

1 Title: The sex-limited effects of mutations in the EGFR and TGF- β signaling pathways on shape and size
2 sexual dimorphism and allometry in the *Drosophila* wing.

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32 **Abstract:** Much of the morphological diversity in nature—including among sexes within a species—is a
33 direct consequence of variation in size and shape. However, disentangling variation in sexual dimorphism
34 for both shape (SShD), size (SSD) and their relationship with one another remains complex. Understanding
35 how genetic variation influences both size and shape together, and how this in turn influences SSD and
36 SShD is challenging. In this study we utilize *Drosophila* wing size and shape as a model system to
37 investigate how mutations influence size and shape as modulated by sex. Previous work has demonstrated
38 that mutations in Epidermal Growth Factor Receptor (EGFR) and Transforming Growth Factor - β (TGF-
39 β) signaling components can influence both wing size and shape. In this study we re-analyze this data to
40 specifically address how they impact the relationship between size and shape in a sex-specific manner, in
41 turn altering the pattern of sexual dimorphism. While most mutations influence shape overall, only a subset
42 have a genotypic specific effect that influences SShD. Furthermore, while we observe sex-specific patterns
43 of allometric shape variation, the effects of most mutations on allometry tend to be small. We discuss this
44 within the context of using mutational analysis to understand sexual size and shape dimorphism.
45

45 **Introduction:**

46
47 In spite of our wealth of knowledge about the natural world, biologists continue to be fascinated by the
48 prevalence of sexual dimorphism. Where sexual dimorphism is often found, it is most often subtle, despite
49 important exceptions of sex-limited characteristics (Bonduriansky & Day 2003), or traits that are highly
50 exaggerated in one sex, but not the other (Lavine et al. 2015). This is particularly evident for morphological
51 traits that demonstrate sexual size (SSD) or sexual shape (SShD) dimorphism (Kijimoto et al. 2012).
52 Within evolutionary biology, explanations for sexual dimorphism have focused on a number of
53 mechanisms that are likely responsible for the origin and maintenance of sexual dimorphism (Reeve &
54 Fairbairn 2001; Allen et al. 2011; Bonduriansky & Chenoweth 2009; Mank 2009; Cox & Calsbeek 2010;
55 Hedrick & Temeles 1989; Shine 1989; Fairbairn & Blanckenhorn 2007) including sexual conflict,
56 differences among the sexes in the variance of reproductive success leading to sexual selection (Fairbairn
57 2005), and sex specific aspects of natural selection (Preziosi & Fairbairn 2000; Ferguson & Fairbairn
58 2000). Despite this, our understanding of the genetic mechanisms that contribute to variation in sexual
59 shape and size dimorphism is still lacking (Mank 2009; Blanckenhorn et al. 2007; Fairbairn & Roff 2006;
60 Fairbairn 1990).

61
62 There is considerable experimental evidence demonstrating that patterns of SSD and SShD can be altered
63 by influencing the condition of individuals (Bonduriansky & Chenoweth 2009; Bonduriansky 2007). There
64 has unfortunately been less success on directly experimentally evolving consistent changes SSD or SShD,
65 with some notable exceptions where dimorphism evolved in response to selection on fecundity (Reeve &
66 Fairbairn 1999) or due to experimental manipulation in the degree of sexual conflict (Prasad et al. 2007).
67 There are even fewer instances where experimental evolution has been able to alter existing size/shape
68 (allometry) relationships (Bolstad et al. 2015).

69
70 Despite previous difficulties with directly selecting for SSD or SShD, we still find evidence for genetic
71 variation in SSD within a number of species (David et al. 2003; Merila et al. 2011). Several studies have
72 utilized induced mutations (Carreira et al. 2011) or defined genomic deletions to examine patterns of SSD
73 (Takahashi & Blanckenhorn 2015). They find that, in general, mutations tend to attenuate differences in
74 SSD and sexual developmental timing difference. Interestingly, while ~50% of the random insertion
75 mutations influenced size and shape, only half of those were consistent between males and females,
76 suggesting considerable sex limitation of the mutational effects (Carreira et al. 2011).

77
78 With respect to the influence of mutations on sexual dimorphism, one important consideration is whether
79 the mutations themselves are directly influencing aspects of sexual dimorphism. Alternatively, mutations
80 may be influencing size and shape of the organism, but are modulated in a sex-limiting fashion. Arguably,
81 it is difficult to distinguish between these possibilities, although for the purposes of this study, we consider
82 a mutation to be modulated by the influence of sex if it influences size or shape as well as having an
83 additional influence on sex (i.e. a sex-by-genotype interaction). The extent to which such mutations
84 influence SSD and SShD remains poorly understood.

85
86 To address these questions, we examined the influence of characterized induced mutations that influence
87 two signaling pathways important for wing development, Epidermal Growth Factor Receptor (EGFR), and
88 Transforming Growth Factor - β , (TGF- β). The *Drosophila* wing is an excellent model for the study of
89 SSD and SShD. First, as it is a premiere model system for the study of development, and as such a great
90 deal is known and understood about the mechanisms governing overall growth and patterning (García-
91 Bellido et al. 1994; Weinkove et al. 1999; Day & Lawrence 2000; Weatherbee et al. 1998). Additionally,
92 *Drosophila melanogaster* and closely related species have a strong pattern of sexual size dimorphism for
93 many traits (and overall body size), with wing size demonstrating some of the greatest degree of overall
94 dimorphism (Testa et al. 2013; Abbott et al. 2010; Gidaszewski et al. 2009). There is extensive variation
95 for size and shape within and between *Drosophila* species, and for the extent of SSD and SShD as well
96 (Gidaszewski et al. 2009). Importantly, the mutational target size for wing shape (Weber 2005) is high
97 (~15% of the genome), thus providing plenty of opportunity for mutations to influencing shape, and
98 potentially those modulated by sex.

99

100 In this study we utilize a previously published data set that examine the influence of 42 mutations in the
101 EGFR and TGF- β signaling pathways when examined in a heterozygous state. We re-analyze this data set
102 to examine the extent to which the mutations have sex-limited phenotypic effects that influence SSD or
103 SShD. Furthermore we examine how patterns of allometric variation between size and shape are altered by
104 both sex and wild type genetic background of the mutations. Despite most mutations having substantial
105 phenotypic effects on either size, shape or both, only a small subset of them appear to have their effects
106 modulated by sex, with respect to both direction and magnitude of effects. Furthermore, we demonstrate
107 that the allometric relationship between size and shape is only subtly influenced by sex and genetic
108 background for these alleles. We discuss these results within the context of sex-limited effects of mutations
109 and their influence on SSD and SShD, and how to interpret allometric relationships between size and shape
110 in *Drosophila*.
111
112

112 **Materials and Methods:**

113

114 ***Provenance of Samples***

115 The data used for this study was originally published by (Dworkin & Gibson 2006). We compared wings
116 from flies across several treatment groups, including: sex, wild type genetic background (Oregon-R and
117 Samarkand), progenitor line and genotype (mutant vs. wild type allele). Fifty different p-element insertion
118 lines, each marked with w^+ , were introgressed into two common wild type backgrounds (Samarkand and
119 Oregon-R), were used along with their respective controls. All wing data, in the form of landmarks, were
120 collected from digital images, as detailed in Dworkin and Gibson (2006). For a more detailed description
121 on the source of these strains and the experimental design, please refer to Dworkin and Gibson (2006).

122

123 Insertional mutations were selected from the Bloomington Stock Center and subsequently introgressed into
124 two wild-type lab strains, Samarkand (Sam) and Oregon-R (Ore). Introgressions were performed by
125 repeated backcrossing of females bearing the insertion to males of Sam and Ore-R. Females from replicate
126 vials within each generation were pooled for the subsequent generation of backcrossing. Since both
127 backgrounds contain a copy of the mini-white transgene, eye color for all flies lacking p-elements was
128 white. Selection was therefore based entirely on the presence of the eye color marker, precluding unwitting
129 selection for wing phenotypes. While the introgression procedure (14 generations of backcrossing) should
130 make the genome of the mutant largely identical to that of the isogenic wild types, some allelic variation in
131 linkage disequilibrium with the insertional element may remain. All experimental comparisons of mutant
132 individuals were therefore made with wild-type siblings from a given cross and should share any remaining
133 segregating alleles unlinked to the p-element. We separated mutants and their wild-type siblings by their
134 corresponding mutant “line” number (supplementary Table 1) to avoid these and potential “vial effects”.
135 All crosses were performed using standard media, in a 25°C incubator on a 12/12-hr light/dark cycle.

136

137 Two vials for each line were set up carefully to result in low to moderate larval density. The temperature of
138 the incubator was monitored cautiously for fluctuations, and vial position was randomized daily to reduce
139 any edge effects. After eclosion and sclerotization, flies from each cross were then separated into mutant
140 and wild type individuals—those with and without the p-element-induced mutations, respectively—based
141 on eye color and stored in 70% ethanol. A single wing from each fly was dissected and mounted in glycerol
142 (see supplementary table 1B for sample sizes). Images of the wings were captured using a SPOT camera
143 mounted on a Nikon Eclipse microscope. Landmarks (as shown in Figure 1) were digitized using tpsDig (v.
144 1.39, Rohlf 2003) software.

145

146 Our analysis necessitated that there be flies from each representative treatment group; those lines with flies
147 missing (e.g. from one background or sex) were left out of the analysis. Of the original 50, 42 lines were
148 ultimately used.

149

150 ***Analysis of Sexual Size Dimorphism (SSD)***

151

152 Centroid size (i.e., the square root of the sum of the squared distances from each landmark to the centroid
153 of the configuration) was used as the size variable in our analyses. Individual size values for male and
154 female within each line and background were taken from the coefficients of a linear model where centroid
155 size was modeled as a function of genotype, sex and their interaction.

156

157 SSD was then calculated based on a common index, wherein the dimorphism is represented as the
158 proportion of female size to male size (Lovich & Gibbons 1992; Smith 1999):

159

160
$$\frac{size_F}{size_M} - 1$$

161

162 The resulting index represents the relative size difference between males and females where 0 indicates a
163 complete lack of dimorphism and 1 indicates that females are 100% larger than males. Negative values
164 represent male-biased dimorphism.

165

166 *Analysis of Sexual Shape Dimorphism (SShD)*

167

168 Generalized Procrustes Analysis (GPA) was used to super-impose landmark configurations after correcting
169 for position and scaling each configuration by its centroid size. This procedure removes non-shape
170 variation from the data—size, orientation and position. From the nine two dimensional landmarks, we are
171 left with 14 dimensions of variation, and thus applied a Principal Components Analysis (PCA) to the
172 Procrustes coordinates (i.e., the shape coordinates after GPA) and the first 14 PC scores were used as shape
173 variables in subsequent shape analyses.

174

175 Two different shape scores were used in this study: one to examine sexual shape dimorphism and one to
176 assess the strength of the allometric relationship of shape on size. First, SShD was estimated using the
177 tangent approximation for Procrustes distance (i.e. Euclidian distance) between the average of male and
178 female wing shape for a given treatment. Additionally, we calculated shape scores from the multivariate
179 regression of shape onto size based on Drake and Klingenberg (2008). Specifically we projected the
180 observed shape data onto the (unit) vector of regression coefficients from the aforementioned multivariate
181 regression. We used these shape scores and regressed them onto centroid size to approximate allometric
182 relationships. Confidence intervals for SSD and SShD as well as allometric coefficients were generated
183 with random non-parametric bootstraps, using 1000 iterations.

184

185 All significance testing for the analyses involving shape data was done with Randomized Residual
186 Permutation Procedure (RRPP) as implemented in the *geomorph* library in R. (Collyer et al. 2015). This
187 method differs from the analyses in the original paper in two important ways. First, the linear model is
188 based upon Procrustes distances, and second the resampling procedure more easily enables inferences
189 within nested models (Collyer et al. 2015) with interaction terms. Specifically, this approach samples
190 (without replacement), the residuals from the “simple” model under comparison, adding these to fitted
191 values, and refitting under the “complex” model. We used the following models to assess the difference in
192 shape dimorphism for each line and wild type background:

193

194 *Model1: Shape ~ Sex + Genotype*

195 *Model2: Shape ~ Sex + Genotype + Sex:Genotype*

196

197 We then performed such analysis for increasing degrees of interactions for the influence of sex, genotype,
198 genetic background and size (for models of shape variables).

199

200 SShD was calculated with one of two methods: the advanced.procD.lm() function in the *geomorph* package
201 (v.2.1.8) in R (v. 3.2.2) and standard Euclidean distances among treatment groups using the lm() function;
202 both approaches yielded equivalent results. To evaluate the mean shape difference caused by sex, we used
203 linear models based upon Procrustes distance (with RRPP) to compare models where sex is and is not a
204 predictor of shape using the procD.lm and advanced.procD.lm functions in *geomorph*. These analyses were
205 randomized (by individual) and repeated 1000 times per treatment group to assess whether the magnitude
206 of effect was greater than expected by chance.

207

208 Despite having separate and independent “control” (wild type) lineages for each cross (to control for any
209 potential vial effects or residual segregating variation), we utilized a sequential Bonferroni correction to
210 maintain our “experiment-wide” nominal alpha of 0.05. Given the large number of comparisons being
211 made, it is likely that this will yield extremely conservative results, and we expect this underestimates the
212 number of mutations that influence sexual dimorphism or mutational effects of allometry of shape on size.

213

214 *Vector Correlations*

215

216 While the above linear model assesses the magnitude of the effects, for shape it is also important to
217 examine the direction of effects. Specifically, whether the mutations influenced the direction of SShD. To
218 examine this, the vector of SShD was calculated within each genotypic group (wild type VS. mutant). We
219 then estimated the vector correlation between the vectors of SShD for the wild type and mutant as follows:

220

221

222

$$r_{VC} = \frac{|SShD_{wt} \cdot SShD_{mt}|}{\|SShD_{wt}\| \times \|SShD_{mt}\|}$$

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Statistical Analysis

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All statistical analyses were conducted using R statistical software (version 3.2.2). Significance testing (specifically those involving RRPP) was conducted using functions within the *geomorph* package (v. 2.1.8) and with custom functions. All error bars are 95% Confidence intervals generated by non-parametric bootstraps. All scripts including custom functions are available on github (<https://github.com/DworkinLab/TestaDworkin2016DGE>).

238 **Results:**

239

240 ***Different wild type strains vary for Sexual Size Dimorphism (SSD) and Sexual Shape Dimorphism***
241 ***(SShD) in wing morphology, for both direction and magnitude.***

242

243 As each mutation was repeatedly backcrossed into two distinct wild type strains—Oregon-R (Ore) and
244 Samarkand (Sam)—we first examined patterns of sexual size and shape dimorphism between these two
245 strains.

246

247 We observed considerable, and highly significant, differences in both SSD and magnitude of SShD
248 between the wild type strains (Fig.1A). Further, with respect to the vector of SShD, both wild-type
249 backgrounds were somewhat divergent (Fig.1B). The computed vector correlation for SShD between both
250 backgrounds falls within the same range as those calculated for SShD by genotype (0.937, 95% CI 0.92, -
251 0.95), suggesting only subtle changes in direction. Additionally, the allometric relationship between shape
252 and size differs between the two wild type backgrounds. While Ore has a stronger overall slope than Sam,
253 the magnitude of both males and females slopes are reversed by background; for females, shape has
254 stronger association with size relative to males in Ore (F 0.113, 95% CI 0.105, 0.122; M 0.099, 95% CI
255 0.091, 0.107), whereas the opposite is true for Sam (F 0.105, 95% CI 0.097, 0.113; M 0.120, 95% CI
256 0.112, 0.129). These differences in size, shape and allometry are all significant based on the randomized
257 resampling permutation procedure (see methods).

258

259 Despite tight control of experimental variables (food, temperature) we observed a surprising amount of
260 residual environmental variation for SSD and SShD among each replicate of the two wild type lineages. In
261 the design of the experiment, where for each mutation, within each background, wild-type controls were
262 generated from the cross that shared the environment (vials) with their otherwise co-isogenic mutant
263 sibling. As all of these offspring across the vials are genetically co-isogenic, and only differ in the subtle
264 aspects of rearing environment across vials, this allows us to assess some aspects of how environmental
265 variation influences SSD and SShD. As shown in Figure 1A, in addition to differences between the two
266 wild type strains for SSD and SShD, there is also variation around the mean estimates for each. Since each
267 data point in Figure 1A corresponds to each mutant's wild-type siblings from a given cross, these points
268 largely reflect variation among “vial” effects. Indeed, models based on Procrustes distance suggest that
269 there are significant vial effects ($P = 0.009$) and vial by sex ($P = 0.001$) even within the background control
270 populations, which are largely attributable to micro-environmental variation. This is somewhat surprising
271 as external sources of variation such as food (all from a common batch) and rearing temperature (all vials
272 reared in a common incubator, with daily rotation of vials to minimize edge effects) were highly controlled
273 in the experiment. This suggests that the magnitude of SSD and SShD for wing form is influenced by subtle
274 environmental changes, suggesting that high levels of replication to control for these factors is generally
275 necessary.

276

277 ***Despite many mutations having substantial effects on overall shape, a relatively small number influence***
278 ***SSD and SShD.***

279

280 As demonstrated in the original study (Dworkin & Gibson 2006) and confirmed here, the vast majority of
281 mutations have a significant influence on shape when measured in the heterozygous state (supplementary
282 table 1). Of the subset of 42 mutations used in the current study (from the original 50), all but 10 had a
283 significant effect for genotype (most surviving even a conservative Bonferroni correction) using the
284 Residual permutation (Collyer et al. 2015). Of those 10, most had significant genotype-by-background
285 effects, consistent with the earlier study (despite a different underlying inferential approach). Despite this,
286 only 18 of the mutations showed evidence for “significant” sex-limited genotypic effects (based on the sex-
287 by-genotype effects), of which 2 survived sequential Bonferroni correction. Additionally, another 12 show
288 evidence for significant effects of sex-by-genotype in combination with other factors in the model (size
289 and/or background). Only one of these 12 survived correction for multiple comparisons. While inferences
290 based on significance alone is quite limited (see below), these results suggest that only a small subset of
291 mutations appear to have sex-limited influences on shape (Table 1).

292

293 To understand these results more fully, we next focused on the magnitudes of SShD and the SSD index,
294 using non-parametric bootstraps to generate confidence intervals on our estimates. We performed the
295 analyses separately for each wild type genetic background given that they can differ for both magnitude
296 and direction of SShD. As shown in Fig.2, while several mutants show significant effects for either SSD,
297 SShD or both in one or both of the backgrounds, the magnitudes of these effects are small, especially
298 considering the relatively large amount of environmental variation in SSD and SShD observed within
299 strains (Fig.1A). Interestingly, while only a few mutations showed evidence for an overall effect on size,
300 these tend to have sex-limited effects (Fig.2).

301
302 In addition to examining the magnitude of effects, we also examined the direction of effects, and whether
303 the mutations substantially changed the direction of SShD relative to their co-isogenic wild type. As shown
304 in Fig.3, the mutations examined in this study generally do not substantially influence the direction of
305 SShD, with several notable exceptions such as the mutation in the *Omb* gene, as well as more subtle effects
306 from mutations such as *sax*, *pnt*, *drk* (among others). Even when the bootstrap confidence intervals do not
307 approach 1, the estimated vector correlation are still generally greater than ~0.9, suggesting only modest
308 changes in the direction of SShD.

309 ***Mutations do not substantially alter directions of SShD, nor patterns of allometry.***

310
311 One important aspect of assessing variation in shape, and in particular in situations where there is either (or
312 both) SSD or SShD, is to account for the allometric effects of size on shape when computing the magnitude
313 and direction of SShD. One important approach is to assume a common allometric relationship between
314 size and shape across the sexes (after adjusting for mean differences in size and shape), and regressing out
315 the effects of size. Then using either the residuals or predicted values of shape (after accounting for size) to
316 compute an “allometry corrected” measure of SShD (Gidaszewski et al. 2009). To utilize such an approach
317 requires that the assumption of a common allometric relationship be valid, as has been observed across
318 *Drosophila* species for the wing shape and size relationship (Gidaszewski et al. 2009).

319
320 Prior to computing the allometry-corrected measure we examined this assumption among the mutations
321 used in this study. Of the 42 independent mutations (with their independent controls), 13 had a significant
322 interaction of sex-by-size on the influence of shape (with 3 surviving the sequential Bonferroni correction).
323 Another 8 of them had a sex-by-size interaction imbedded within a higher-order interaction term. Despite
324 this the overall magnitudes of effects and directions of allometric relationships appear to be highly similar,
325 with a few important exceptions (Fig.4). Thus it is unclear whether using an allometry-free correction is
326 warranted within the context of this study. It is worth noting that making the assumption of a shared
327 allometric relationship, and computing the allometry-corrected measure of SShD did not substantially alter
328 our findings (Supplementary Figure 1; Supplementary Table 2).
329

330 Discussion

331
332 While previously underappreciated, it is clear that mutations in genes in several growth factor pathways can
333 act in a sex-specific manner. Of the 42 mutations analyzed, 12 had a significant sex-by-genotype
334 interaction on size, shape or both (Fig.2). Only a few mutant alleles had the ability to affect the sexual
335 dimorphism in allometry, the relationship between shape and size (Fig.4). Furthermore, nearly all of the
336 mutants appear to act in a background-dependent manner, affecting shape or size in one genotype, but not
337 the other (Figure 2).

338
339 Previous research has demonstrated the ability of growth pathways to respond to various perturbations,
340 including: individual mutation (Palsson & Gibson 2004; Gao & Pan 2001; Tatar et al. 2001), genetic
341 background (Chandler et al. 2013; Dworkin & Gibson 2006; Paaby & Rockman 2014) and environment
342 (Ghosh et al. 2013; Shingleton et al. 2009; de Moed et al. 1997). Our results are unique in that they allow
343 us to directly assess the effects of these perturbations on relative growth based on sex for both direction and
344 magnitude. Relative differences between male and female growth patterns due to these mutations are
345 ultimately responsible for the generation of SSD and/or SShD.

346 *The importance of multiple independent control lineages*

347
348 As expected, different wild type strains vary in magnitude and direction of effects for SSD and SShD
349 (Figure 1). The Oregon-R wild type background displays greater dimorphism in both size and shape
350 compared to Sam. Implicit in our results is the understanding that genetic background itself has a profound
351 effect on the underlying wild-type growth pathways and all of the downstream consequences this can have.
352

353
354 Somewhat more surprising is that both SSD and SShD appear quite environmentally sensitive (despite the
355 genotypic effects being relatively insensitive based on our previous work). While great care was taken to
356 reduce the effects of microclimactic variation, edge effects, nutritional variation and even genotypic
357 variation, our results demonstrate that size and shape dimorphism remain highly variable (Figure 1).

358
359 There always remains the possibility that environmental variation does not entirely account for the wild-
360 type variation observed. For each backcrossed line, a small amount of genetic information surrounding
361 each p-element insertion site is unavoidable, especially during recombination in final cross with mutants
362 and wild-types. This effect is somewhat unlikely, however, due to the fact that these recombination events
363 are rare and affect only single measured individuals. Regardless, such a large amount of variation in trait
364 values within “isogenic” lines is unexpected. Most studies attribute any such variation within genetically
365 (and environmentally) identical lines to stochastic variation in gene expression (Rea et al. 2005; Kirkwood
366 et al. 2005; Raj & van Oudenaarden 2008). Such claims are, however, outside of the scope of our current
367 study.

368 *Rare sex-limited effects on wing form among mutations in EGFR and TGF- β signaling*

369
370 While much is known about the development of wing size and shape (Shingleton et al. 2005; García-
371 Bellido et al. 1994; Weinkove et al. 1999; Day & Lawrence 2000; Prober & Edgar 2000), comparatively
372 little is known about the sex-specific effects of the genes involved (Horabin 2005; Abbott et al. 2010;
373 Gidaszewski et al. 2009). While these mutations represent only a subset of the almost innumerable potential
374 mutations within and among genes, they serve as a lens through which we can view the sex-limited effects
375 of mutations. It is now clear that only a handful of genes associated with growth may be acting in a sex-
376 dependent manner. Indeed, these results call for a further investigation of the formerly understudied sex-
377 effects of growth pathways.

378
379 One such study confirms a link between many of the patterning mutations used in the current study and the
380 development of SSD in the wing (Horabin 2005). In her 2005 paper, Horabin demonstrated that
381 components of the sex-determination pathway (specifically, *Sxl*) were responsible for activating size-
382 regulating genes within the Hedgehog signaling pathway. In fact, of the handful of genes to display sex-
383 limited effects on SSD or SShD, a few were associated with this pathway, including: *Omb*, *dad* and *Dpp*
384 (Horabin 2005; Abu-Shaar & Mann 1998). This does not appear to be coincidence as these are the only
385

386 mutants in this pathway that we utilized for this study. Since these mutants only represent a subset of those
387 with sex-limiting effects, we cannot assign causality to this pathway. Instead, this demonstrates that sex-
388 limiting effects of genes interact with more complexity than previously understood; no one pathway
389 appears to be acting in a sex-dependent manner to generate shape/size.

390
391 Another candidate pathway involved in the generation of SSD is the Insulin and Insulin-like growth factor
392 (IIS)/Target of Rapamycin (TOR) pathway. Evidence suggests that components of this pathway, such as
393 *InR* (Testa et al. 2013; Shingleton et al. 2005) and *foxo* (Carreira et al. 2011) can contribute to SSD and/or
394 SShD.

395
396 Further studies, such as Takahashi & Blanckenhorn (2015) have found that most mutations appear to
397 decrease the SSD of wing form. Our data appear to yield an interesting trend for the direction of SSD based
398 on genetic background. Ostensibly, growth-pathway mutants in the Ore wild type background tend to
399 decrease SSD, whereas mutants that affect SSD in Sam tend to increase it. At this point it is impossible to
400 say if this trend is biologically meaningful, but given that Ore has a greater underlying magnitude of SSD
401 (and is already in conflict with Rensch's rule), these mutations may be interfering with genetic mechanisms
402 influencing sexual dimorphism in the Ore background.

403
404 Our data is somewhat inconsistent with the findings of another previous mutation screen study, namely
405 those of Carreira et al. (2011), wherein the authors found a much greater proportion of random insertion
406 mutations appeared to have sex-specific effects on wing shape. The reasons for this are as of yet unclear,
407 but may reflect methodology, magnitude of mutational effects used or that in the current study all mutations
408 were limited to two signaling pathways. First, our methods allowed us to effectively tease apart the sex-
409 limited interactions of sex for each genotype pair by plotting them in a size-shape space. Second, the
410 authors used a different wild type genetic background than either that were used in this study (Canton-S). It
411 is clear from this study and others (Dworkin & Gibson 2006; Chandler et al. 2013) that genetic background
412 has an appreciable effect on gene function. At least part of the variation in the number of genes affecting
413 wing SSD must necessarily be due to genetic background effects; however, genetic background effects
414 cannot wholly account for the differences observed. Third, we cannot rule out the effects of dominance
415 when discussing the effects of gene function. The genotype of flies in the study by Carreira et al. (2011)
416 was homozygous for all mutants used. Their lines were chosen specifically for their non-lethal homozygous
417 phenotype, whereas mutation used in our study were chosen irrespective of lethality. Because of this, our
418 flies necessarily had to be heterozygous in order to avoid lethality associated with the homozygous
419 phenotype. Perhaps not all loss-of-function mutants within our study were sufficient to alter the phenotype
420 in a sex-limited manner. Finally, because our mutants were deliberately selected based on their association
421 with wing shape morphogenesis, our results are not strictly comparable to those of Carreira et al. (2011).

422 423 ***Disentangling mutant-phenotype relationships*** 424

425 Our findings suggest that in most cases when a mutational analysis is performed to understand the genetic
426 architecture of SSD or SShD, it is important to assess whether the mutation is only affecting SSD/SShD or
427 whether it is instead demonstrating some degree of sex-biased influence. Many genes may therefore appear
428 to alter SSD/SShD, but are instead only affected by sex as one of several variables of its expression. This
429 may seem like an arbitrary distinction, but it is important if we are to fully understand the genetic
430 underpinnings of complex phenotypes. Many mutants, such as those in the EGFR signaling pathway used
431 here, are either lethal or at least partially ablate development of certain organs as homozygotes, indicating
432 that these genes are necessary for the development of the organ itself. If heterozygotes have sex-specific
433 effects on size or shape, we cannot necessarily conclude that this gene affects SSD or SShD, but rather that
434 the gene is important for formation of an organ and has sex-dependent effects. Only in the case of genes
435 such as *Mafl*, a gene that has been demonstrated to directly effect SSD in *Drosophila* (Rideout et al. 2012),
436 can we conclude that said gene is affecting SSD and not simply acting in a sex-limited manner.

437
438 To fully understand the scope of SSD and SShD, one must precisely define what is meant by size and
439 shape. While the definition of size is relatively straightforward to interpret, shape is somewhat more
440 nuanced. For many organs, shape can essentially be broken down into the relative size of component parts
441 of the larger structure (given that all aspects are homologous). For instance, during development in

442 *Drosophila* there are multiple quadrants of the developing wing imaginal disc whose individual sections
443 may grow more or less in relation to the others, thus altering the “shape” of the wing. Mutant phenotypes
444 may manifest as changes to large sections, such as a widening of the entire central portion of the wing (*Ptc*)
445 or they may be subtler in effect, altering the placement of only a single crossvein (*cv-2*) (Dworkin &
446 Gibson 2006). While these mutants may have local effects on size, such that they alter shape, what is less
447 clear is whether these mutants are affecting size in a localized manner or the actual shape itself.

448
449 The effects of each pathway appear relatively consistent despite differences in genetic background. While
450 mutations within the *Egfr* pathway tended to affect primarily SShD, those in the TGF- β pathway had a
451 more mixed effect (more frequently affecting SSD). This pattern suggests that genetic background may
452 only alter a mutation’s quantitative effect, rather than its qualitative effect.

453
454 Ultimately, our results demonstrate the importance of distinguishing between the relative contributions of
455 each mutation to sexual dimorphism for shape, size or both. Of those mutants with sex-limited effects, even
456 fewer exclusively affect either shape or size dimorphism (Fig.2). While some studies have been successful
457 in artificially altering SSD of specific traits through selection (Bird & Schaffer 1972; Emlen et al. 2005;
458 Reeve & Fairbairn 1996), it is unclear whether whole trait size or simply trait shape (e.g. length) has been
459 altered. Our results demonstrate the need to exercise caution when discussing the effect of mutants on size
460 or shape dimorphism.

461 462 *Reassessing the assumption of common allometry*

463
464 One important method for quantifying “shape” changes involves examining allometric relationships,
465 specifically static allometry, which is the relationship among adult individuals between body size and organ
466 size (Huxley 1932; Stern & Emlen 1999). In fact, one of the most obvious way that males and females can
467 differ is through differences in scaling relationships between body parts; these encompass some of the most
468 obvious sources of variation in the natural world (Bonduriansky & Day 2003; Shingleton et al. 2009). By
469 studying the relationship between two traits (e.g. body vs organ size), we can glean important information
470 about the relative growth of traits and, therefore, the underlying mechanisms of differences in the growth of
471 these traits. Consequently, allometry is an important tool for biologists to assess differences in size and
472 shape dimorphism within (and across) a species. Our results support the claim for the importance of
473 studying allometry by demonstrating that, while some mutants may have sex-limited effects on shape
474 and/or size dimorphism (Fig.2), they do not necessarily effect the relationship between trait shape and size
475 (Fig.4). Many mutants cause significant differences in sexual dimorphism of allometry, but do not
476 necessarily alter SSD or SShD. These results may seem counterintuitive, but it is important to remember
477 that, while changes in SSD or SShD may shift the direction of slope of allometry along one or more
478 dimensions (in shape space), this does not necessarily alter the allometric slope itself (Frankino et al. 2005).

479
480 Since D’Arcy Thompson (1917) outlined his approach of how relative changes in body and organ size can
481 be mapped out onto Cartesian coordinates to visualize relative growth, the study of allometry and shape
482 have been closely linked. Modern approaches use similar, albeit much more complicated methods to assess
483 changes in relative landmark positions (Sanger et al. 2013; van der Linde & Houle 2009; Abbott et al.
484 2010). Ostensibly, one of the downfalls of shape analysis is that shape inherently carries information about
485 its underlying relationship to size, despite the fact that geometric morphometric analyses partially separates
486 it from shape (Gidaszewski et al. 2009; Mosimann 1970; Gould 1966; Nevill et al. 1995). More
487 specifically, size itself is a measurement based on some aspect of shape. If size and shape do not scale
488 isometrically (such that unit increase in size is accompanied by an equal increase in shape), then the
489 underlying co-variation will be reflected in estimates of shape that are disproportionately affected by size
490 (Mosimann 1970). This issue is implicit in the geometry of shapes themselves; as absolute size increases,
491 surface area to volume ratios decrease (Gould 1966). This is particularly bad news for studies wishing to
492 analyze induced changes in shape and size, because it means that the degree of independence between these
493 two variables may be difficult to infer. However, by plotting size on shape and using the residuals from this
494 model, Gidaszewski et al. (2009) were able to effectively eliminate the issue of non-independence with size
495 and shape. These residuals represent the total variation in shape that is not due to allometric effects of size.

496

497 Allometric patterns of variation across sex and genotype are necessarily more complicated. While it is
498 known that shape (and shape dimorphism) is strongly influenced by its relationship with size, it is not
499 always clear that the assumption of a common allometric relationship across sexes is met. Previous studies
500 examining patterns of SSD and SShD (Gidaszewski et al. 2009) generally made the assumption of a
501 common allometric relationship between males and females within each *Drosophila* species. This was
502 despite their analysis suggesting that this assumption may not hold for all species. For the data we
503 examined here, we could reject this assumption based on inferences based on statistical significance. Yet, it
504 is clear that the magnitude of such differences were small, and allometric relationships were similar in most
505 cases. Indeed, the allometric influence of size on shape appears to be largely consistent with respect to
506 direction of effects, with a few notable exceptions (Fig.4). Regardless, we erred on the side of caution with
507 this matter and decided to eschew analysis of SSD and SShD under assumption of common allometry. It is
508 worth noting that the assumption of common allometry did not substantially alter the observed results
509 (Supplementary Figure 1). As with other studies, we suggest that a rejection of this assumption simply
510 based upon significance may not be optimal, and future work should determine what the consequences of
511 making such assumptions might be for studies of sexual dimorphism and allometry.

512
513 Our results clearly demonstrate the effects of growth pathway mutants on SSD and SShD. Most notably,
514 we cannot rule out the sex-specific effects of any genes involved in growth. Our results demonstrate the
515 current lack of understanding of how growth-related genes interact with the sex of the individual. By
516 visualizing the effects of each mutation within the framework of size/shape space we gain a previously
517 unrealized understanding of the role each mutant plays in generating a sex's phenotype. While this method
518 is especially powerful for studying sexual dimorphism, its applications are not restricted to it. We therefore
519 present this method as a means for dissecting the contributions of mutants to the development of size and
520 shape.

521
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527
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718 **Figure Legends:**

719

720 **Fig.1** Natural variation in SSD and SShD for two wild type strains. A) SSD and SShD in both wild type
721 background strains are represented three ways: scatterplot (center), SSD histogram with density curve (x-
722 axis, top) and SShD histogram with density curve (y-axis, right). Data points represent mean value for
723 wild-type siblings of each heterozygous mutant cross from a given vial. The Samarkand wild type
724 background has a wider range of SSD, encompassing the low end of the spectrum, whereas Oregon-R tends
725 to be more consistently large in SSD. B) Average direction of SShD in a typical Samarkand (left) and
726 Oregon-R (right) wild type wing. Landmark coordinates are mapped onto a typical wing to demonstrate
727 shape. Arrows represent the vector of shape change (magnified 5x) from female to male wing shapes.
728

729 **Fig.2** Magnitude of SSD and SShD for 42 mutants in Oregon-R (left) and Samarkand (right) wild type
730 backgrounds. The effect of each mutant is mapped out in a size-and-shape dimorphism space. Genotypic
731 means for each mutant are indicated by point style and connected by a solid line. SSD is plotted on each x-
732 axis for all plots and SShD is displayed on the y-axis. The plots above display the entire range of variation
733 observed, while those below display only the area with the highest density of points. Lines with significant
734 sex-by-genotype effects are highlighted as follows: effect on both size and shape, shape only and size only.
735 Only significant genes (after sequential Bonferroni correction) from the linear models are colored. Few
736 mutations in this study alter sexual dimorphism of size or shape. In addition, the effect of mutations also
737 appears to be highly background dependent, as only two lines, *Omb* and *Egfr*, were consistent in both
738 backgrounds. Error bars are 95% confidence intervals (unadjusted alpha). All gene names are displayed
739 lower-case, regardless of dominance.
740

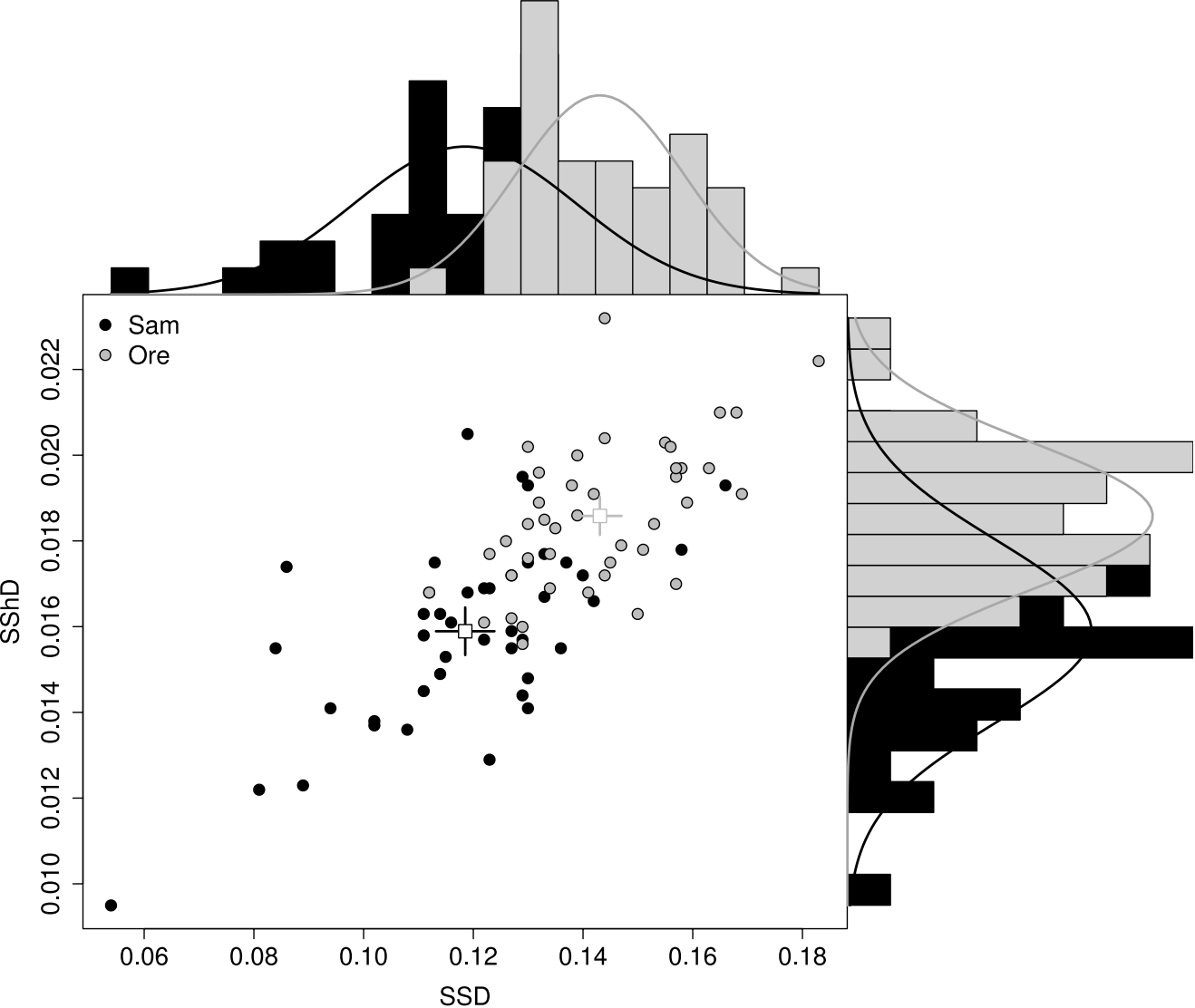
741 **Fig.3** Vector correlations to assess similarity of direction for sexual shape dimorphism (mutant VS. wild
742 type) by background. While genetic background appears to have little effect on the direction of SShD for
743 most mutations, several stand out with more divergent directions of SShD. Those mutations with large
744 background effects are also notable for their large effect on size and/or shape. Error bars are 95%
745 confidence intervals (unadjusted alpha).
746

747

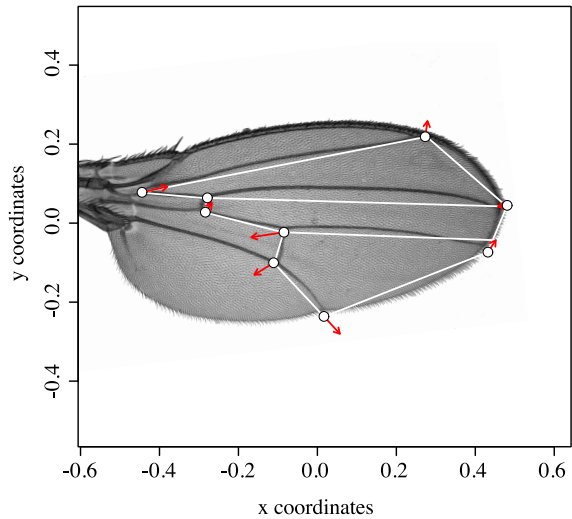
748 **Fig.4** Variation in the magnitude of association between shape and size allometric coefficients among
749 mutations in the Oregon-R (top) and Samarkand (bottom) wild type backgrounds. The “slope” of the
750 allometric relationship for shape on size is displayed by sex and genotype. The magnitude of allometric
751 effects appears to be relatively stable across strains, with few mutants substantially altering the wild type
752 pattern of allometric co-variation. Individual lines whose mutants cause a significant sex-by-size interaction
753 are represented dark in contrast to non-significant (faded) lines. Error bars are 95% confidence intervals
754 (unadjusted alpha) for each individual treatment; significance is assessed based solely the interaction terms
755 from the multivariate linear models.
756

757 **Supplementary Fig.1** Magnitude of SSD and SShD for 42 mutants in Oregon-R (left) and Samarkand
758 (right) wild type backgrounds, after correcting for the influence of allometry (shape on size). A common
759 allometry relationship was assumed across genotype and sex within each background and line combination.
760 Residuals from the allometric model were then used for the analysis. This figure is otherwise identical to
761 figure 2 (which does not correct for allometry). The effect of each mutant is mapped out in a size-and-shape
762 dimorphism space. Genotypic means for each mutant are indicated by point style and connected by a solid
763 line. SSD is plotted on each x-axis for all plots and SShD is displayed on the y-axis. The plots above
764 display the entire range of variation observed, while those below display only the area with the highest
765 density of points. Lines with significant sex-by-genotype effects are highlighted as follows: effect on both
766 size and shape, shape only and size only. Only significant genes (after sequential Bonferroni correction)
767 from the linear models are colored. Error bars are 95% confidence intervals (unadjusted alpha). All gene
768 names are displayed lower-case, regardless of dominance.
769

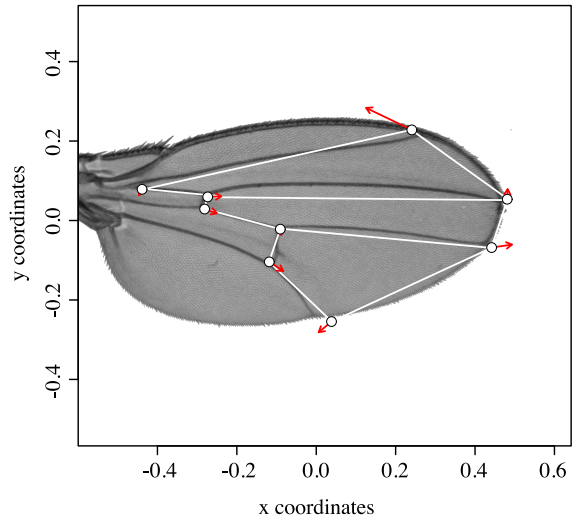
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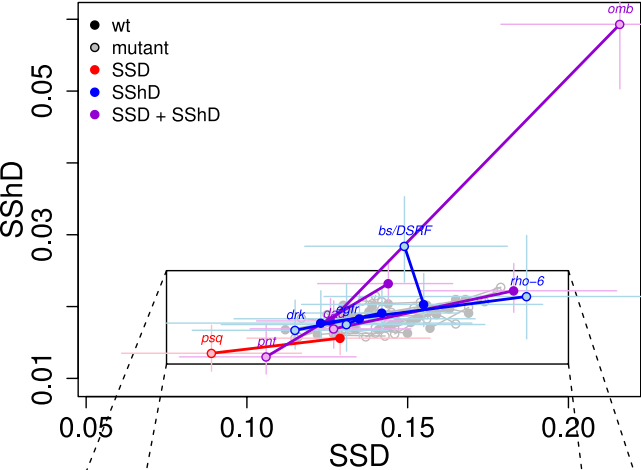
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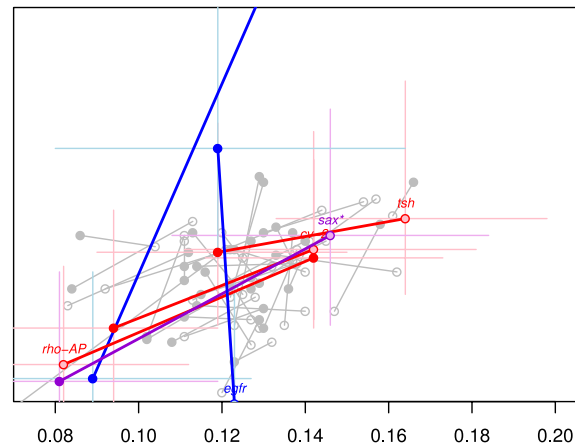
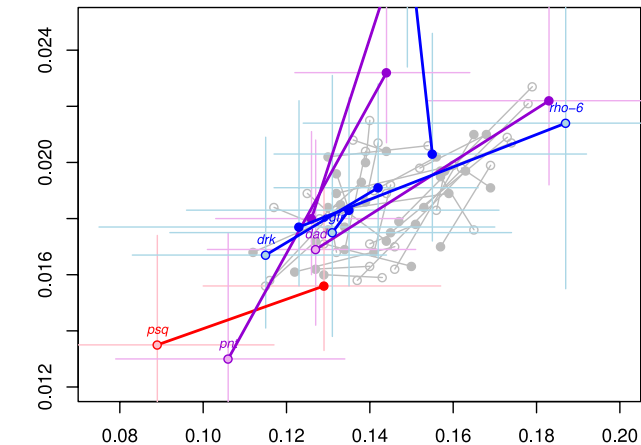
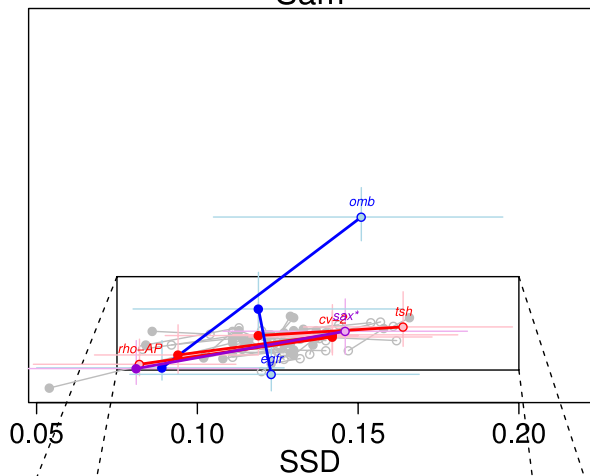
Ore



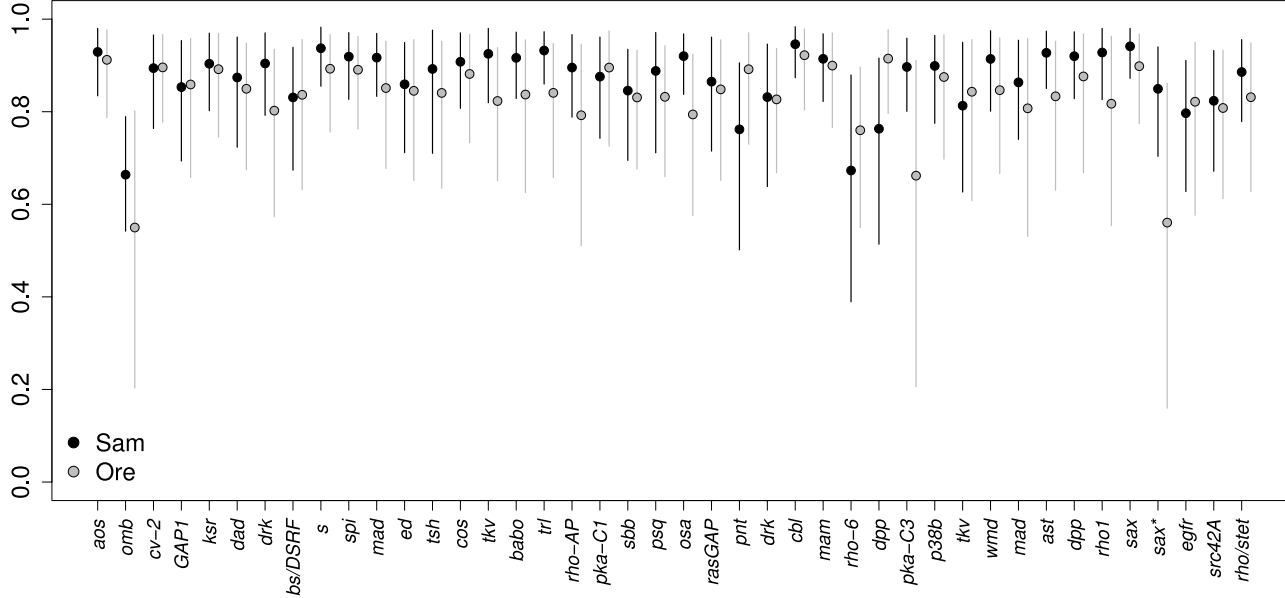
Ore



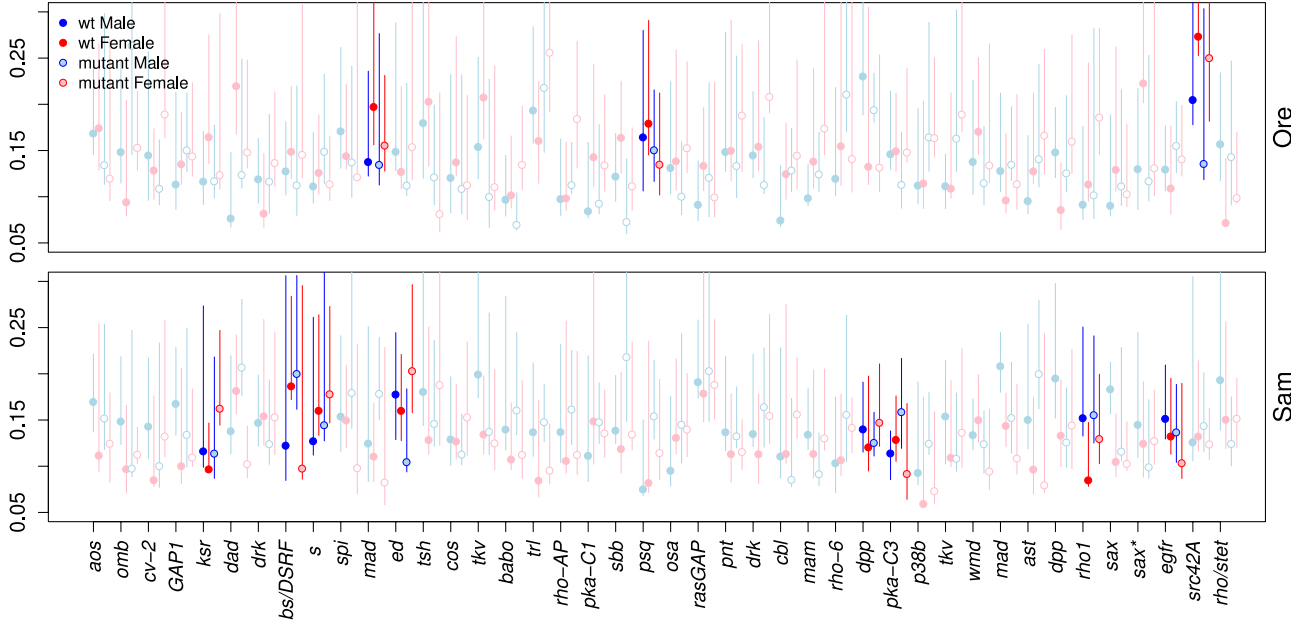
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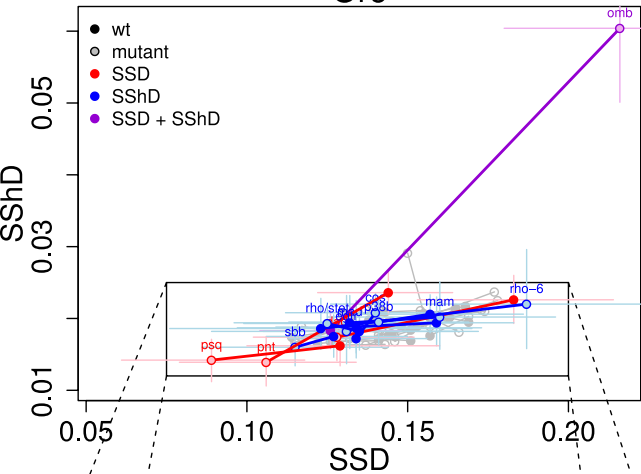
Vector Correlation



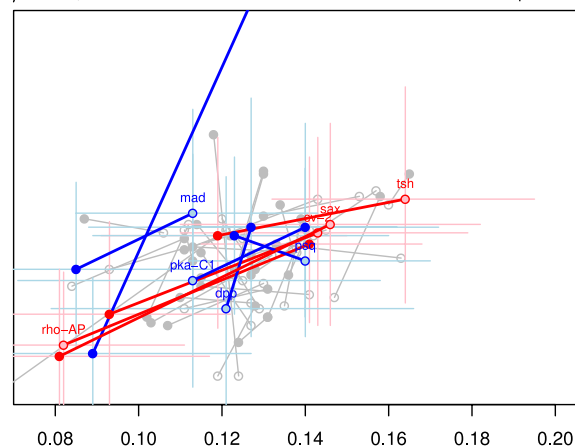
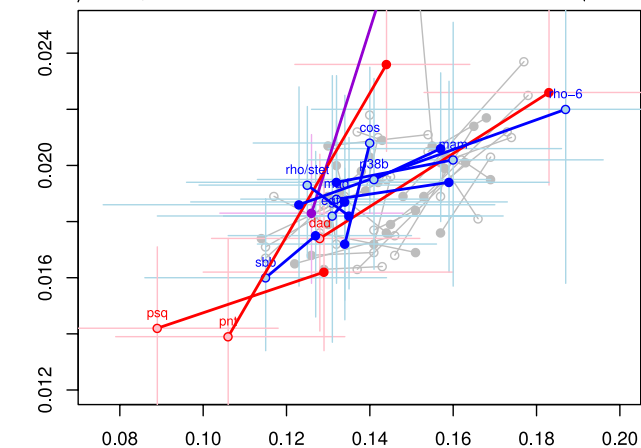
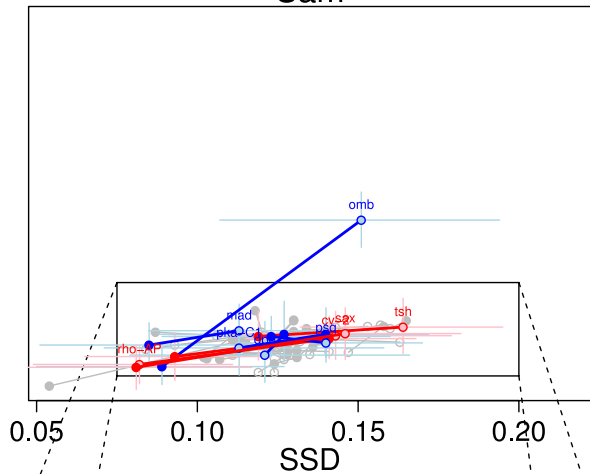
Slope of Allometry



Ore



Sam



Title: The sex-limited effects of mutations in the EGFR and TGF- β signaling pathways on shape and size sexual dimorphism and allometry in the *Drosophila* wing.

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Keywords: Sexual dimorphism, size, shape, allometry, geometric morphometrics, signal transduction, *Drosophila*, mutational analysis

Table 1: Summary table of significant effects by background among mutants. All significant values are taken from Figures 1-4. In the case of vector correlations, 80% was chosen arbitrarily to represent only a small subset of mutants of large effect.

Mutant	Allele	Pathway	Δ SSD	Δ SShD	Δ Vector Correlation	Δ Allometry
<i>aos</i>	W11	Egfr				
<i>omb</i>	md653	TGF-β	Ore	Sam, Ore	Sam, Ore	
<i>cv-2</i>	225-3	TGF-β				
<i>GAP1</i>	mip-w[+]	Egfr				
<i>ksr</i>	J5E2	Egfr				Sam
<i>dad</i>	J1E4	TGF-β	Ore	Ore		
<i>drk</i>	k02401	Egfr		Ore		
<i>bs/DSRF</i>	k07909	Egfr		Ore		Sam
<i>s</i>	k09530	Egfr				Sam
<i>spi</i>	s3547	Egfr				
<i>mad</i>	k00237	TGF-β				Ore
<i>ed</i>	k01102	Egfr				Sam
<i>tsh</i>	A3-2-66	TGF-β	Sam			
<i>cos</i>	k16101	Hh				
<i>tkv</i>	k19713	TGF-β				
<i>babo</i>	k16912	TGF-β/Hh				
<i>trl</i>	S2325	TGF-β				
<i>rho-AP</i>	BG00314	?	Sam		Sam	
<i>pka-C1</i>	BG02142	Hh				
<i>sbb</i>	BG01610	TGF-β				
<i>psq</i>	kg00811	Egfr	Ore			Ore
<i>osa</i>	kg03117	Chromatin Remodeling			Sam	
<i>rasGAP</i>	kg02382	Egfr				
<i>pnt</i>	kg04968	Egfr	Ore	Ore	Ore	
<i>drk</i>	k02401	Egfr				
<i>cbl</i>	kg03080	Egfr				
<i>mam</i>	kg02641	N/Egfr				
<i>rho-6</i>	kg05638	Egfr		Ore	Sam, Ore	
<i>dpp</i>	kg04600	TGF-β			Ore	Sam
<i>pka-C3</i>	kg00222	Hh			Sam	Sam
<i>p38b</i>	kg01337	TGF-β/Egfr				
<i>tkv</i>	kg01923	TGF-β				
<i>wmd</i>	kg07581	Unknown				
<i>mad</i>	kg00581	TGF-β				
<i>ast</i>	kg07563	Egfr				
<i>dpp</i>	kg08191	TGF-β				
<i>rho1</i>	kg01774	Egfr?				Sam
<i>sax</i>	kg07525	TGF-β				
<i>sax*</i>	sax4	TGF-β	Sam	Sam	Sam	
<i>egfr</i>	k05115	Egfr		Sam, Ore	Ore	Sam
<i>src42A</i>	kg02515	Egfr				Ore
<i>rho/stet</i>	kg07115	Egfr				

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Supplementary Table 1a: Significance Table (raw p values) for effects on wing shape where G= genotype, S= sex, B= background and Cs= (centroid) size

Mutant	Line	Allele	Pathway	G	G x S	G x B	Cs x G x S	Cs x G x B	G x S x B	Cs x G x S x B
<i>aos</i>	2513	W11	Egfr	0.001	0.671	0.14	0.863	0.827	0.324	0.093
<i>omb</i>	3045	md653	TGF- β	0.001	0.001	0.003	0.004	0.001	0.001	0.086
<i>cv-2</i>	6342	225-3	TGF- β	0.092	0.363	0.093	0.023	0.822	0.027	0.677
<i>GAP1</i>	6372	mip-w[+]	Egfr	0.001	0.034	0.001	0.674	0.881	0.405	0.428
<i>ksr</i>	10212	J5E2	Egfr	0.001	0.935	0.193	0.057	0.89	0.364	0.248
<i>dad</i>	10305	J1E4	TGF- β	0.001	0.054	0.593	0.24	0.058	0.098	0.296
<i>drk</i>	10372	k02401	Egfr	0.001	0.046	0.482	0.478	0.544	0.57	0.796
<i>bs/DSRF</i>	10413	k07909	Egfr	0.001	0.111	0.001	0.157	0.163	0.437	0.165
<i>s</i>	10418	k09530	Egfr	0.001	0.463	0.019	0.849	0.23	0.518	0.002
<i>spi</i>	10462	s3547	Egfr	0.001	0.518	0.003	0.495	0.52	0.438	0.573
<i>mad</i>	10474	k00237	TGF- β	0.001	0.56	0.001	0.542	0.464	0.125	0.094
<i>ed</i>	10490	k01102	Egfr	0.001	0.88	0.012	0.07	0.051	0.542	0.046
<i>tsh</i>	10842	A3-2-66	TGF- β	0.095	0.458	0.397	0.947	0.775	0.165	0.262
<i>cos</i>	11156	k16101	Hh	0.011	0.228	0.001	0.267	0.096	0.288	0.36
<i>tkv</i>	11191	k19713	TGF- β	0.001	0.121	0.001	0.358	0.813	0.26	0.161
<i>babo</i>	11207	k16912	TGF- β /Hh	0.054	0.428	0.047	0.465	0.51	0.429	0.1
<i>trl</i>	12088	S2325	TGF- β	0.001	0.136	0.315	0.046	0.82	0.997	0.23
<i>rho-AP</i>	12413	BG00314	?	0.523	0.006	0.005	0.983	0.815	0.196	0.098
<i>pka-C1</i>	12752	BG02142	Hh	0.225	0.946	0.356	0.032	0.89	0.825	0.601
<i>sbb</i>	12772	BG01610	TGF- β	0.001	0.203	0.038	0.323	0.647	0.02	0.539
<i>psq</i>	12916	kg00811	Egfr	0.011	0.38	0.223	0.979	0.732	0.03	0.01
<i>osa</i>	12945	kg03117	Chromatin Remodeling	0.001	0.188	0.036	0.876	0.373	0.043	0.123
<i>rasGAP</i>	13311	kg02382	Egfr	0.541	0.371	0.001	0.037	0.038	0.286	0.307
<i>pnt</i>	13535	kg04968	Egfr	0.002	0.063	0.021	0.173	0.752	0.504	0.609
<i>drk</i>	13943	k02401	Egfr	0.095	0.223	0.001	0.514	0.22	0.107	0.222
<i>cbl</i>	13944	kg03080	Egfr	0.003	0.459	0.018	0.765	0.57	0.947	0.195
<i>mam</i>	14189	kg02641	N/Egfr	0.001	0.908	0.577	0.298	0.035	0.245	0.066
<i>rho-6</i>	14208	kg05638	Egfr	0.809	0.35	0.814	0.448	0.72	0.152	0.25
<i>dpp</i>	14268	kg04600	TGF- β	0.006	0.898	0.022	0.285	0.058	0.912	0.025
<i>pka-C3</i>	14345	kg00222	Hh	0.177	0.045	0.035	0.587	0.276	0.1	0.018
<i>p38b</i>	14364	kg01337	TGF- β /Egfr	0.247	0.59	0.031	0.566	0.906	0.771	0.902
<i>tkv</i>	14403	kg01923	TGF- β	0.041	0.111	0.009	0.222	0.835	0.501	0.269
<i>wmd</i>	14541	kg07581	Unknown	0.093	0.264	0.04	0.651	0.157	0.039	0.378
<i>mad</i>	14578	kg00581	TGF- β	0.002	0.651	0.153	0.815	0.199	0.216	0.753
<i>ast</i>	14638	kg07563	Egfr	0.014	0.972	0.074	0.211	0.934	0.666	0.781
<i>dpp</i>	14694	kg08191	TGF- β	0.002	0.822	0.074	0.162	0.878	0.421	0.424
<i>rho1</i>	14901	kg01774	Egfr?	0.082	0.059	0.353	0.083	0.677	0.082	0.03
<i>sax</i>	14920	kg07525	TGF- β	0.046	0.24	0.003	0.31	0.519	0.5	0.389
<i>sax*</i>	5404	sax4	TGF- β	0.04	0.001	0.026	0.205	0.701	0.808	0.795
<i>egfr</i>	10385	k05115	Egfr	0.001	0.098	0.023	0.12	0.112	0.077	0.316
<i>src42A</i>	13751	kg02515	Egfr	0.001	0.057	0.023	0.054	0.212	0.617	0.13
<i>rho/stet</i>	14321	kg07115	Egfr	0.001	0.653	0.305	0.064	0.558	0.102	0.327

Supplementary Table 1b: Sample size of treatments, where

M= male, F= female, w= wild-type, m= mutant, O= Oregon-R background, S= Samarkand background

Mutant	Line	M, w, O	F, w, O	M, w, S	F, w, S	M, m, O	F, m, O	M, m, S	F, m, S
aos	2513	21	20	21	20	20	20	20	22
omb	3045	19	18	20	20	12	19	19	20
cv-2	6342	10	11	20	20	10	10	20	20
GAP1	6372	19	18	20	19	18	19	20	20
ksr	10212	17	20	19	20	20	20	22	20
dad	10305	20	20	18	20	20	20	20	20
drk	10372	20	20	20	20	20	20	20	21
bs/DSRF	10413	19	19	20	20	20	19	20	20
s	10418	20	20	18	21	20	20	20	20
spi	10462	18	20	20	20	20	19	21	21
mad	10474	19	20	20	22	20	20	18	22
ed	10490	20	20	19	20	20	20	21	21
tsh	10842	19	21	20	20	20	19	22	19
cos	11156	19	20	19	20	22	19	20	18
tkv	11191	20	20	20	21	20	21	18	17
babo	11207	19	21	20	20	20	20	20	20
trl	12088	20	20	21	20	20	21	20	20
rho-AP	12413	20	20	20	20	20	19	20	19
pka-C1	12752	20	20	18	20	20	20	21	21
sbb	12772	20	20	20	19	20	22	20	21
psq	12916	21	20	21	20	20	20	20	21
osa	12945	31	30	20	20	30	24	20	20
rasGAP	13311	19	19	20	19	21	21	20	20
pnt	13535	20	20	20	19	20	19	20	20
drk	13943	20	19	20	21	16	17	21	21
cbl	13944	20	20	21	20	20	20	20	20
mam	14189	10	8	21	21	10	10	20	20
rho-6	14208	19	21	20	20	19	20	20	21
dpp	14268	21	20	19	20	20	18	22	18
pka-C3	14345	21	20	20	20	21	20	20	20
p38b	14364	10	10	20	20	10	6	20	20
tkv	14403	11	10	10	12	10	11	20	20
wmd	14541	20	20	20	21	20	21	21	20
mad	14578	20	19	22	21	21	19	20	20
ast	14638	20	20	20	20	20	18	20	20
dpp	14694	20	19	19	20	20	20	20	20
rho1	14901	20	20	20	21	20	20	20	20
sax	14920	19	19	20	20	20	20	21	20
sax*	5404	20	21	18	19	20	20	20	20
egfr	10385	20	19	20	20	20	20	20	20
src42A	13751	19	20	19	20	19	20	19	20
rho/stet	14321	19	19	19	20	19	21	19	19

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Keywords: Sexual dimorphism, size, shape, allometry, geometric morphometrics, signal transduction, *Drosophila*, mutational analysis

Supplementary Table 2: Significance Table (raw p values) for effects on wing shape after the effects of allometry are removed where G= genotype, S= sex, B= background and Cs= (centroid) size.

Mutant	Line ID	G	G x S	G x B	Cs x G x S	Cs x G x B	G x S x B	Cs x G x S x B
<i>aos</i>	2513	0.098	0.221	0.001	0.664	0.31	0.236	0.625
<i>omb</i>	3045	0.001	0.072	0.001	0.6	0.311	0.002	0.264
<i>cv-2</i>	6342	0.066	0.215	0.171	0.494	0.412	0.619	0.208
<i>GAP1</i>	6372	0.002	0.235	0.001	0.075	0.012	0.177	0.299
<i>ksr</i>	10212	0.008	0.005	0.002	0.165	0.24	0.165	0.526
<i>dad</i>	10305	0.16	0.009	0.797	0.158	0.003	0.962	0.205
<i>drk</i>	10372	0.005	0.174	0.031	0.115	0.021	0.021	0.607
<i>bs/DSRF</i>	10413	0.001	0.126	0.001	0.204	0.021	0.005	0.427
<i>s</i>	10418	0.001	0.002	0.106	0.377	0.188	0.009	0.107
<i>spi</i>	10462	0.001	0.527	0.002	0.558	0.371	0.025	0.359
<i>mad</i>	10474	0.001	0.019	0.005	0.594	0.656	0.005	0.746
<i>ed</i>	10490	0.001	0.778	0.591	0.998	0.225	0.393	0.164
<i>tsh</i>	10842	0.008	0.216	0.002	0.837	0.828	0.051	0.988
<i>cos</i>	11156	0.001	0.019	0.037	0.025	0.03	0.036	0.375
<i>tkv</i>	11191	0.001	0.004	0.006	0.404	0.947	0.301	0.135
<i>babo</i>	11207	0.048	0.427	0.02	0.208	0.517	0.721	0.092
<i>trl</i>	12088	0.001	0.001	0.04	0.776	0.891	0.005	0.38
<i>rho-AP</i>	12413	0.075	0.444	0.017	0.805	0.69	0.194	0.009
<i>pka-C1</i>	12752	0.001	0.227	0.107	0.081	0.513	0.002	0.204
<i>sbb</i>	12772	0.001	0.537	0.004	0.703	0.001	0.396	0.069
<i>psq</i>	12916	0.004	0.283	0.143	0.677	0.127	0.048	0.823
<i>osa</i>	12945	0.001	0.255	0.02	0.699	0.492	0.728	0.368
<i>rasGAP</i>	13311	0.027	0.666	0.031	0.565	0.587	0.792	0.607
<i>pnt</i>	13535	0.016	0.199	0.111	0.439	0.199	0.659	0.293
<i>drk</i>	13943	0.002	0.005	0.001	0.414	0.905	0.081	0.051
<i>cbl</i>	13944	0.138	0.07	0.329	0.148	0.002	0.754	0.518
<i>mam</i>	14189	0.001	0.15	0.043	0.001	0.109	0.043	0.386
<i>rho-6</i>	14208	0.086	0.091	0.433	0.664	0.959	0.009	0.099
<i>dpp</i>	14268	0.017	0.322	0.08	0.798	0.109	0.871	0.822
<i>pka-C3</i>	14345	0.019	0.024	0.567	0.151	0.237	0.345	0.23
<i>p38b</i>	14364	0.003	0.498	0.171	0.542	0.001	0.466	0.6
<i>tkv</i>	14403	0.021	0.225	0.023	0.403	0.673	0.454	0.395
<i>wmd</i>	14541	0.028	0.118	0.435	0.248	0.599	0.001	0.172
<i>mad</i>	14578	0.001	0.038	0.026	0.639	0.456	0.357	0.11
<i>ast</i>	14638	0.078	0.091	0.002	0.995	0.317	0.007	0.132
<i>dpp</i>	14694	0.001	0.299	0.045	0.476	0.027	0.56	0.801
<i>rho1</i>	14901	0.221	0.362	0.436	0.366	0.329	0.394	0.244
<i>sax</i>	14920	0.002	0.394	0.38	0.758	0.272	0.135	0.338
<i>sax*</i>	5404	0.007	0.007	0.047	0.442	0.929	0.299	0.845
<i>egfr</i>	10385	0.001	0.125	0.03	0.742	0.393	0.014	0.211
<i>src42A</i>	13751	0.031	0.37	0.002	0.384	0.195	0.411	0.586
<i>rho/stet</i>	14321	0.002	0.156	0.19	0.02	0.12	0.207	0.421