wing size plasticity ( $F_{192,192} = 1.13, P=0.37$ ), or leg size plasticity ( $F_{192,192} =$   
318  $1.05, P=0.74$ ).

319

320 **Discussion**

321 Evolution of morphological scaling dominates the generation of morphological diversity among  
322 species, and yet we know little of how selection targets the developmental-genetic mechanisms  
323 that regulate trait and body size to create this diversity. Theoretical studies that distinguish  
324 between population and individual scaling relationships have hypothesized that the distribution  
325 of cryptic individual scaling relationships (seesaw versus speedometer versus broomstick)  
326 determines how the population-level scaling relationship will respond to selection (Dreyer *et*  
327 *al.*, 2016). Our study explores variation among individual scaling relationships derived from  
328 isogenic *D. melanogaster* lineages, and how this variation relates to nutritionally induced size  
329 plasticity of two traits and the body. We find that, across the full range of body sizes generated  
330 by variation in developmental nutrition, the distribution of individual scaling relationships is  
331 approximately a seesaw for both wing-body and leg-body scaling. Further and somewhat  
332 surprisingly, we find that variation in the slope of the individual scaling relationships, which  
333 reflects the relative nutritional plasticities of trait and body size, is primarily a consequence of  
334 variation in the relative plasticity of body size. These data provide important insight into the  
335 genetic basis of variation in morphological scaling, and how this variation may respond to  
336 selection to generate morphological diversity through evolution of the population-level scaling  
337 relationship.

338

339 *Evolvability and the Genetic Architecture of Scaling*

340 While the slopes of morphological scaling relationships can vary dramatically among species,  
341 particularly for exaggerated traits used to attract or compete for mates (Baker and Wilkinson,  
342 2001), this seems to be the exception rather than the rule: For most species and most traits, the  
343 slopes of morphological scaling relationships tend to be evolutionarily invariant (Gould, 1966;  
344 Pelabon *et al.*, 2014). These observations have led to the hypothesis that morphological scaling  
345 relationships are evolutionarily constrained by developmental or physiological mechanisms  
346 (Pelabon *et al.*, 2014). Developmental studies in *Drosophila*, however, reveal that simple  
347 changes in the expression of a single gene are sufficient to substantially alter the slope of trait-  
348 body scaling for traits that otherwise maintains a near constant allometric coefficient across  
349 species, such as the wing or genitalia (Tang *et al.*, 2011; Shingleton and Tang, 2012).  
350 Subsequent studies have changed gene expression to alter the slope of genital-body scaling in  
351 dung beetles (Casasa and Moczek, 2018), mandible-body scaling in male flour beetles (Okada *et*  
352 *al.*, 2019), and horn-body scaling in rhinoceros beetles (Ohde *et al.*, 2018). Consequently, it  
353 does not appear that the evolutionary invariance of morphological scaling relationship slope is  
354 rooted in developmental or physiological constraint, at least mechanistically.

355

356 An alternative explanation for the evolutionary conservation of scaling is that there is little  
357 genetic variation in the developmental mechanisms that regulate morphological scaling  
358 relationships, and upon which selection can act. This would be evident as a lack of genetic  
359 variation in the slopes of individual morphological scaling relationships within a population.  
360 Hitherto, there have been almost no data on the genetic variation of either the slope or

361 intercept of morphological scaling (Frankino *et al.*, 2019). However, our study reveals  
362 considerable variation in the slope of both the wing-body and leg-body individual scaling  
363 relationships, with coefficients of variation comparable to that for overall body size (Lafuente *et al.*, 2018). A similar study on the scaling relationship between wing-vein length and wing size  
364 *al.*, 2018). A similar study on the scaling relationship between wing-vein length and wing size  
365 also revealed considerable genetic variation in the slope of individual scaling relationships  
366 (Houle *et al.*, 2019). Thus, any observed inertia in the evolution of trait-body scaling  
367 relationship slope does not appear to result from lack of genetic variation, at least in  
368 *Drosophila*. An important caveat, however, is that the variation in slope detected in both this  
369 and previous studies (Frankino *et al.* 2019; Houle *et al.* 2019) is among isogenic lineages and  
370 may not reflect the level of additive genetic variation for the slope of individual scaling  
371 relationships in natural populations.

372

373 If the slopes of morphological scaling relationships are not developmentally or physiologically  
374 constrained, and if they possess levels of genetic variation comparable to that for body size,  
375 which responds rapidly to artificial selection (Hillesheim and Stearns, 1991; Partridge and  
376 Fowler, 1993; Turner *et al.*, 2011), we should expect morphological scaling to also respond  
377 rapidly to artificial selection. However, artificial selection on the slope of the wing-body scaling  
378 relationship in *Drosophila* revealed an erratic and weak response, with an apparent heritability  
379 of less than 0.015 (Stillwell *et al.*, 2016). The imposed selection regime attempted to alter the  
380 slope of the population wing-body scaling relationship without changing either mean wing or  
381 mean body size (i.e., to rotate the scaling relationship approximately about the bivariate mean).  
382 To increase the slope, these investigators selected large-bodied individuals with

383 disproportionately large wings and crossing them with small-bodied individuals with  
384 disproportionately small wings. To decrease the slope, they selected large-bodied individuals  
385 with disproportionately small wings and crossing them with small-bodied individuals with  
386 disproportionately large wings. The authors ascribed the low response to pleiotropy between the  
387 slope and mean trait and body size (Stillwell *et al.*, 2016). However, an alternative explanation  
388 is that the selection regime failed to consider the relationship between the observed  
389 population-level scaling relationship and the underlying distribution of individual cryptic scaling  
390 relationships. That is, the individuals selected because of their disproportionately sized wings  
391 may have possessed individual wing-body scaling relationships that would not facilitate the  
392 desired response to selection. This would occur if the slope distribution of the individual scaling  
393 relationship were of the speedometer or broomstick distribution (Dreyer *et al.*, 2016). The  
394 same reasoning may explain why another selection experiment, which attempted to change the  
395 slope of the scaling relationship between wing-vein length and wing size, had a similarly weak  
396 and erratic response when the full range of body size was produced via diet manipulation  
397 (Bolstad *et al.*, 2015). This latter study also ascribed the relative lack of response to pleiotropic  
398 constraints (Houle *et al.*, 2019), rather than a failure of the selection regime to efficiently target  
399 alleles that regulate the slope of the scaling relationship.

400

401 Our data detailing the distribution of individual scaling relationships in a population – albeit  
402 among homozygous genotypes – will facilitate the design of artificial selection regimes that  
403 most efficiently target the slope of individual scaling relationships. The efficacy of such  
404 selection regimes will provide a nuanced method to test of the pace and extent to which the

405 slopes of morphological scaling relationships can evolve. Indeed, earlier artificial selection  
406 experiments to shift the intercept of morphological scaling relationships in *Drosophila*  
407 (Robertson, 1962) and stalk-eyed flies (Wilkinson, 1993) – by selecting to increase relative wing  
408 size and eye-span – rapidly and indirectly altered the slope of the relationship. A third study,  
409 that applied directional selection on body size in the tobacco hornworm *Manduca sexta* also  
410 indirectly altered the slope of the wing-body scaling relationship (Tobler and Nijhout, 2010).  
411 Our data (Figure 3) may reveal why such selection will be effective, at least with respect to the  
412 wing-body scaling relationship in *Drosophila*: Selection to increase relative wing size in large  
413 well-fed flies will indirectly select to increase the slope of the wing-body scaling relationship  
414 (Figure 4 B' and C').

415  
416 Why then, given the apparent extent of genetic variation underlying the slope of population-  
417 level morphological scaling relationships, do their slopes appear to be evolutionarily  
418 constrained? Our data, along with those of Houle et al. (Houle *et al.*, 2019), support the  
419 hypothesis that the evolutionary conservatism of morphological scaling relationship slopes is a  
420 consequence of natural selection, which will favor proportions that enable ecological  
421 performance. This may be particularly true for appendages that are involved in mobility, such as  
422 wings and legs, where changes in loading – that is total body mass divided by appendage  
423 dimensions (Gilchrist and Huey, 2004; David *et al.*, 2011) – may have substantial energetic or  
424 functional consequences. An alternative, non-exclusive, hypothesis is that changes in the slope  
425 of scaling relationships reduce fitness due to pleiotropic effects, for example by altering the  
426 scaling relationship between other traits and body size (Houle *et al.*, 2019). While this may be

427 the case for the scaling relationships among traits in a highly integrated organ, for example the  
428 veins of the wing (Houle *et al.*, 2019), this does not appear to be true for the relationship  
429 among appendages: Developmentally altering the scaling relationship between wing and body  
430 size, for example, does not affect the scaling relationships between body size and other traits  
431 (Tang *et al.*, 2011). Nevertheless, our data indicate a tight genetic correlation in size plasticity  
432 among traits independent of body size plasticity. Because linkage disequilibrium breaks down  
433 over short distances in the population of flies used in our study (Mackay *et al.*, 2012), this  
434 correlation likely arises from pleiotropy, which would need to be broken for natural selection to  
435 change the slope of one trait's morphological scaling relationship with body size independently  
436 of another. Exploring the fitness of flies that have been allometrically engineered to have  
437 atypical scaling relationships, generated using either transgenics or artificial selection, will help  
438 resolve these questions (Wilkinson and Reillo, 1994; Frankino *et al.*, 2005, 2007; Houle *et al.*,  
439 2019).

440

#### 441 *Size Plasticity and the Genetic Architecture of Scaling*

442 The slope of nutritionally-generated individual scaling relationships reflects the relative  
443 nutritional plasticity of trait and body size (Shingleton *et al.*, 2007). Variation among the slopes  
444 of these scaling relationships can result from genetic variation in relative body-size plasticity,  
445 trait-size plasticity, or both. From a developmental perspective, both trait and body size  
446 plasticity is a response to developmental nutrition, mediated through systemic growth-  
447 regulatory mechanisms, canonically the IIS and TOR signaling pathways (Vea and Shingleton,  
448 2020). Autonomous changes in a trait's growth-sensitivity to variation in either IIS or TOR

449 signaling is sufficient to alter the slope of the trait-body scaling relationship (Tang *et al.*, 2011;  
450 Shingleton and Tang, 2012; Luo *et al.*, 2013; Casasa and Moczek, 2018; Okada *et al.*, 2019). If  
451 there were genetic variation in the growth-sensitivity of individual traits to changes in IIS or  
452 TOR signaling, this would generate genetic variation in the slope of the trait-body size  
453 morphological scaling relationship. Further, developmental studies suggest that the distribution  
454 of slopes (seesaw, speedometer, broomstick) would depend on the locus of genetic variation.  
455 For example, changes in the expression of the *Forkhead Transcription Factor (FOXO)*, which  
456 suppresses growth when nutrition is low but is not active when nutrition is high, generate a  
457 broomstick distribution of scaling relationships (Shingleton and Tang, 2012). In contrast,  
458 changes in the expression of the *Insulin Receptor (InR)*, which promotes growth when nutrition  
459 is high but is not active when nutrition is low, generate a speedometer distribution (Shingleton  
460 and Tang, 2012).

461

462 We found that in both males and females, variation in the slope of both the wing-body and leg-  
463 body individual scaling relationships correlated most strongly with genetic variation in body size  
464 plasticity, rather than size plasticity of the individual traits. This suggests that it is variation in  
465 the sensitivity of the body to changes nutrition, independent of the sensitivity of individual  
466 traits, that generates variation in the slope of individual scaling relationships. How this is  
467 achieved seems paradoxical, since the size of the body ostensibly reflects the collective size of  
468 its constituent parts. In *Drosophila*, as with all fully metamorphic insects, the external  
469 appendages, such as wings, legs, genitalia, and mouthparts, develop as imaginal discs within the  
470 larval body. Pupal size, which we used a proxy for overall body size, is determined by the size of

471 the larva when it stops feeding approximately 24 hours before pupariation. The imaginal discs,  
472 however, continue to grow until approximately 24 hours after pupariation (Bryant and Schmidt,  
473 1990). Consequently, the developmental mechanisms that regulate body size are potentially  
474 distinct from those that regulate the size of individual traits (Tang *et al.*, 2011). Thus, genetic  
475 variation in the plasticity of body size and the plasticity of trait size can be distinct from each  
476 other. This explanation is supported by the observation that variation in the size plasticities of  
477 both the wing and the leg are more tightly correlated with each other than with the body as a  
478 whole (Table 6). Even though body size plasticity is at least partially independent from trait size  
479 plasticities, genetic variation in the former will inevitably result in coordinated changes in the  
480 slope of morphological scaling across the traits.

481

482 While we have a extensive knowledge of the developmental mechanisms that regulate  
483 nutritionally-induced size plasticity of both the body and of individual traits (Nijhout *et al.*,  
484 2014), it remains a hypothesis that genetic variation in this plasticity, and by extension in the  
485 slope of individual nutritional scaling relationships, lies within these mechanisms. This  
486 hypothesis appears to be supported by GWAS studies on thermotolerance and thermally  
487 induced body size plasticity, which have identified and functionally validated genes that are  
488 involved in the response to environmental change in general and thermal change in particular  
489 (Gerken *et al.*, 2015; Lafuente *et al.*, 2018; Lecheta *et al.*, 2020). Consequently, we may expect  
490 that genetic variation for nutritionally-induced size variation lies within the developmental  
491 pathways involved in the response to nutritional change. The next step is therefore to identify  
492 the genes that underlie the observed slope variation in nutritional scaling relationships, and to

493 functionally test their role in regulating the response of body and trait size to variation in  
494 developmental nutrition.

495

#### 496 *Conclusion*

497 Our data reveal the distribution of previously cryptic individual scaling relationships for wing  
498 and leg size against body size in *Drosophila*, and explore their relationship with variation in  
499 nutritionally induced plasticity of trait and body size. These data not only provide insight into  
500 the genetic architecture of the wing-body and leg-body population scaling relationships, but  
501 they also allow us to predict how the population scaling relationship will respond to selection  
502 for changes in slope and intercept. Further, future analysis promises to identify the  
503 developmental mechanisms that are responsible for the observed genetic variation in individual  
504 scaling relationships – the mechanisms that may be targeted by selection to alter population  
505 scaling.

506

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511

#### 512 **Author Contributions**

513 AWS and WAF designed the study; ASW and IMV oversaw the collection of the data; ASW, IMV,  
514 WAF and AWS contributed to the data analysis and in preparing the manuscript for publication.

515

## 516 **Competing Interests**

517 None of the authors have any competing financial interests in relation to the work described.

518

## 519 **Data Archiving**

520 All the data and the R scripts used to analyze them are deposited on Dryad.

521

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628

## 629 **Figure Captions**

630 **Figure 1:** Individual and population scaling relationships. **(A)** Individual scaling relationships  
631 (thin grey lines) result from variation in body size due to variation in a single environmental or  
632 genetic factor, with all other size regulatory factors held constant. However, because each  
633 individual has a single genotype and is exposed to a single combination of environmental  
634 factors, it occupies only a single point on its individual scaling relationship (white circles). The  
635 observed population scaling relationship (red line) is the scaling relationship among individuals  
636 in a population. **(B)** The distribution of individual scaling relationships determines how the  
637 population scaling relationship responds to selection (Frankino *et al.*, 2019), and can be  
638 *speedometer*, *broomstick*, or *seesaw*, depending on where the median point of intersection (red  
639 circle) lies relative to the bivariate mean of trait sizes (blue circle).

640

641 **Figure 2:** The population scaling relationships of (A) wing-body and (B) leg-body size in female  
642 (gray points, broken line) and male (black points, solid line) *Drosophila*. Points show the mean  
643 wing/leg/body size of all flies in each lineage. Lines show the mean population scaling  
644 relationship, generated by sampling a single individual from each lineage, fitting the MA  
645 regression and repeating 10,000 times by sex. For both wing-body and leg-body scaling  
646 relationships, there is a significant difference in intercept but not slope between females (gray)  
647 and males (black) (Table 1). The measurements taken are shown in red on the images of the  
648 wing, leg and pupa.

649

650 **Figure 3:** Distribution of individual scaling relationships among isogenic fly lineages. **(A, A')** The  
651 distribution of wing-body individual scaling relationships in females (A) and males (A'). Males  
652 have proportionally smaller wings and shallower slopes than females (Table 1). **(B, B')** The  
653 distribution of leg-body individual scaling relationships in females (B) and males (B'). Males  
654 have proportionally larger legs and steeper slopes than females (Table 1). The steepness of the  
655 slope is indicated by color (green = shallow, blue = steep). The blue circle shows the bivariate  
656 mean of trait-body size. The red circle shows the median point of intersection (MPI) for the  
657 lines. The black line is the mean individual scaling relationship across all lineages. All the scaling  
658 relationships extend two standard deviations above and below the mean body size for each  
659 lineage. Dashed lines indicate bivariate mean (blue) and MPI (red) for body size and trait size.

660

661 **Figure 4:** The relationship between mean relative wing/leg size and the slope of individual  
662 scaling relationships among isogenic fly lineages. **(A)** For a seesaw distribution of individual

663 cryptic scaling relationships (dashed lines) for the largest individuals (black lines) there should  
664 be a positive correlation between their mean relative wing size in each lineage and the slope of  
665 the lineage's individual scaling relationship, whereas this relationship should be negative  
666 among the smallest individuals (gray lines). **(B,C)** For both females (B, B') and males (C, C'),  
667 there was a significant positive relationship between relative wing size for the largest 25% of  
668 individuals in each lineage, and slope of the lineage's individual wing-body scaling relationship  
669 (OLS regression: slope = relative wing size,  $F_{1,192} > 8.98$ ,  $P < 0.003$  for both), and a significant  
670 negative relationship between relative wing size for the smallest 25% of individuals in each  
671 lineage, and slope (OLS regression: slope = relative wing size,  $F_{1,192} > 26.79$ ,  $P < 0.001$  for both).  
672 **(D)** For a speedometer distribution of individual scaling relationships (dashed lines), for the  
673 largest individuals (black lines) there should be a positive correlation between their mean  
674 relative wing size in each lineage and the slope of the lineage's individual scaling relationship,  
675 whereas this relationship should be weaker or absent among the smallest individuals (gray  
676 lines). **(D, E)** For both females (D, D') and males (E, E'), there was a significant positive  
677 relationship between relative leg size for the largest 25% of individuals in each lineage, and  
678 slope of the lineage's individual leg-body scaling relationship (OLS regression: slope = relative  
679 leg size,  $F_{1,192} > 7.17$ ,  $P < 0.008$  for both), but no relationship between relative wing size for the  
680 smallest 25% of individuals in each lineage, and slope (OLS regression: slope = relative leg size,  
681  $F_{1,192} < 0.65$ ,  $P > 0.42$  for both).

682

683 **Figure 5:** The relationship between wing-, leg-, and body-size plasticity in a lineage and the  
684 slope of the lineage's wing-body and leg-body scaling relationship. The  $R^2$  for the relationship

685 between wing-, leg-, and body-size plasticity and the slope of the wing/leg-body size scaling  
686 relationship, among lineages, captures the proportion of variation in slope that is due to  
687 variation in wing/leg- or body-size plasticity. **(A, B)** Variation in the slope of the wing-body  
688 scaling relationship is due to variation in both wing- and body-size plasticity in both females and  
689 males, although variation in body-size plasticity is more important in males. **(C, D)** In both males  
690 and females, variation among lineages in the slopes of the leg-body individual scaling  
691 relationships is due primarily to variation in body-size plasticity. <sup>ns</sup> = non-significant, \*  $P < 0.05$ ,  
692 \*\*\*  $P < 0.0001$ . Grey shading is 95% confidence interval of the slope. All relationships were fit  
693 using OLS linear regression.

694

695

696

697 **Table 1:** The slope and intercept of the wing-body and leg-body population and individual  
 698 scaling relationships in males and females

Level of Scaling	Traits	Sex	Slope <sup>a</sup>	Intercept <sup>a</sup>
Population scaling relationship	Wing-Body	Female	0.991 (0.912–1.074)	0.051 (0.041–0.063)
		Male	0.962 (0.875–1.067)	-0.043 (-0.054–0.033)
	Leg-Body	Female	1.011 (0.923–1.102)	-0.007 (-0.23–0.006)
		Male	1.072 (0.969–1.184)	0.020 (0.007–0.035)
Individual scaling relationship	Wing-Body	Female	1.008 (0.984–1.033)	0.053 (0.044–0.062)
		Male	0.946 (0.925–0.967)	-0.043 (-0.051–0.033)
	Leg-Body	Female	1.034 (0.998–1.069)	-0.005 (-0.015–0.005)
		Male	1.065 (1.037–1.093)	0.020 (0.007–0.035)

699 <sup>a</sup> Estimates of the slope/intercept and their 95% confidence intervals (in parentheses) are based on 10,000  
 700 population scaling relationships, each constructed by randomly sampling one individual of each sex from each  
 701 lineage (genotype) and fitting an MA regression to the sampled data.

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708 **Table 2:** Variances (diagonal), Covariances (below diagonal) and Pearson's Correlations (above  
 709 diagonal) of trait and body size plasticity within and between sexes <sup>a</sup>

710

		male			female		
		wing	leg	pupa	wing	leg	pupa
male	wing	<b>0.0084</b>	0.80	0.64	0.57	0.43	0.50
	leg	0.0081	<b>0.0122</b>	0.64	0.50	0.48	0.46
	pupa	0.0049	0.0059	<b>0.0070</b>	0.47	0.39	0.63
female	wing	0.0051	0.0054	0.0038	<b>0.0095</b>	0.76	0.63
	leg	0.0044	0.0060	0.0037	0.0083	<b>0.0127</b>	0.64
	pupa	0.0045	0.0050	0.0052	0.0061	0.0071	<b>0.0097</b>

711 <sup>a</sup> The darker color the higher the covariance/correlation. All correlations are significant at  $P < 0.0001$ .

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## Figures

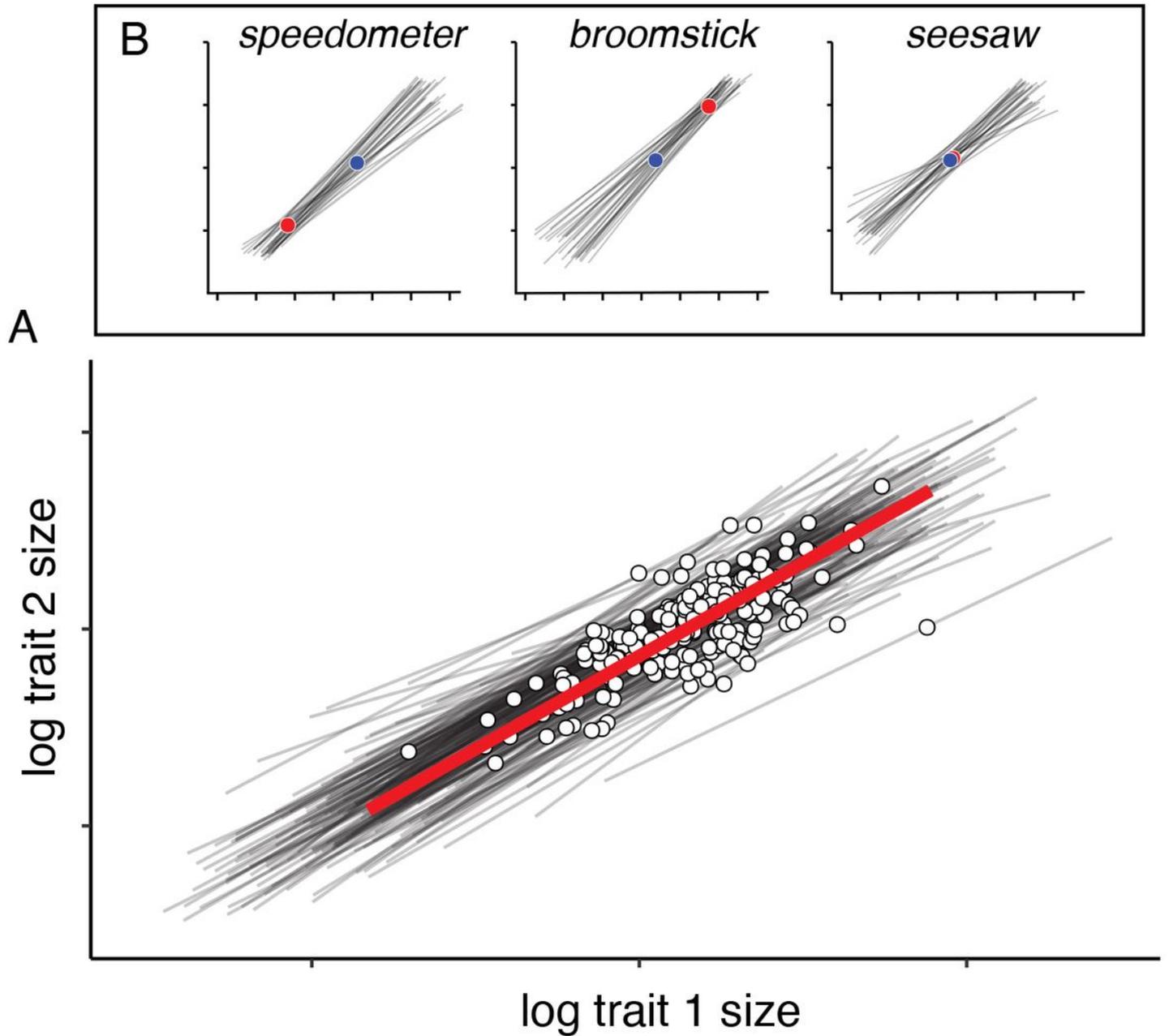
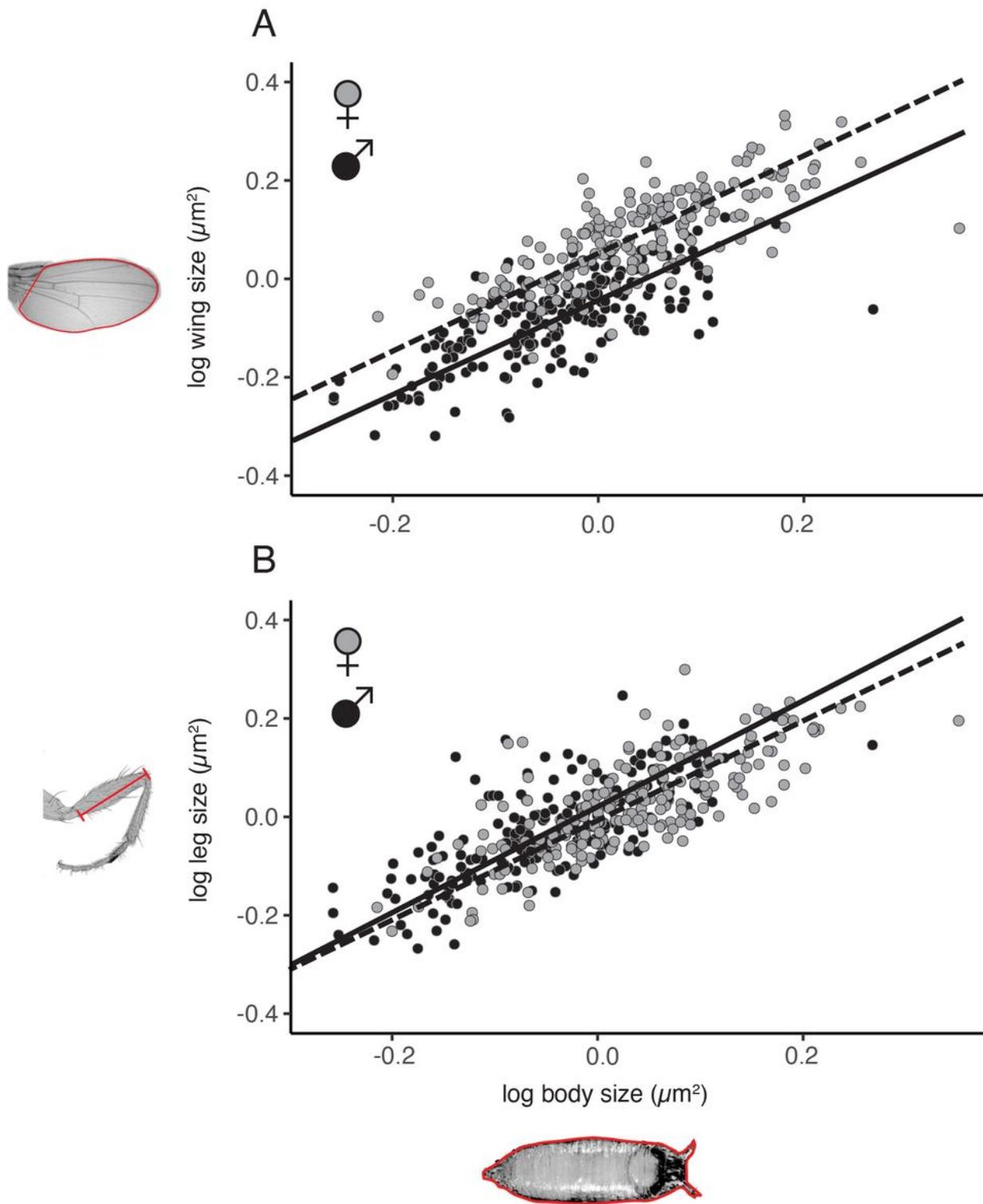


Figure 1

Individual and population scaling relationships. (A) Individual scaling relationships (thin grey lines) result from variation in body size due to variation in a single environmental or genetic factor, with all other size regulatory factors held constant. However, because each individual has a single genotype and is exposed to a single combination of environmental factors, it occupies only a single point on its individual scaling relationship (white circles). The observed population scaling relationship (red line) is the scaling relationship among individuals in a population. (B) The distribution of individual scaling relationships determines how the population scaling relationship responds to selection (Frankino et al., 2019), and can

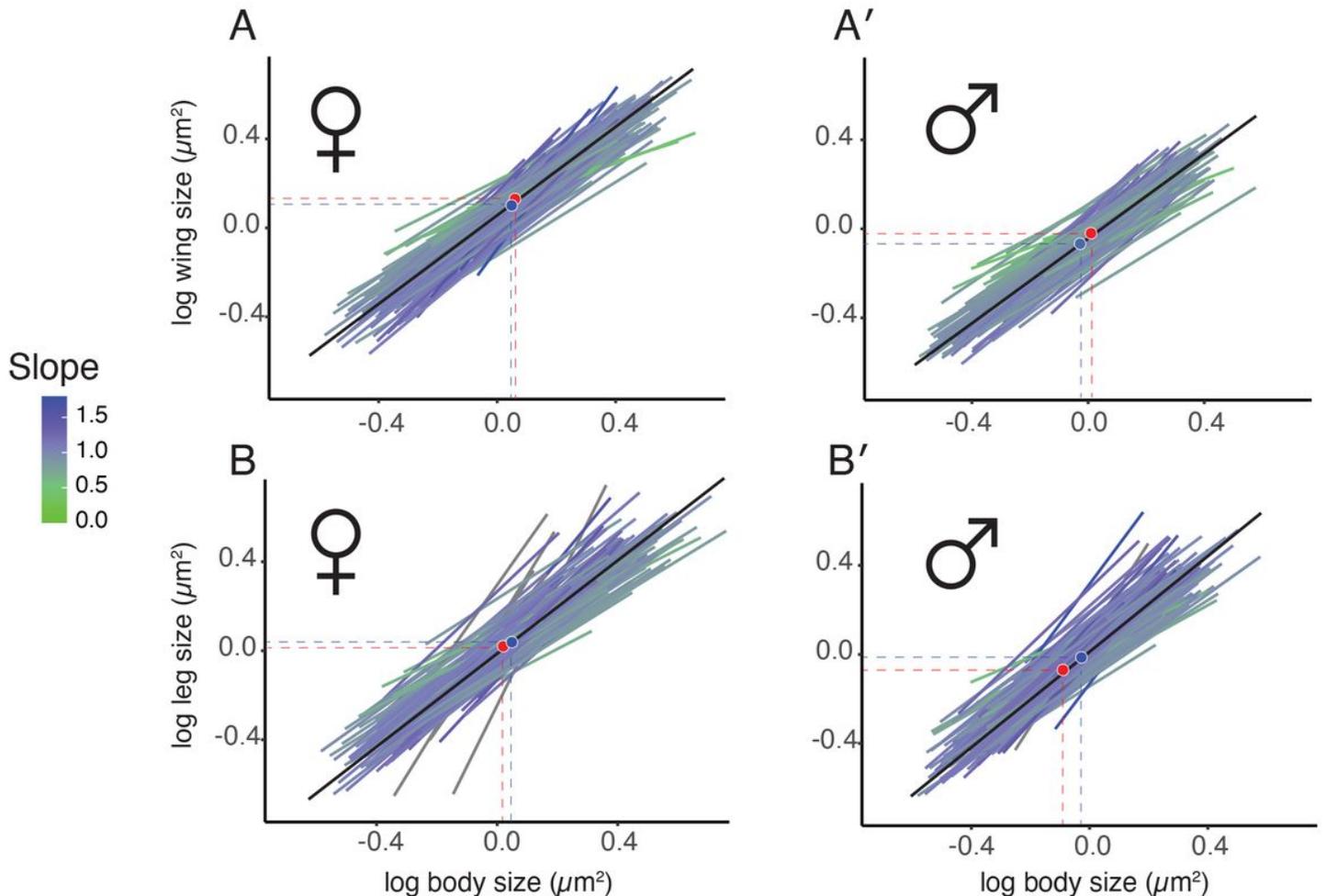
be speedometer, broomstick, or seesaw, depending on where the median point of intersection (red circle) lies relative to the bivariate mean of trait sizes (blue circle).



**Figure 2**

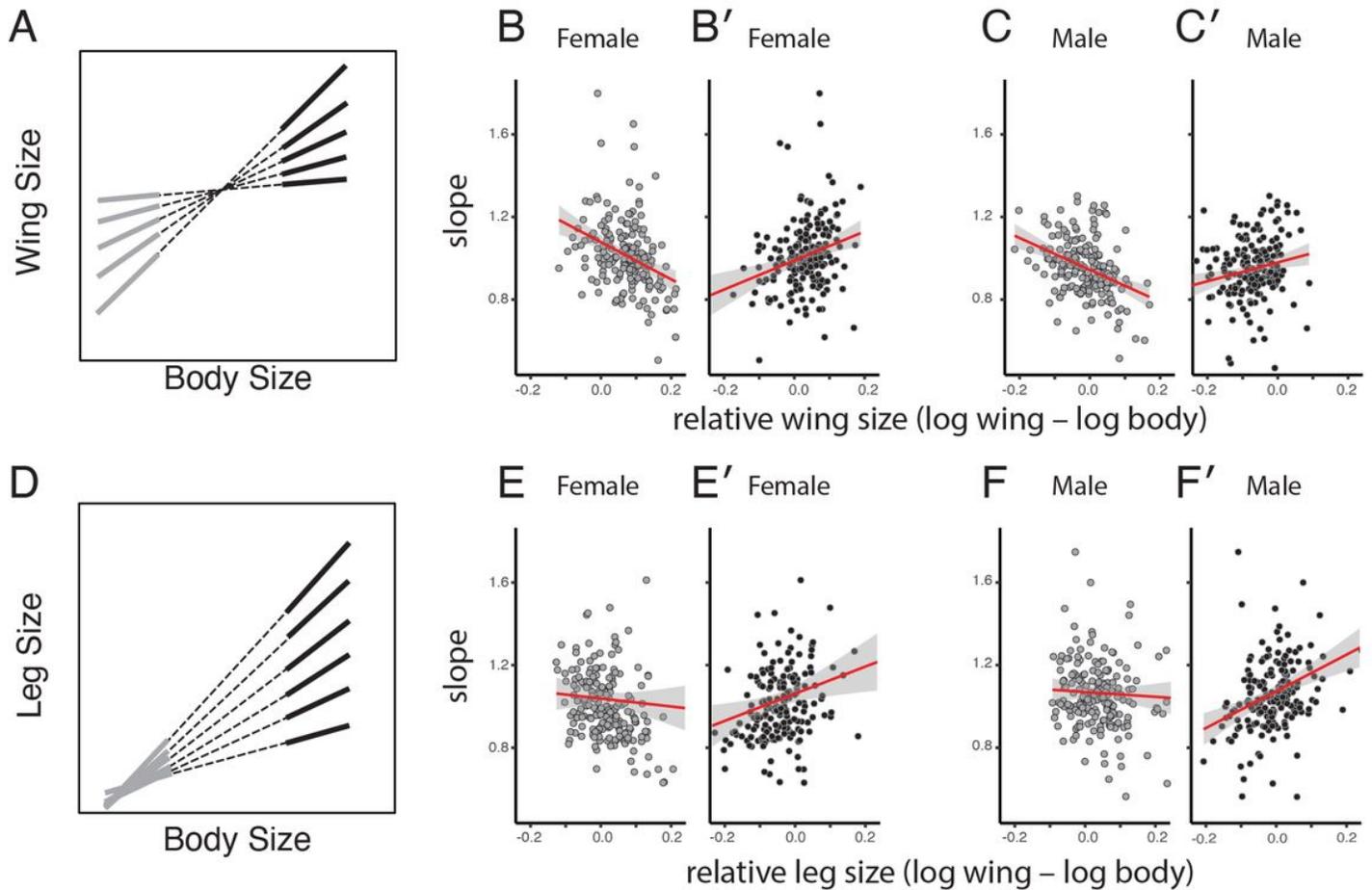
The population scaling relationships of (A) wing-body and (B) leg-641 body size in female (gray points, broken line) and male (black points, solid line) *Drosophila*. Points show the mean wing/leg/body size of

all flies in each lineage. Lines show the mean population scaling relationship, generated by sampling a single individual from each lineage, fitting the MA regression and repeating 10,000 times by sex. For both wing-body and leg-body scaling relationships, there is a significant difference in intercept but not slope between females (gray) and males (black) (Table 1). The measurements taken are shown in red on the images of the wing, leg and pupa.



**Figure 3**

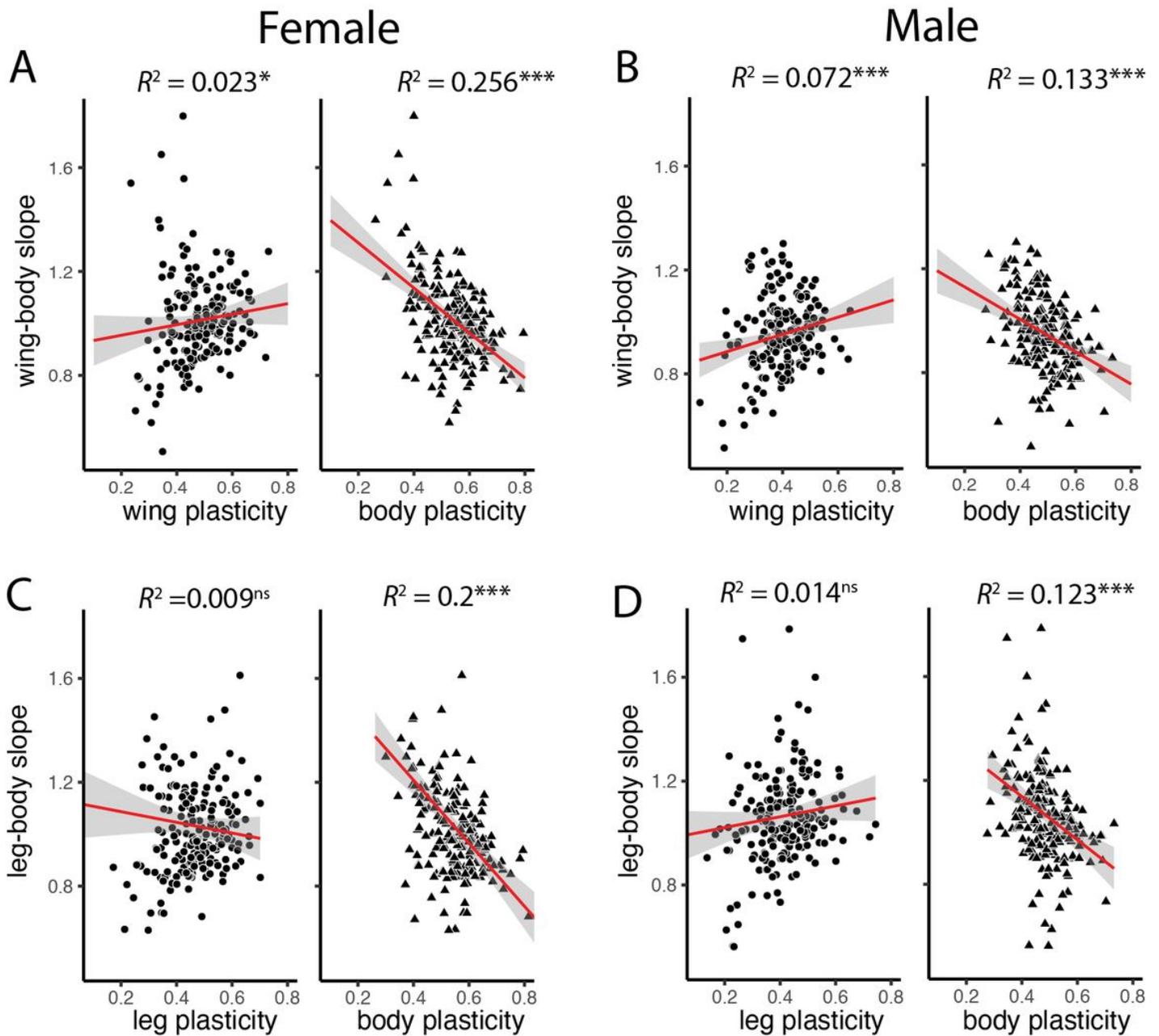
Distribution of individual scaling relationships among isogenic fly lineages. (A, A') The distribution of wing-body individual scaling relationships in females (A) and males (A'). Males have proportionally smaller wings and shallower slopes than females (Table 1). (B, B') The distribution of leg-body individual scaling relationships in females (B) and males (B'). Males have proportionally larger legs and steeper slopes than females (Table 1). The steepness of the slope is indicated by color (green = shallow, blue = steep). The blue circle shows the bivariate mean of trait-body size. The red circle shows the median point of intersection (MPI) for the lines. The black line is the mean individual scaling relationship across all lineages. All the scaling relationships extend two standard deviations above and below the mean body size for each lineage. Dashed lines indicate bivariate mean (blue) and MPI (red) for body size and trait size.



**Figure 4**

The relationship between mean relative wing/leg size and the slope of individual scaling relationships among isogenic fly lineages. (A) For a seesaw distribution of individual cryptic scaling relationships (dashed lines) for the largest individuals (black 663 lines) there should be a positive correlation between their mean relative wing size in each lineage and the slope of the lineage's individual scaling relationship, whereas this relationship should be negative among the smallest individuals (gray lines). (B,C) For both females (B, B') and males (C, C'), there was a significant positive relationship between relative wing size for the largest 25% of individuals in each lineage, and slope of the lineage's individual wing-body scaling relationship (OLS regression: slope = relative wing size,  $F_{1,192} > 8.98$ ,  $P < 0.003$  for both), and a significant negative relationship between relative wing size for the smallest 25% of individuals in each lineage, and slope (OLS regression: slope = relative wing size,  $F_{1,192} > 26.79$ ,  $P < 0.001$  for both). (D) For a speedometer distribution of individual scaling relationships (dashed lines), for the largest individuals (black lines) there should be a positive correlation between their mean relative wing size in each lineage and the slope of the lineage's individual scaling relationship, whereas this relationship should be weaker or absent among the smallest individuals (gray lines). (D, E) For both females (D, D') and males (E, E'), there was a significant positive relationship between relative leg size for the largest 25% of individuals in each lineage, and slope of the lineage's individual leg-body scaling relationship (OLS regression: slope = relative leg size,  $F_{1,192} > 7.17$ ,  $P < 0.008$  for both), but no relationship between relative wing size for the smallest 25% of

individuals in each lineage, and slope (OLS regression: slope = relative leg size,  $F_{1,192} < 0.65$ ,  $P > 0.42$  for both).



**Figure 5**

The relationship between wing-, leg-, and body-size plasticity in a lineage and the slope of the lineage's wing-body and leg-body scaling relationship. The  $R^2$  for the relationship between wing-, leg-, and body-size plasticity and the slope of the wing/leg-body size scaling relationship, among lineages, captures the proportion of variation in slope that is due to variation in wing/leg- or body-size plasticity. (A, B) Variation in the slope of the wing-body scaling relationship is due to variation in both wing- and body-size plasticity in both females and males, although variation in body-size plasticity is more important in males. (C, D) In both males and females, variation among lineages in the slopes of the leg-body

individual scaling relationships is due primarily to variation in body-size plasticity. <sup>ns</sup> = non-significant, \* P < 0.05, \*\*\* P < 0.0001. Grey shading is 95% confidence interval of the slope. All relationships were fit using OLS linear regression.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.pdf](#)