



HAL
open science

Pleiotropy promotes male exaggerated weapon and its associated fighting behaviour in a water strider

William Toubiana, David Armisen, Decaras Amélie, Abderrahman Khila

► To cite this version:

William Toubiana, David Armisen, Decaras Amélie, Abderrahman Khila. Pleiotropy promotes male exaggerated weapon and its associated fighting behaviour in a water strider. 2020. hal-03064873

HAL Id: hal-03064873

<https://hal.science/hal-03064873v1>

Preprint submitted on 16 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Pleiotropy promotes male exaggerated weapon and its associated fighting behaviour**
2 **in a water strider**

3
4 **Authors:** William Toubiana¹, David Armisén^{1,2}, Decaras Amélie¹ and Abderrahman Khila¹

5
6 **Author affiliations:**

7 ¹ Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Université Claude Bernard Lyon
8 1, CNRS UMR 5242, Ecole Normale Supérieure de Lyon, 46, allée d'Italie, 69364 Lyon Cedex 07,
9 France.

10
11 ² Present address:

12
13 * **Corresponding author:** abderrahman.khila@ens-lyon.fr ; william.toubiana@ens-lyon.fr
14
15

16 **Keywords:** Weapons, scaling relationships, pleiotropy, reproductive behaviour

17
18 **Abstract**

19 Exaggerated sexually selected traits, often carried by males, are characterized by the evolution of
20 hyperallometry, resulting in their disproportionate growth relative to the rest of the body¹⁻³. While the
21 evolution of allometry has attracted much attention for centuries, our understanding of the
22 developmental genetic mechanisms underlying its emergence remains fragmented^{4,5}. Here we show
23 that the hyperallometric legs in the males of the water strider *Microvelia longipes* are associated with a
24 specific signature of gene expression during development. Using RNAi knockdown, we demonstrate
25 that a broadly expressed growth factor, Bone Morphogenetic Protein 11 (BMP11, also known as
26 Growth Differentiation Factor 11), regulates leg allometries through increasing the allometric
27 coefficient and mean body size in males. In contrast, BMP11 RNAi reduced mean body size but did
28 not affect slope in females. Furthermore, our data show that a tissue specific factor, Ultrabithorax
29 (Ubx), increases intercept without affecting mean body size. This indicates a genetic correlation
30 between mean body size and variation in allometric slope, but not intercept. Strikingly, males treated
31 with BMP11 RNAi exhibited a severe reduction in fighting frequency compared to both controls and
32 Ubx RNAi-treated males. Overall, we demonstrate a genetic correlation between male body size, the
33 exaggerated weapon, and the intense fighting behaviour associated with it in *M. longipes*. Our results
34 provide evidence for a role of pleiotropy in the evolution of allometric slope.
35
36
37
38
39
40
41
42
43
44
45
46
47

48 **Introduction**

49 Extravagant ornaments and weapons, often carried by males in a wide range of lineages, represent
50 some of the most striking outcomes of directional sexual selection driven by female choice or male
51 competition^{2,3,6,7}. Exaggerated sexually selected traits are often characterized by their extreme growth
52 and hypervariability among individuals of the same population^{8,9}. These features derive from changes
53 in scaling relationships, especially the elevation of the allometric coefficient (i. e. hyperallometry),
54 which results in certain structures growing disproportionately larger relative to the rest of the body⁸⁻¹¹.
55 Despite the longstanding interest in the study of allometry, the genetic architecture underlying
56 variation in allometric coefficient is still largely unknown^{9,12-15}. Allometric slope is known to be
57 relatively stable during evolution and several studies suggest that this stasis could be the consequence
58 of genetic correlation with other traits^{4,12-14,16,17}. In the context of trait exaggeration (e. g.
59 hyperallometry), Fisher predicted a genetic correlation between male exaggerated ornaments and
60 female preference for the exaggeration – a process known as runaway selection². Other studies, for
61 example in the ruffs or horned beetles, also suggest a genetic link between the elaboration of male
62 exaggerated traits and other secondary sexual traits such as male body size and reproductive behaviour
63¹⁸⁻²⁰. However, empirical tests of these predictions remain difficult to achieve. Determining the genetic
64 architecture of allometric slope and testing its correlation with other traits would therefore greatly
65 advance our understanding of the evolution of scaling relationships^{9,12-15}.

66
67 We address these questions in the water strider *Microvelia longipes*, where some males exhibit
68 extremely long third legs due to a hyperallometric relationship with body size²¹. Among the over 200
69 known species in this genus, *M. longipes* is the only species found where males simultaneously exhibit
70 large body size, and high variation in both body and leg length^{21,22}. Males of other *Microvelia* species
71 exhibit some but not all of these features, suggesting that the evolution of hyperallometry may be
72 linked to the evolution of increased mean and variation in body size²¹. In addition, males of *M.*
73 *longipes* use the exaggerated legs to fight and dominate egg-laying sites where they intercept and mate
74 with gravid females²¹. Compared to its closest related species *M. pulchella*, *M. longipes* males fight
75 over an order of magnitude more frequently, thus indicating that the evolution of the exaggerated
76 weapon is also associated with the increase in competition intensity between males²¹. Here we use
77 comparative transcriptomics, along with studies of gene function and behaviour, to determine the
78 genetic basis of third leg exaggeration in this species. We show that the developmental gene *Bone*
79 *Morphogenetic Protein 11 (BMP11)*, also known as *Growth Differentiation Factor 11 (GDF11)*, plays
80 a pleiotropic role both in the hyperallometry of the third leg, increase body size and fighting intensity
81 in males, thus revealing a strict genetic correlation between these traits in the male of *M. longipes*.

82 83 **Results**

84 **A burst of leg growth during the last nymphal instars**

85 In *M. longipes*, third leg length is highly variable among males and this variability is higher in males
86 compared to females (Figure 1A)²¹. A developmental growth curve (Figure 1B; Supplementary figure
87 1) revealed that leg length is identical between individuals at the 1st to 3rd instars, but starts to diverge
88 between the sexes and also among males at the 4th instar (Figure 1B). A dramatic increase in leg length
89 was observed between 5th instar and adult males (Figure 1B). These differences in leg length between
90 individuals are independent of any variation in developmental time from 1st instar to adult (ANCOVA,
91 F-value= 1.965, p-value= 0.1712; supplementary figure 2). Therefore, the growth burst and its
92 fluctuation at the end of post-embryonic development in male exaggerated legs accounts for both the
93 sexual dimorphism and the hypervariability among males (Figure 1A-C). Ontogenetic allometry also
94 confirmed the exaggerated growth rate of male third legs during development (Supplementary table 1).
95 The first and second legs in males and females showed similar growth dynamics, although not as
96 extreme as the third legs (Figure 1B). Based on these observations, we hypothesize that the striking
97 variation in leg growth dynamics within and between the sexes is attributable to variation in gene
98 expression at the end of nymphal development.

99

100 **Leg exaggeration is associated with a specific signature of leg-biased gene expression**

101 Insect legs are serial homologs that share most of their developmental genetic program due to common
102 origin^{23,24}. Therefore, we sequenced leg RNA, at the developmental stage where we detected a burst
103 of growth, to match the observed morphological divergence in leg growth with differences in gene
104 expression between serial homologs of each sex (Supplementary table 2). Although the differences in
105 length between serial homologs were more pronounced in males than in females (Figure 1)²¹, we
106 failed to detect any correlation between such variation in morphology and variation in gene expression
107 (Figure 2A). This is reflected in the first major axis of variation, which separated the legs with similar
108 contribution (about 35% of the total variation) in both males and females (Figure 2A). This result
109 suggests that morphological divergence in serial homologs between the sexes may not result from a
110 global change in gene expression but rather from specific sets of genes.

111 To identify the genes underlying the growth differences in serial homologs, we examined the
112 differentially expressed genes between the first and the third leg (leg-biased genes), and compared the
113 divergence in expression between these two legs in males and females. We found that the third legs
114 diverged more from the first legs in males than in females, both in terms of number of differentially
115 expressed genes and in the degree of differential expression (Figure 2B; Supplementary figure 3).
116 Similar results were observed for the second legs in males, which are moderately exaggerated
117 (Supplementary figures 3 & 4). A hierarchical clustering analysis showed that the up-regulated genes
118 restricted to male third legs (489 genes) are similarly expressed between the first and the third legs of
119 females. Similar results were also observed for biased genes in female third legs (Figure 2B),
120 indicating that a large proportion of up-regulated genes in the third legs are sex-specific. In the first
121 legs, which have more similar scaling relationship between males and females, we found that about

122 63% of leg-biased genes were common between the sexes (Figure 2C). Moreover, hierarchical
123 clustering analyses revealed that genes which were up-regulated in the first legs specifically in males
124 showed similar expressions with the first legs of females (Figure 2C). Likewise, female-restricted
125 genes showed similar expressions with the first legs of males (Figure 2C). Finally, leg-biased genes
126 shared among sexes do not show any average differences in expression between males and females
127 (Supplementary figure 5). Altogether, these data suggest that the morphological divergence of serial
128 homologs is associated with up-regulation of a unique set of genes in the third legs of both sexes, with
129 a higher magnitude, both in number and degree of differential expression, in male exaggerated third
130 legs. We note also that the third legs express a considerable set of common genes between males and
131 females, possibly underlying more global regulators of leg growth and allometry.

132

133 **Key developmental genes control the scaling relationship of male third legs.**

134 Extravagant signals and weapons, such as *M. longipes* third legs, are predicted to occur in good
135 condition individuals because of the high fitness cost they impose^{7,25,26}. Proposed mechanisms for the
136 development of exaggerated phenotypes include increased sensitivity or the production of growth
137 factors by the exaggerated organ^{5,7}. To test this hypothesis, we examined the developmental genetic
138 pathways that are enriched in the exaggerated legs through analyses of gene ontology in leg-biased
139 genes (Supplementary table 3). Genes preferentially up-regulated in the third legs of males were
140 enriched in metabolic processes, transmembrane transport and growth signalling pathways. Genes
141 preferentially up-regulated in the third legs of females were enriched in signalling and metabolic
142 pathways as well as response to stimuli and cell communication processes. Among the genes and
143 pathways that were hypothesized to play a role in the regulation of growth-related exaggerated traits³,
144 many were identified in our datasets as differentially expressed between serial homologs within the
145 same sex or between the sexes (Supplementary figure 6; Supplementary table 4; see Toubiana et al.
146 companion paper for sex-biased genes).

147 To test the role of these genes in trait exaggeration, we conducted an RNA interference (RNAi) screen
148 targeting about 30 candidates representing transcripts that were leg-biased, sex-biased, broadly
149 expressed or tissue-specific (Supplementary table 5). This functional screen identified two genes, *Bone*
150 *Morphogenetic Protein 11* (BMP11, also known as *Growth Differentiation Factor 11*) and
151 *Ultrabithorax* (Ubx), with unambiguous and reproducible effect on leg exaggeration. BMP11 is a
152 known secreted growth regulator with a systemic effect in vertebrates^{27,28} (Supplementary Figure 7),
153 whereas Ubx is a tissue-specific Hox protein known to be confined to the second and third thoracic
154 segments in water striders²⁹. In our dataset, *BMP11* is expressed in all legs but was up-regulated in the
155 third legs of males, whereas *Ubx* is absent from the first legs, lowly expressed in the second legs, and
156 highly expressed in the third legs of males (Supplementary tables 2 and 5).

157

158 RNAi depletion of *BMP11* at the fourth nymphal instar significantly decreased the mean and variance
159 of adult body and leg length (Figure 3A-B; Supplementary table 6 for statistical analyses).
160 Importantly, RNAi against *BMP11* also significantly reduced the allometric coefficient of male adult
161 legs (Figure 3C; Supplementary table 6). In comparison to the controls, *BMP11* knockdown
162 individuals showed around 12% reduction in body length, and the allometric coefficient significantly
163 dropped from 3.70 to 1.96 (anova/likelihood ratio tests, p-values < 0.05) (Figure 3B-C; Supplementary
164 table 6; also see material and methods). The allometric coefficients in the first and second legs of
165 males were also lower compared to their corresponding controls, but to a lower degree than in the third
166 legs (Supplementary figure 8; Supplementary table 6). Therefore, *BMP11* increases both the allometric
167 coefficient of all legs and mean body size in the males (Figure 3C; Supplementary figure 8;
168 Supplementary table 6). Despite the high and differential expression of *BMP11* in the legs of females
169 (Supplementary table 6), *BMP11* knockdown in females reduced body size but did not affect the
170 scaling relationship between leg and body length (Figure 3A-C; Supplementary table 6). Interestingly,
171 the effect of *BMP11* RNAi knockdown brings body size and scaling relationship of *M. longipes* males
172 closer to its sister species *M. pulchella* (Figure 3A-C, Supplementary figure 8). Altogether, these
173 results indicate that *BMP11* is a key growth regulator in *M. longipes*, playing a role in the regulation
174 of both leg allometry and body size in males during development.

175 Contrary to *BMP11*, *Ubx* knockdown did not affect the slope but rather shifted the intercept due to a
176 16% decrease in males' third leg length (t-test: $t = -3.5135$, $df = 62.904$, p-value < 0.05) (Figure 3A-C;
177 Supplementary table 6). We also detected a small effect in the second legs and no effect in the first
178 legs, consistent with the expression of *Ubx* in these tissues (Figure 3A; Supplementary figure 8;
179 Supplementary table 6). Contrary to *BMP11*, *Ubx* knockdown did not affect average body length
180 (Figure 3B-C; Supplementary table 6). *Ubx* knockdown in females also altered the intercept in the
181 third leg length allometry, but to a lower extent than in males despite the similar expression of *Ubx*
182 (Figure 3C; Supplementary table 2 and 5; Supplementary table 6). This result first demonstrates that,
183 in *M. longipes*, changes in leg length can be genetically decoupled from changes in body length.
184 Second, this tissue-specific gene is associated with a change in intercept but not in allometric
185 coefficient.

186

187 ***BMP11* knockdown males fight less frequently than control males**

188 We have shown that the evolution of exaggerated third legs in *M. longipes* was accompanied with
189 increased body size and increased male fighting intensity compared to its sister species *M. pulchella*
190 (Figure 3; ²¹). To test whether *BMP11* also influences male fighting behaviour in *M. longipes*, we
191 compared the frequency of fights between controls and individuals treated with *BMP11* RNAi in
192 male-male competition assays (see Material and methods; Supplementary videos 1 and 2). Similar to
193 the controls, *BMP11* RNAi-treated males attempted to dominate egg-laying sites, mated, and guarded
194 females (Supplementary video1&2; ²¹). However, unlike the controls, these *BMP11* RNAi-treated

195 males avoided fighting when other males approached egg-laying sites. Instead, they adopted a
196 sneaking behaviour by focusing on the female rather than trying to chase rival males away, or by
197 abandoning the site altogether without a fight (Supplementary video 2). We quantified this “docile”
198 behaviour by calculating the frequency of fights on floaters and found that *BMP11* males fought on
199 average twelve times less than control males (Figure 4A; t-test: $t = 3.6134$, $df = 3.0559$, $p\text{-value} <$
200 0.05). *BMP11* females were attracted by male’s signals on floaters, but we failed to observe any
201 mating events during our trials (Supplementary video 2). We further observed that *Ubx* RNAi-treated
202 males retained aggressive fighting behaviour (Figure 4A), although the fight between two rival males
203 lasted significantly longer compared to controls (Figure 4B; Supplementary video 3). These results
204 suggest that *BMP11*, but not *Ubx*, increases fighting frequency in *M. longipes* males. Collectively, our
205 results show that in *M. longipes* males, *BMP11* regulates the development of the exaggerated weapon
206 and the associated fighting behaviour.

207

208 **Discussion**

209 We have shown that leg exaggeration in *M. longipes* males is associated with a specific
210 signature of gene expression. Among the genes up-regulated in the exaggerated trait, we demonstrated
211 that a broadly expressed growth factor, BMP11, increases slope, whereas a tissue specific factor, *Ubx*,
212 increases intercept. Finally, we have shown that BMP11 regulates the growth of the body, the
213 disproportionate growth of the legs, and also increases aggressiveness in males. This indicates that
214 these three male traits are genetically correlated through the pleiotropic effect of BMP11. How
215 BMP11 changes fighting behaviour in *M. longipes* males is unknown. A possible explanation is that
216 the docile behaviour of BMP11-RNAi treated males can be due to a plastic response whereby males
217 recognize their small body size and change reproductive strategy. Another possible explanation could
218 involve a role of BMP11 in the development of nervous system components associated with the
219 fighting behaviour.

220 Exaggerated traits represent striking cases of differential growth between various organs, and
221 we are just beginning to understand the underlying developmental genetic processes^{5,30}. Studies in
222 various insects suggest that traits would achieve exaggeration via their increased sensitivity to
223 systemic growth factors such as Juvenile Hormone or Insulin^{7,10,11,31,32}. Our data also show that
224 exaggeration, as seen in the third legs of *M. longipes* males, can be induced by growth regulating
225 molecules other than hormones, such is the case for BMP11 and *Ubx*. These molecules are produced
226 in excess by the exaggerated tissue, indicating an alternative path to trait exaggeration where over-
227 production of the growth factor occurs locally rather than through heightened sensitivity to a systemic
228 factor. Whether and how this increased tissue-specific expression of growth factors is connected to
229 systemic growth pathways remains to be tested¹¹.

230 Our experiments describe a common effect of BMP11 on mean body length in both sexes. By
231 contrast to males, the effect of BMP11 on body length is decoupled from its effect on leg length in

232 females (Figure 5C). It is possible that increased body size has been favoured in both sexes, through
233 increased competitiveness in males and increased fertility in females^{33,34}. Another explanation could
234 be that smaller females were disfavoured due to reproductive incompatibility as body size increased in
235 males. On the other hand, the lack of correlation in leg length between the sexes may be a
236 consequence of sexual conflict resolution through dimorphism³⁵.

237 Studies of scaling relationships reported a strong stasis of the allometric coefficient at the
238 microevolutionary time scale¹²⁻¹⁴. Even at the macro-scale, slope is known to evolve slower compared
239 to other scaling parameters such as intercept^{12,13}. Several hypotheses were formulated to explain such
240 evolutionary stasis, including a lack of genetic variation, strong stabilizing selection acting on slope,
241 or a constraining effect of pleiotropy¹²⁻¹⁵. Our results, implicating BMP11 as a master regulator of
242 growth in both body size and all three legs lend support to pleiotropy as a major driver of evolutionary
243 stasis in allometric coefficient. In contrast, Ubx, which regulates the size of the third legs but not body
244 size, has a role in changing the intercept. This suggests that the genetic architecture of slope may have
245 more pleiotropic effect than that of intercept, possibly explaining the observed differences in
246 evolutionary stasis between these two scaling parameters.

247 An important question is how trait exaggeration evolves despite the evolutionary stasis of
248 slope. We have shown that the evolution of hyperallometry in *M. longipes* is associated with increased
249 mean body size and male aggressiveness compared to its sister species *M. pulchella*²¹. These changes
250 are also associated with strong selection on males due to intense competition for access to females²¹.
251 In this context, selection is expected to favour traits that increase male competitiveness. Strikingly in
252 *M. longipes*, BMP11 seems to regulate mean body size, allometric slope, and male aggressiveness, all
253 of which increase male competitiveness. In this context, it is possible that directional selection
254 favoured genotypes that regulate all three traits at the same time leading to the co-evolutionary process
255 observed in *M. longipes*. Pleiotropy would therefore become both a promoting factor for the evolution
256 of exaggerated weapons and a constraining factor involved in the stasis of slope.

257

258 **Material and methods**

259

260 **Growth curves**

261 *M. longipes* is a hemimetabolous insect that develops through successive moults which represent an
262 exuvia of the previous nymphal instar. We raised each individual separately from 1st nymphal instar to
263 adulthood. Throughout this process, each individual produced five exuvia corresponding to the five
264 nymphal instars. We then collected the exuvia and the adult from each individual and measured their
265 leg lengths (Supplementary figure 1).

266

267 **Comparative transcriptomics: analyses of variance**

268 Sample collection, transcriptome assembly and read mapping/normalization are described in a
269 companion paper (Toubiana et al.). We performed an analysis of variance on sex separately (Figure 2).
270 Here we took in consideration both line and replicate effects (See Toubiana et al. companion paper).
271 The latter effect matched the days where RNA was extracted from each sample. We corrected for both
272 effects, using Within Class Analysis, in subsequent analyses.

273

274 **Identification of leg-biased genes**

275 The number of reads per “gene” was used to identify differences in expression among the different
276 legs using DESeq2³⁶. We first filtered transcripts for which expression was lower than 2 FPKM in
277 more than half of the samples after combining the two inbred populations (12 samples total).
278 Transcripts with average expression that was lower than 2 FPKM in the two comparing legs were also
279 discarded. Finally, we ran the differential expression analysis by taking into account the line and
280 replicate effects. We called differentially expressed genes any gene with a fold-change > 1.5 and a
281 $P_{adj} < 0.05$. All differential expression analyses were performed on the two lines combined as we
282 aimed to identify genes involved in allometric slope, which is a common feature of both lines.

283

284 **Hierarchical clustering**

285 Average expressions of leg-biased genes in the different sexes were clustered using Euclidean
286 clustering in the R package PVCLUST version 1.3-2³⁷ with 1000 bootstrap resampling. Heatmaps and
287 clustering were performed using the $\log_2(\text{TPM})$ average expression of each gene from each tissue.
288 Heatmaps were generated using the R package GPLOTS version 3.0.1.1.

289

290 **Nymphal RNAi**

291 Double stranded RNA (dsRNA) was produced for *BMP11* and *Ultrabithorax*. T7 PCR fragments of
292 the two genes were amplified from cDNA template using forward and reverse primers both containing
293 the T7 RNA polymerase promoter. The amplified fragments were purified using the QIAquick PCR
294 purification kit (Qiagen) and used as a template to synthesize the dsRNA as described in³⁸. The
295 synthesized dsRNA was then purified using an RNeasy purification kit (Qiagen) and eluted in
296 Spradling injection buffer³⁹ at a concentration of $6\mu\text{g}/\mu\text{l}$. For primer information, see Supplementary
297 table 5. Nymphal injections were performed in the line selected for long legged males²¹ at the 4th instar
298 as described in⁴⁰. Injected nymphs were placed in water tanks, for which the water was changed every
299 day, and fed daily with nine fresh crickets until they developed into adults. We measured adult legs,
300 body, pronotum and mesonotum on a dissecting SteREO Discovery.V12 Zeiss stereomicroscope using
301 the Zen Pro 2011 software. *BMP11* knock-down individuals had a significantly shorter pronotum
302 compared to controls. This is evident in that the mesonotum is no longer covered by the pronotum
303 (Supplementary figure 9). We used this effect on the pronotum to detect injected individuals that
304 displayed wild type-like phenotypes, as is characteristic to the RNAi technique, by looking at the

305 effect on the ratio pronotum-mesonotum (Supplementary figure 10). To further confirm that these
306 individuals were unaffected by *BMP11* RNAi, we compared their scaling relationships to normal
307 individuals and found that they display wild type-like phenotypes in all traits measured
308 (Supplementary figure 11). We therefore removed those individuals from the final analyses.

309

310 **Behavioural assays**

311 Male competition assays were performed using artificial puddles containing five egg-laying floaters.
312 Each replicate corresponded to a population of five wild type females from the long-legged selected
313 line²¹ and ten males from a treatment (either ten control or ten *BMP11*-RNAi males). Analyses were
314 performed on four replicates for each condition. Male and female interactions were recorded on a
315 Nikon digital camera D7200 with an AF-S micro Nikkor 105 mm lens. Video acquisitions were taken
316 a couple of hours after the bugs were transferred to the puddle. We defined a fight as an interaction
317 between two males going back-to-back and kicking with their third legs on or near a floater. If two
318 contestants stopped fighting for more than five seconds, we counted the new interaction as a separate
319 fight.

320

321 **Statistical analyses**

322 All statistical analyses were performed in RStudio 0.99.486. Comparisons for the mean trait size and
323 trait variance were performed on raw data, whereas log-transformed data were used for scaling
324 relationship comparisons. We used standardized major axis (SMA) as well as linear models to assess
325 differences in scaling relationships ('smatr' package and anova in R respectively, ⁴¹).

326

327 **Acknowledgements**

328 We thank Francois Leulier, Severine Viala, Rajendhran Rajakumar for comments on the manuscript
329 and Gael Yvert, Kevin Parsons and Roberto Arbore for discussions. This work was supported by and
330 ERC Co-G WaterWalking #616346 and Labex CEBA grants for AK and a BMIC PhD fellowship to
331 WT.

332

333 **Author contributions:** AK and WT conceived the work, WT collected samples for sequencing, WT
334 and DA performed analyses of gene expression, WT and AD performed RNAi screen, WT performed
335 behavioural and statistical test, WT and AK wrote the manuscript.

336

337 **References**

- 338 1 Darwin, C. *The descent of man, and selection in relation to sex*. (J. Murray, 1871).
- 339 2 Fisher, R. A. *The genetical theory of natural selection*. (The Clarendon Press, 1930).
- 340 3 Lavine, L., Gotoh, H., Brent, C. S., Dworkin, I. & Emlen, D. J. Exaggerated Trait Growth in Insects.
341 *Annu Rev Entomol* **60**, 453-472, doi:10.1146/annurev-ento-010814-021045 (2015).

- 342 4 huxley, J. S. *Problems of Relative Growth*. (1932).
- 343 5 Shingleton, A. W. & Frankino, W. A. The (ongoing) problem of relative growth. *Curr Opin Insect Sci* **25**,
344 9-19, doi:10.1016/j.cois.2017.10.001 (2018).
- 345 6 Emlen, D. J. The Evolution of Animal Weapons. *Annu Rev Ecol Evol S* **39**, 387-413,
346 doi:10.1146/annurev.ecolsys.39.110707.173502 (2008).
- 347 7 Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I. & Lavine, L. C. A mechanism of extreme growth and
348 reliable signaling in sexually selected ornaments and weapons. *Science* **337**, 860-864,
349 doi:10.1126/science.1224286 (2012).
- 350 8 Shingleton, A. W., Frankino, W. A., Flatt, T., Nijhout, H. F. & Emlen, D. J. Size and shape: the
351 developmental regulation of static allometry in insects. *Bioessays* **29**, 536-548, doi:10.1002/bies.20584
352 (2007).
- 353 9 Emlen, D. J. & Nijhout, H. F. The development and evolution of exaggerated morphologies in insects.
354 *Annual Review of Entomology* **45**, 661-708 (2000).
- 355 10 Casasa, S., Schwab, D. B. & Moczek, A. P. Developmental regulation and evolution of scaling: novel
356 insights through the study of Onthophagus beetles. *Curr Opin Insect Sci* **19**, 52-60,
357 doi:10.1016/j.cois.2016.11.004 (2017).
- 358 11 Toubiana, W. & Khila, A. The benefits of expanding studies of trait exaggeration to hemimetabolous
359 insects and beyond morphology. *Curr Opin Genet Dev* **39**, 14-20, doi:10.1016/j.gde.2016.05.015 (2016).
- 360 12 Houle, D., Jones, L. T., Fortune, R. & Sztepanacz, J. L. Why does allometry evolve so slowly? *Integr*
361 *Comp Biol* (2019).
- 362 13 Voje, K. L., Hansen, T. F., Egset, C. K., Bolstad, G. H. & Pelabon, C. Allometric Constraints and the
363 Evolution of Allometry. *Evolution* **68**, 866-885, doi:10.1111/evo.12312 (2014).
- 364 14 Bolstad, G. H. *et al.* Complex constraints on allometry revealed by artificial selection on the wing of
365 *Drosophila melanogaster*. *P Natl Acad Sci USA* **112**, 13284-13289, doi:10.1073/pnas.1505357112 (2015).
- 366 15 Harrison, J. F. Evolvability and nonevolvability of allometric slopes. *P Natl Acad Sci USA* **112**, 13426-
367 13427, doi:10.1073/pnas.1517621112 (2015).
- 368 16 Huxley, J. S. Constant differential growth ratios and their significance. *Nature* **114**, 895-896, doi:DOI
369 10.1038/114895a0 (1924).
- 370 17 Tobler, A. & Nijhout, H. F. A Switch in the Control of Growth of the Wing Imaginal Disks of *Manduca*
371 *sexta*. *Plos One* **5**, doi:ARTN e10723
372 10.1371/journal.pone.0010723 (2010).
- 373 18 Moczek, A. P. & Emlen, D. J. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do
374 alternative reproductive tactics favour alternative phenotypes? *Anim Behav* **59**, 459-466,
375 doi:10.1006/anbe.1999.1342 (2000).
- 376 19 Kupper, C. *et al.* A supergene determines highly divergent male reproductive morphs in the ruff. *Nature*
377 *Genetics* **48**, 79-+, doi:10.1038/ng.3443 (2016).
- 378 20 Lamichhaney, S. *et al.* Structural genomic changes underlie alternative reproductive strategies in the ruff
379 (*Philomachus pugnax*). *Nature Genetics* **48**, 84-+, doi:10.1038/ng.3430 (2016).
- 380 21 Toubiana, W. & Khila, A. Fluctuating selection strength and intense male competition underlie variation
381 and exaggeration of a water strider's male weapon. *Proc Biol Sci* **286**, 20182400,
382 doi:10.1098/rspb.2018.2400 (2019).

- 383 22 Andersen, N. M. *The semiaquatic bugs (Hemiptera: Gerromorpha)*. Vol. - Entomograph Vol. 3.
384 (Scandinavian Science Press LTD., 1982).
- 385 23 Akam, M. Arthropods: Developmental diversity within a (super) phylum. *P Natl Acad Sci USA* **97**, 4438-
386 4441 (2000).
- 387 24 Schram, F. R. & Koenemann, S. Developmental genetics and arthropod evolution: part 1, on legs. *Evol*
388 *Dev* **3**, 343-354 (2001).
- 389 25 Zahavi, A. Mate Selection - Selection for a Handicap. *J Theor Biol* **53**, 205-214, doi:Doi 10.1016/0022-
390 5193(75)90111-3 (1975).
- 391 26 Biernaskie, J. M., Grafen, A. & Perry, J. C. The evolution of index signals to avoid the cost of dishonesty.
392 *P R Soc B* **281**, doi:ARTN 20140876
393 10.1098/rspb.2014.0876 (2014).
- 394 27 Andersson, O., Reissmann, E. & Ibanez, C. F. Growth differentiation factor 11 signals through the
395 transforming growth factor-beta receptor ALK5 to regionalize the anterior-posterior axis. *Embo Rep* **7**,
396 831-837, doi:10.1038/sj.embor.7400752 (2006).
- 397 28 Jamaiyar, A. *et al.* The versatility and paradox of GDF 11. *Pharmacol Therapeut* **175**, 28-34,
398 doi:10.1016/j.pharmthera.2017.02.032 (2017).
- 399 29 Khila, A., Abouheif, E. & Rowe, L. Evolution of a novel appendage ground plan in water striders is
400 driven by changes in the Hox gene Ultrabithorax. *PLoS Genet* **5**, e1000583,
401 doi:10.1371/journal.pgen.1000583 (2009).
- 402 30 Zinna, R. *et al.* Sexual dimorphism and heightened conditional expression in a sexually selected weapon
403 in the Asian rhinoceros beetle. *Molecular Ecology* **27**, 5049-5072, doi:10.1111/mec.14907 (2018).
- 404 31 Gotoh, H. *et al.* Developmental Link between Sex and Nutrition; doublesex Regulates Sex-Specific
405 Mandible Growth via Juvenile Hormone Signaling in Stag Beetles. *Plos Genetics* **10**, doi:ARTN
406 e1004098
407 10.1371/journal.pgen.1004098 (2014).
- 408 32 Gotoh, H. *et al.* Juvenile Hormone Regulates Extreme Mandible Growth in Male Stag Beetles. *Plos One*
409 **6**, doi:ARTN e21139
410 10.1371/journal.pone.0021139 (2011).
- 411 33 Berger, D., Walters, R. & Gotthard, K. What limits insect fecundity? Body size- and temperature-
412 dependent egg maturation and oviposition in a butterfly. *Funct Ecol* **22**, 523-529, doi:10.1111/j.1365-
413 2435.2008.01392.x (2008).
- 414 34 Mclain, D. K., Lanier, D. L. & Marsh, N. B. Effects of Female Size, Mate Size, and Number of
415 Copulations on Fecundity, Fertility, and Longevity of *Nezara-Viridula* (Hemiptera, Pentatomidae). *Ann*
416 *Entomol Soc Am* **83**, 1130-1136, doi:DOI 10.1093/aesa/83.6.1130 (1990).
- 417 35 Arnqvist, G. & Rowe, L. *Sexual conflict*. (Princeton University Press, 2005).
- 418 36 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq
419 data with DESeq2. *Genome Biol* **15**, 550, doi:10.1186/s13059-014-0550-8 (2014).
- 420 37 Suzuki, R. & Shimodaira, H. Pvcust: an R package for assessing the uncertainty in hierarchical
421 clustering. *Bioinformatics* **22**, 1540-1542, doi:10.1093/bioinformatics/btl117 (2006).
- 422 38 Refki, P. N., Armisen, D., Crumiere, A. J. J., Viala, S. & Khila, A. Emergence of tissue sensitivity to Hox
423 protein levels underlies the evolution of an adaptive morphological trait. *Developmental Biology* **392**,
424 441-453, doi:10.1016/j.ydbio.2014.05.021 (2014).

- 425 39 Rubin, G. M. & Spradling, A. C. Genetic transformation of *Drosophila* with transposable element vectors.
426 *Science* **218**, 348-353 (1982).
- 427 40 Khila, A., Abouheif, E. & Rowe, L. Comparative Functional Analyses of Ultrabithorax Reveal Multiple
428 Steps and Paths to Diversification of Legs in the Adaptive Radiation of Semi-Aquatic Insects. *Evolution*,
429 doi:10.1111/evo.12444 (2014).
- 430 41 Warton, D. I., Duursma, R. A., Falster, D. S. & Taskinen, S. smatr 3-an R package for estimation and
431 inference about allometric lines. *Methods Ecol Evol* **3**, 257-259, doi:10.1111/j.2041-210X.2011.00153.x
432 (2012).
433
434

435 **Figure legends**

436
437 **Figure 1:** Growth dynamics of male exaggerated third legs in *M. longipes*. **(A)** Adult large and small
438 males and a female showing final growth. **(B)** Leg length variation during post-embryonic
439 development (nymphal development) in males and females. Each line represents leg growth dynamics
440 of a single individual during nymphal development until adulthood. **(C)** Static allometries between
441 body length and the three pairs of legs in males and females. Power law regressions were fitted to the
442 raw data to represent the static allometric equation $y=bx^a$. Differences in static allometry parameters
443 were tested on log-transformed data in²¹.
444

445 **Figure 2:** Signature of trait exaggeration among leg-biased genes. **(A)** PCA analysis of expressed
446 genes in male and female legs separately. **(B-C)** Venn-diagrams illustrate the number of leg-biased
447 genes shared in females (purple) and males (blue), both for up-regulated genes in the third **(B)** and first
448 legs **(C)**. The size of the diagrams is proportional to the number of leg-biased genes (fold-change >
449 1.5). Heatmaps and hierarchical clustering show similarity in average expression between legs and
450 sexes for the set of leg-biased genes specific to females (purple) and males (blue).
451

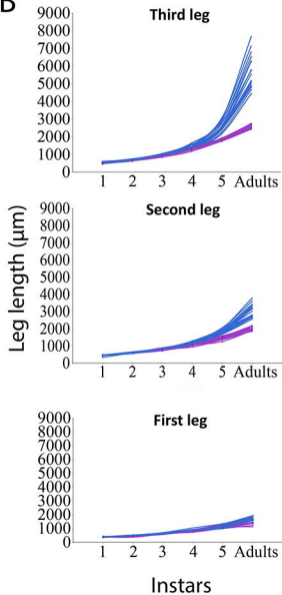
452 **Figure 3:** Effect of RNAi against BMP11 and Ubx on adult leg length (μm), body length (μm) and
453 scaling relationships. **(A)** Effect of BMP11 and Ubx RNAi in adult male and female leg length (mean
454 and variance) across the three legs. Statistical analyses can be found in Supplementary table 6. **(B)**
455 Effect of BMP11 and Ubx RNAi on adult male and female body length. Statistical analyses can be
456 found in Supplementary table 6. **(C)** Comparisons of adult static allometries between controls (grey),
457 BMP11 RNAi (red) and Ubx RNAi (blue) in *M. longipes* males and females. Male and female static
458 allometries of *M. pulchella* natural population (black) is also shown for evolutionary comparison.
459 Power law regressions were fitted to the raw dataset. Statistical analyses were also performed on log-
460 transformed data and can be found in Supplementary table 6.
461

462 **Figure 4:** BMP11 and Ubx defects on male reproductive behaviour. **(A)** Defects of BMP11 and Ubx
463 knockdowns on male fighting frequency compared to control individuals. **(B)** Defects of BMP11 and
464 Ubx knockdowns on male fighting duration compared to control individuals.
465
466
467
468
469

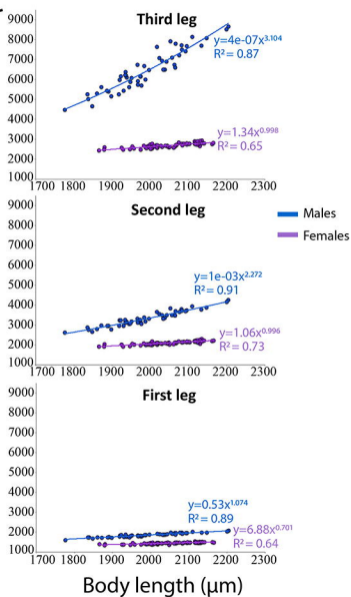
A

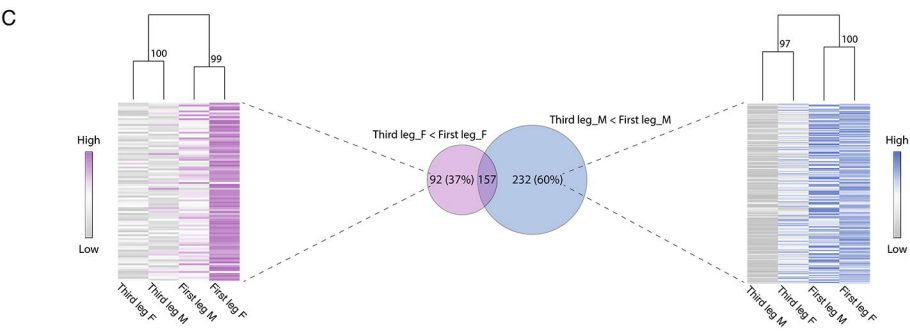
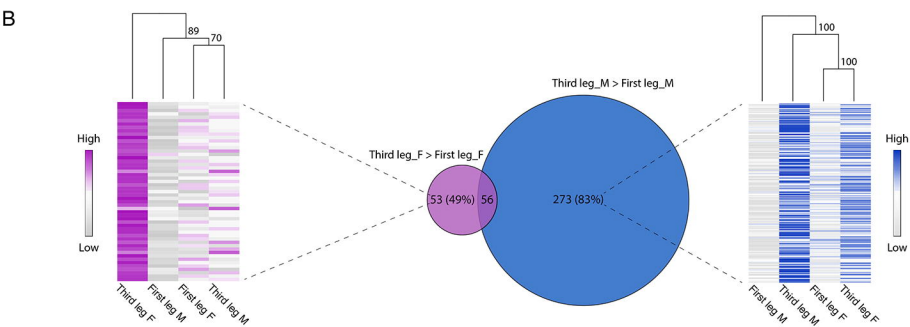
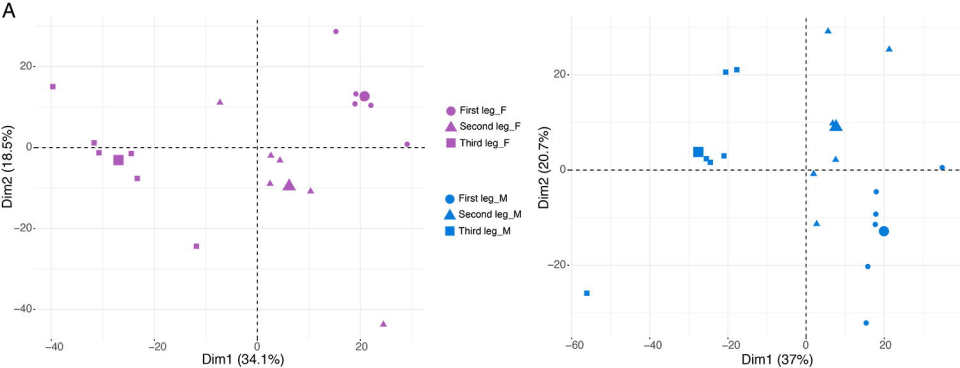


B

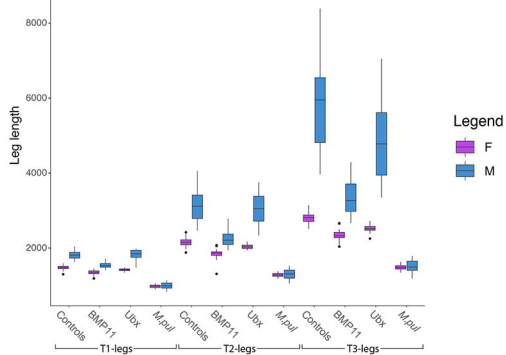


C

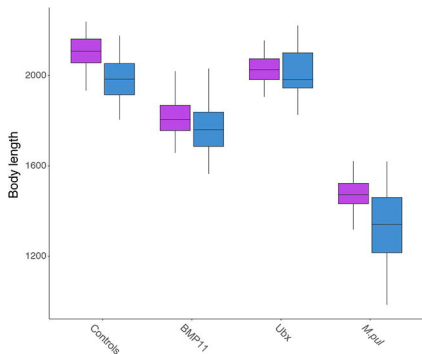




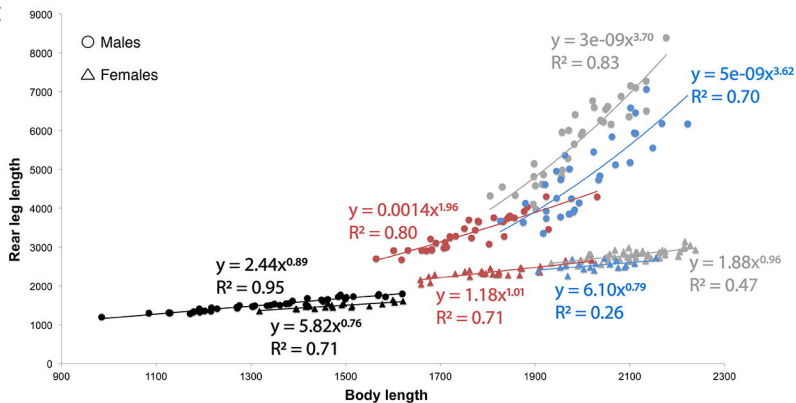
A



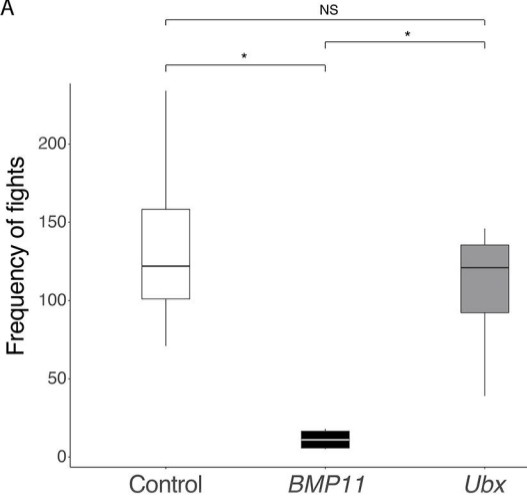
B



C



A



B

