

1 **Fluctuating selection strength and intense male competition underlie variation and**
2 **exaggeration of a water strider's male weapon**

3
4
5

6 **Authors:** William Toubiana¹ and Abderrahman Khila^{1*}

7

8 *** Corresponding author:** abderrahman.khila@ens-lyon.fr

9

10

11

12 **Author affiliations:**

13 ¹ Institut de Génomique Fonctionnelle de Lyon, Université de Lyon, Université Claude
14 Bernard Lyon 1, CNRS UMR 5242, Ecole Normale Supérieure de Lyon, 46, allée d'Italie, 69364
15 Lyon Cedex 07, France

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33 **Abstract:**

34 Sexually selected traits can reach high degrees of expression and variation under directional
35 selection. A growing number of studies suggest that such selection can vary in space, time
36 and form within and between populations. However, the impact of these fluctuations on
37 sexual trait expression is poorly understood. The water strider *Microvelia longipes* displays a
38 striking case of exaggeration and phenotypic variation where males display extreme
39 differences in the size of their rear legs. To study the origin and maintenance of this
40 exaggerated trait, we conducted comparative behavioral and morphometric experiments in
41 a sample of *Microvelia* species. We uncovered differences both in the mating behavior and
42 the degree of sexual dimorphism across these species. Interestingly, *M. longipes* evolved a
43 specific mating behavior where males compete for egg-laying sites, consisting of small
44 floating objects, to intercept and copulate with gravid females. Field observations revealed
45 rapid fluctuation in *M. longipes* habitat stability and the abundance of egg-laying sites.
46 Through male-male competition assays, we demonstrated that male rear legs are used as
47 weapons to dominate egg-laying sites and that intense competition is associated with the
48 evolution of rear leg length exaggeration. Paternity tests using genetic markers
49 demonstrated that small males could only fertilize about 5% of the eggs when egg-laying
50 sites are limiting, whereas this proportion increased to about 20% when egg-laying sites
51 become abundant. Furthermore, diet manipulation and artificial selection experiments also
52 showed that the exaggerated leg length in *M. longipes* males is influenced by both genetic
53 and nutritional factors. Collectively, our results highlight how fluctuation in the strength of
54 directional sexual selection, through changes in the intensity of male competition, can drive
55 the exaggeration and phenotypic variation in this weapon trait.

56
57
58
59
60
61
62
63
64

65 Introduction

66 Phenotypic variation is central to the process of evolution [1], and understanding the
67 mechanisms of its emergence and persistence in natural populations remains at the
68 forefront of evolutionary biology studies [2]. Sexually selected traits represent some of the
69 primary examples illustrating both intra- and interspecies phenotypic variation [3, 4]. Males
70 in both vertebrates and invertebrates are known to wield extravagant phenotypes that can
71 differ in their nature, location, size, and shape [3-5]. Examples include deer antlers, beetle
72 horns, eyestalks in some flies, pseudoscorpion antennae and harlequin beetle legs. Some
73 males of these species can develop degrees of trait expression so high that they appear
74 exaggerated compared to other body parts or other homologous structures in the other sex
75 [6]. A central prediction for these exaggerated traits to evolve is that only large individuals
76 can afford to bear them, which can be a good indicator of body size and thus act as an
77 honest signal for male quality [7-9]. Under this prediction, females will favor males with the
78 highest trait expression, thus imposing strong directional selection in favor of trait
79 exaggeration [3, 10]. In other situations, the trait is used as a weapon in male-male
80 competition with its size being a good predictor for the outcome of the contest over access
81 to females [11-14].

82 In these examples, sexual selection is thought to be directional and persistent over
83 time [9, 12, 15]. These traits are also known to be subject to survivorship costs, which
84 constrain their degree of expression resulting in a net stabilizing selection. These
85 observations raise important questions regarding the maintenance of phenotypic variation
86 in natural populations [3, 9, 15-21]. A growing number of studies suggest that selection may
87 not be as consistent over time and space, and that environmental changes may influence the
88 strength, direction, and form of sexual selection [22-26]. These fluctuations in selection may,
89 in turn, favor the elevated plastic response and genetic variation observed in sexual traits,
90 possibly influencing their variation and evolution over time [9, 21-23]. Studies assessing the
91 interplay between selection, genetics and plasticity, within the context of a changing
92 environment are therefore crucial to the general understanding of the origin and
93 maintenance of highly variable exaggerated sexual traits.

94 Here we focus on a novel model system, the water strider *Microvelia longipes*, that
95 displays a strong sexual dimorphism where males have evolved both longer and more
96 variable rear legs than females [27]. The genus *Microvelia* (Heteroptera, Gerromorpha,

97 Veliidae) comprises some 170 species of small water striders distributed worldwide and
98 occupying various fresh water habitats including temporary rain puddles and stable large
99 water bodies [27]. First we reconstructed phylogenetic relationships of five *Microvelia*
100 species and compared their degree of dimorphism, scaling relationships between leg and
101 body length, and various aspects of mating behavior. We report a clear association between
102 the intensity of male competition and the evolution of trait exaggeration in *M. longipes*
103 males. We then determined the fitness advantages of these exaggerated legs through
104 fertilization success performed under selective conditions reflecting fluctuations in their
105 natural environment. Finally, we assessed the contribution of the strength of sexual
106 selection, genetic variation, and phenotypic plasticity to the variation of exaggerated rear
107 legs in *M. longipes* males.

108

109 **Results and discussion**

110 **Sexual Dimorphism and scaling relationships in *Microvelia* species**

111 We found a considerable inter-species variation in the degree of sexual dimorphism
112 within the *Microvelia* genus (Figure 1). Measurements of various body parts revealed
113 dimorphism in average body length, leg length, and the scaling relationship between these
114 two traits (Figure 1B; Supplementary table 1). In some species, such as *M. americana* and *M.*
115 *paludicola*, the dimorphism in leg and body length is small, whereas in others such as *M.*
116 *longipes*, the dimorphism is spectacular (Figure 1A). The extreme leg elongation found in *M.*
117 *longipes* males originates from the evolution of hyperallometry where the allometric
118 coefficient is significantly higher than 1 and reaches a value of 3.2 – one of the highest
119 known (Figure 1B; Supplementary table 1) [5, 28]. In contrast, *M. longipes* females and both
120 sexes of all other species show scaling relationships between leg and body length that are
121 isometric or near isometry (Figure 1B; Supplementary table 1). Taking the two traits
122 individually, *M. longipes* male legs are both significantly longer and more variable than
123 female legs (Figure 2A, B; Supplementary table 1). In contrast, *M. longipes* body size is
124 significantly more variable in males than in females, but average body length is not
125 significantly different between the sexes (Figure 2A, C; Supplementary table 1). Despite
126 these major differences, both sexes presented leg and body length distributions that were
127 not significantly different from normality (Supplementary table 2).

128 Finally, we found that the males of three *Microvelia* species (*Microvelia* sp., *M. americana*
129 and *M. paludicola*) evolved prominent spikes on the rear legs indicative of a function in
130 grasping females during pre-mating struggles [29] (Figure 1A). Overall, these analyses
131 indicate that the evolution of hypervariable exaggerated legs in *M. longipes* males results
132 from the high variance in body length and the associated hyperallometric relationship with
133 leg length (Figure 1 and 2). In *M. pulchella*, despite the high variation in male body length,
134 the near isometric relationship between leg and body length makes their legs less
135 exaggerated and less variable than *M. longipes* males (Figure 1B; Supplementary table 1).
136 Moreover, the diversity in sexual dimorphism between *Microvelia* species does not seem to
137 follow any particular phylogenetic pattern (Figure 1), suggesting that variation in the
138 ecology, behavior, or mating systems may play a role in the divergence of the sexes in these
139 species.

140

141 **Mating systems in *Microvelia* species.**

142 We characterized mating systems and sexual interactions in all five species to better
143 understand the differences in sexual dimorphism (Supplementary figure 2). In nature, the
144 *Microvelia* genus comprises species that occupy a wide variety of habitats [27]. Most species
145 live nearshore, in stagnant, large water bodies [27]. Some species, like *M. longipes*, *M.*
146 *pulchella* or *Microvelia* sp. are gregarious and specialize in small temporary puddles filled
147 with rainwater in tropical South America [27, 30]. Behavioral observations both in the wild
148 and in laboratory-recreated puddles revealed that *M. longipes* males are highly territorial
149 and tend to aggressively guard floating objects consisting of small twigs or pieces of dead
150 leaves (Supplementary figure 3). These are egg-laying sites where males signal to attract
151 females, by vibrating their rear-legs and pounding with their genitalia on the water surface
152 producing ripples (Supplementary videos 1 and 2). We hereafter refer to these objects as
153 egg-laying floaters. When a female approaches the floater, the dominating male switches
154 from signaling to a courtship behavior. The female inspects the floater and either leaves or
155 mates without any resistance with the courting male and immediately lays 1 to 4 eggs (n=26
156 mating events) (Supplementary figure 3; Supplementary video 2). The male then initiates an
157 aggressive guarding behavior by turning around the egg-laying female and chasing other
158 approaching males to (Supplementary video 2). After egg-laying the female leaves and the
159 male initiates another cycle of signaling on the same floater. During this entire process,

160 other males constantly challenge the signaling male in an attempt to dominate the floater.
161 During these contests, the dominant and the challenging male fight back-to-back by kicking
162 each other with their rear-legs until one of them is chased away (Supplementary video 2).
163 We also observed that females could lay eggs in the mud at the margin of the puddle and
164 that males attempt to mate outside floaters by jumping on female's back randomly in the
165 puddle.

166 *M. pulchella*, the sister species of *M. longipes* (Figure 1A), is also found in small
167 temporary puddles [30] and displays a highly similar mating behavior despite the lack of
168 rear-leg exaggeration (Figure 1C). Males of *M. pulchella* compete for egg-laying floaters,
169 fight with their rear-legs, and generate ripples to attract females. Also like *M. longipes*,
170 females of *M. pulchella* lay their eggs both on floaters and in the mud. In spite of similarities
171 in their mating behavior, these two sister species display significant morphological
172 differences, raising the question as to which factors drove the evolution of trait exaggeration
173 in *M. longipes*.

174 In the three other species, *M. americana*, *M. paludicola*, and *Microvelia sp.*, males
175 possess grasping spines on their rear-leg femurs (Figure 1A) and actively harass females in an
176 attempt to mate. Females consistently struggle through vigorous shaking, frequently
177 resulting in the rejection of the male. Males of these three species also fight occasionally but
178 the fights do not seem to result in the dominance of any particular localized resource (Figure
179 1C; Supplementary figure 2). *M. americana* and *M. paludicola* females lay eggs exclusively on
180 water margins while females in *Microvelia sp.* lay eggs randomly either on floaters or water
181 margins, but do not do so immediately after mating (Figure 1C; Supplementary figure 2).
182 Altogether, these data show that the behavior, consisting of contests using the rear-legs,
183 predates the origin of exaggerated leg length and could therefore be necessary but not
184 sufficient for its evolution. Moreover, differences in egg-laying habits may have driven the
185 diversity in male mating strategies and sexual dimorphism in the *Microvelia* genus. In small
186 temporary habitats, eggs laid in the mud are at high risk of desiccation when water levels go
187 down, and nymphs tend to drown at hatching when water levels go up, something we
188 frequently observe in laboratory conditions. Egg-laying behavior on floating objects, which
189 remain on the surface despite fluctuating water levels, is likely an adaptation to the fast-
190 changing state of the habitat. Interestingly, male behavior consisting of dominating these
191 egg-laying floaters is observed only in species where females lay eggs just after mating,

192 indicative of the high fitness value in accessing them. This behavior is also associated with
193 the high body length variation in *M. longipes* and *M. pulchella* males (Figure 1B), suggesting
194 a link between body size variation and competition for oviposition sites.

195

196 **Intensity of male competition in *M. longipes* compared to *M. pulchella***

197 In order to evaluate the contribution of exaggerated leg length to male mating
198 success, we tested whether a correlation existed between male leg length and their ability to
199 dominate egg-laying sites. We found increased rear leg length to be strongly correlated with
200 the fighting outcome (ANOVA, $F(1, 13) = 144.6$, $p < 0.01$), where the males with longer legs
201 won 97% of the fights ($n = 75$ fights) and dominated the floater (Figure 3A). We also observed
202 this male dominance over egg-laying sites in *M. pulchella*, which did not evolve leg
203 exaggeration. We therefore hypothesized that male phenotypic differences between *M.*
204 *longipes* and *M. pulchella* could be driven by differences in the intensity of male
205 competition. When we measured the intensity of male competition in standardized space
206 conditions, we found that *M. longipes* males fought on average 8 times more frequently
207 than *M. pulchella* males in a period of 1 hour (Figure 3B, Supplementary table 3). This
208 indicates that male competition is significantly higher in *M. longipes* than in *M. pulchella*.
209 More importantly, 81 % of *M. longipes* fights occurred on the floaters (Figure 3B,
210 Supplementary table 3) whereas *M. pulchella* males' fights occurred randomly on and away
211 from floaters (Figure 3B, Supplementary table 3). The same result was reached when we
212 repeated this experiment in standardized density conditions taking into account size
213 differences between the two species (Supplementary table 3). These data demonstrate first
214 that increased rear leg length in *M. longipes* males favors male dominance over egg-laying
215 sites to better intercept gravid females. While both *M. longipes* and *M. pulchella* males
216 intercept females and compete on those egg-laying sites, competition intensity for egg-
217 laying sites is almost an order of magnitude higher in *M. longipes*. A primary difference
218 between the ecology of these two species is that *M. longipes* specializes in rainwater-filled
219 small puddles while *M. pulchella* is a generalist that can be found in both temporary and
220 more stable water bodies ([31, 32] and personal field observations). This difference in niche
221 specialization has two major impacts on *M. longipes* population structure. First, *M. longipes*
222 populations can reach very high densities confined in a small space, something we observed
223 frequently in the wild and which is not the case for *M. pulchella*. Second, because the water

224 level in the puddle can change rapidly (Supplementary figure 4), floaters represent the safest
225 substrate in terms of survival of the progeny. This may explain why females bounce the
226 floater up and down before they copulate and lay eggs (Supplementary video 2), and why *M.*
227 *longipes* males are so aggressive in dominating these floaters. The situation is different for
228 *M. pulchella* due to the higher stability of the habitat, making floaters less critical and the
229 survival of eggs in the mud more likely. These ecological conditions favoring high-density
230 populations and floating objects as the more suitable egg-laying substrate may have at least
231 contributed to the high competitiveness observed in *M. longipes*, and thus acted as a driving
232 force for the evolution of the exaggerated leg length for use as a weapon. Both empirical
233 and theoretical models suggest that population density can influence aggressiveness and the
234 intensity of sexual selection [33], and our data show how increased competitiveness can
235 drive secondary sexual traits to reach dramatic levels of expression.

236

237 **Effect of exaggerated leg length on male reproductive fitness in *M. longipes***

238 Post-mating competition is widespread in insects [34], including water striders [35-
239 38], and can strongly alter the outcome of pre-mating strategies [34, 39]. Field observations
240 also indicate that the state of the habitat occupied by *M. longipes* can fluctuate rapidly and,
241 sometimes, the water can evaporate entirely in days (Supplementary figure 4). Moreover,
242 the amount of egg-laying resources is highly variable from one puddle to another and can
243 additionally fluctuate with water level (personal observations from the field). We
244 hypothesized that these rapidly changing conditions will influence competition and mating
245 success across the distribution of male phenotypes. To test this hypothesis, we conducted
246 paternity tests using *M. longipes* lines that are homozygous for distinct microsatellite
247 markers that can reveal the identity of the parents (see methods for more details). We set
248 the experiment such that heterozygous progeny could only originate from eggs fertilized by
249 small males. Because egg-laying floaters represent the primary resource that males
250 dominate to intercept gravid females, we designed a first treatment where floaters were
251 limiting (3 floater for 6 large and 6 small males) and another treatment where floaters were
252 abundant (20 floaters for 6 large and 6 small males). We also genotyped the progeny from
253 eggs laid in the mud to determine mating success of different male phenotypes in contexts
254 other than the dominance of floaters. In all replicates of each treatment, females laid the
255 majority of their eggs on floaters regardless of whether floaters are limiting (91% of a total

256 of 512 eggs) or abundant (71% of a total of 500 eggs) (Figure 3D). However, females laid on
257 average three times more eggs on the mud when floaters were limiting (Supplementary
258 table 4). In the condition where floaters were limiting, small males fertilized 4.6% (15 eggs
259 of a total of 357 eggs) of the eggs laid on floaters and 25% of the eggs laid in the mud (35
260 eggs of a total of 143 eggs) on average (Figure 3D; Supplementary table 4). Interestingly, the
261 number of eggs sired by small males was more than twice higher in the mud than on floaters
262 (Figure 3D; Supplementary table 4). This suggests that when the dominance of floaters by
263 small males is limited, they primarily achieve egg fertilization by mating outside floaters. In
264 the condition of abundant floaters, the proportion of eggs fertilized by small males on
265 floaters increased significantly to 19% (96 eggs of a total of 468 eggs) (Figure 3D;
266 Supplementary table 4), while that outside floaters remained unchanged (11 eggs of a total
267 of 44 eggs) (Figure 3D; Supplementary table 4). In contrast to the treatment with limiting
268 floaters, here the number of eggs fertilized by small males is almost nine times higher on
269 floaters than in the mud (Figure 3D; Supplementary table 4). These results show that small
270 males can sire significantly more progeny when egg-laying sites are abundant but can also
271 mate outside these egg-laying sites when floaters are limiting. Therefore, sexual selection is
272 strong in favor of large males with long legs but can become relaxed in conditions where
273 egg-laying sites are abundant. Rapid changes in water level and high heterogeneity between
274 puddles are intrinsic to the life history of this species and are expected to cause variation in
275 the amount of accessible egg-laying floaters over time and space. This fluctuating selection is
276 therefore likely to influence the strength of competition and mating success and contribute
277 to the high phenotypic variation found in *M. longipes* natural populations.

278

279 **Environmental and genetic contributions to male rear leg variation**

280 We have shown that possible fluctuation in the strength of sexual selection may
281 favor phenotypic variation, however its impact on the mechanistic underpinnings of
282 phenotypic variation in *M. longipes* males is unknown. We therefore tested how genetic
283 variation and phenotypic plasticity contribute to the maintenance of high variation in *M.*
284 *longipes* male leg length. Artificially selected large and small male lines, generated through
285 15 sib-sib successive crosses from a natural population, showed a shifted distribution of
286 male leg length towards the respective extreme phenotypes of the distribution (Figure 4A).
287 The difference between these two lines held for both absolute and relative leg length, but

288 the allometric coefficient remained, nonetheless, unchanged (Supplementary figure 5;
289 Supplementary table 5). This shows that genotypic variation contributes to the variation in
290 both rear leg length and body size.

291 Next, we tested the reaction norm of one of these inbred lines in poor and rich
292 nutritional condition. Despite near identical genotype, individuals reared in poor condition
293 developed shorter legs than individuals reared in rich condition such that the distributions of
294 the two treatments were almost non-overlapping (Figure 4B, Supplementary figure 6;
295 Supplementary table 5). Importantly, this difference in leg length between the two
296 treatments resulted mostly from differences in overall body size (t-test body length: $t =$
297 10.5643 , $df = 25.274$, $p\text{-value} = 9.244e\text{-}11$) but not in the scaling relationship as we failed to
298 detect any significant difference in the allometric coefficient or the intercept between rich
299 and poor conditions (Supplementary figure 6; Supplementary table 5). The same result was
300 reached when we tested condition dependence in a laboratory population where no specific
301 selection has been applied, although some statistical tests detected a small but significant
302 difference in intercept between the two conditions (Supplementary figure 7; Supplementary
303 table 5). This difference was nonetheless not significant when using a linear model (ANOVA,
304 $F(1,88) = 2.6202$, $p\text{-value} = 0.1076$). We therefore conclude that, in *M. longipes*, male body
305 size is highly condition-dependent but the rear legs are not or they are to a small extent
306 after body size correction. Altogether, these results suggest that male leg length variation in
307 nature results from the contribution of both genetic variation and strong condition
308 dependence. The fluctuations in the amount of egg-laying floaters, combined with
309 phenotypic plasticity, is expected to result in the maintenance of a certain degree of genetic
310 variation in the population through the incomplete removal of alleles of small leg and body
311 size. However, episodes of relaxed selection are not only known to increase genetic variation
312 in the population, but also to favor the evolution of reaction norms and therefore increase
313 phenotypic plasticity [40, 41].

314

315 **Conclusions**

316 This study provides a good example of how various ecological factors influence the
317 intensity of sexual selection and ultimately the mechanisms and patterns of phenotypic
318 variation. In the genus *Microvelia*, mating systems are diverse and are likely to influence the
319 diversification of male-specific secondary sexual traits used in pre-mating copulatory

320 strategies. The intense male competition to dominate egg-laying sites in *M. longipes*, unlike
321 other *Microvelia* species, underlies the evolution of exaggerated leg length used as a
322 weapon. Dominating males that intercept and copulate with gravid females on egg-laying
323 sites gain a significant increase in their reproductive fitness by siring the majority of the eggs.
324 This intense selection on increased leg length can, however, be relaxed when egg-laying sites
325 are abundant thus allowing small males to fertilize a significant number of eggs. We have
326 also shown that plasticity in response to nutritional condition along with genetic variation
327 both contribute to the high phenotypic variation we observe in body and leg length. It is
328 possible that fluctuating selection, combined with phenotypic plasticity, both facilitate the
329 dramatic increase and maintenance of phenotypic variation in *M. longipes* compared to
330 other *Microvelia* species. It is also important to note that the fluctuating selection described
331 here (availability of egg-laying floaters) is independent of the individual condition. Therefore
332 its influence on phenotypic variation cannot be the consequence of a pre-existing increase of
333 condition-dependence, as it would be the case for fluctuating selection on food resources
334 for example. Altogether, these results point to two ways in which alleles for small male body
335 and leg size will be maintained in the population. First, because small males can sire a
336 significant number of progeny due to possible episodes of relaxed selection. Second,
337 because males with allelic combinations for low trait expression can develop larger body and
338 leg size if they experience higher nutritional condition during development. Therefore
339 condition dependence causes a non-linear relationship between genotypes and phenotypes,
340 making directional selection less efficient in depleting genetic variation. In their opinion
341 paper, Cornwallis and Uller [23] refer to this process as a “feedback loop between
342 heterogeneity, selection and phenotypic plasticity”.

343 The findings outlined here open important research avenues to gain a general
344 understanding of how sexual selection can impact phenotypic evolution. *Microvelia longipes*
345 as a new hemimetabolous insect model with an exaggerated secondary sexual trait offers
346 the opportunity to complete the substantial literature in holometabolous insects such as
347 beetles or various flies [8, 42-45]. Males of many species of water striders employ water
348 surface ripples as mating calls, and it is unknown whether females can deduce the size of the
349 male from the ripple pattern and whether this would influence female choice [27, 46-49]. In
350 addition, the number and the frequency of allelic variants underlying this trait and how they
351 may interact with the environment remains to be tested. The ease of rearing and the relative

352 short generation time make *Microvelia longipes* a powerful future model to study the extent
353 to which genetic variation and environmental stimuli influence gene expression and
354 ultimately phenotypic variation.

355

356 **Acknowledgements**

357 We thank Emília Santos and Antonin Crumière for help with collecting bugs, Felipe Moreira
358 for help with species identification, Russell Bondurianski, Locke Rowe, Kevin Parsons, Gaël
359 Yvert, François Leulier, Augustin Le Bouquin, Amélie Decaras, Cédric Finet, Aidamalia Vargas,
360 Roberto Arbore for helpful discussions and comments on the manuscript, and David Armisén
361 and Antoine Melet for help with genetic markers identification and data measurements. This
362 work was supported by an ERC-CoG# 616346 and labex CEBA to AK, and a PhD fellowship
363 from Ecole Doctorale BMIC de Lyon to W.T.

364

365 **Author contributions:** A.K. and W.T. designed research; W.T. performed research; A.K. and
366 W.T. analyzed data; and A.K. and W.T. wrote the paper

367

368 **Material & methods**

369 *Population sampling and culture*

370 *Microvelia* populations were collected during fieldwork in French Guyana in Crique Patate
371 near Cayenne. These populations were maintained at 25°C and 50% humidity. The bugs were
372 fed on either frozen or freshly euthanized crickets. Adults laid eggs on Styrofoam floaters
373 and the hatched nymphs were raised in separate tanks to avoid cannibalism.

374

375 *Measurement of *Microvelia* species and statistics*

376 Rear leg and body lengths of all *Microvelia* species were measured with a SteREO Discovery
377 V12 (Zeiss) using the Zen software. All statistical analyses were performed in RStudio
378 0.99.486. Comparisons for mean trait size and trait distributions were performed on raw
379 data whereas log-transformed data were used for scaling relationship comparisons. We used
380 Major-Axis (MA) regression to assess differences in scaling relationships (“smatr” package in
381 R, [50]). Differences in intercepts were estimated using a Wald statistic test and we used
382 Likelihood ratio test for differences in slopes [50].

383

384 *Behavioral observations and video acquisition*

385 Male and female interactions of all *Microvelia* species were observed in a recreated small
386 puddle, using local mud, and were filmed with a Nikon digital camera D7200 with an AF-S
387 micro nikkor 105mm lens. Observations and video acquisitions were taken a couple of hours
388 after the bugs were transferred to the puddle. In *M. longipes* and *M. pulchella* male and
389 female interactions were also observed in the field.

390

391 *Microvelia phylogenetic reconstruction*

392 The phylogenetic relationships between the five *Microvelia* species used in the behavioral
393 assays was generated using the Geneious software version 7.1.9 using plugins MrBayes
394 version 3.2.6 and PhyML version 3.0, as described in [51]. The phylogenetic reconstruction
395 was performed using 14 molecular markers retrieved from in house transcriptome
396 databases: *12S RNA*; *16S RNA*; *18S RNA*; *28S RNA*; *Cytochrome Oxydase subunit I (COI)*;
397 *Cytochrome Oxydase subunit II (COII)*; *Cytochrocme Oxydase subunit III (COIII)*; *Cytochrome b*
398 *(cyt b)*; *Ultrabithorax (Ubx)*; *Sex combs reduced (Scr)*; *Gamma interferon inducible thiol*
399 *reductase (gilt)*; *Antennapedia (Antp)*; *Distal-less (dll)*; *Doublesex (dsx)*. All these markers can
400 be retrieved in GenBank using the following accession numbers: (Will be provided before
401 publication). Phylogenetic reconstruction was performed using MrBayes version 3.2.6 and
402 PhyML version 3.0 in Geneious 7.1.9 as described in [52]. Concatenation of sequence
403 alignments and phylogenetic tree in Newick format are also available in the Dryad Digital
404 Repository.

405

406 *Fight frequency assay*

407 To compare the number of fights between males of *M. longipes* and *M. pulchella*, we
408 isolated twenty-five adult males and females over a period of two days. Both sexes were
409 then mixed together in the puddle during 30 minutes before observation. The number of
410 fights on and outside floaters was counted for a period of one hour. We repeated the
411 experiment the following day with the same males and females kept mixed together
412 overnight. Finally, in order to account for population density, because of size differences
413 between the two species, we calculated the number of fights in a reduced sample of ten
414 males and ten females in *M. longipes*.

415

416 *Artificial selection experiment, phenotyping and line sequencing*

417 We assessed the genetic contribution of rear leg length variation in males by performing an
418 artificial selection experiment for long versus short-legged males. Individual males from the
419 French Guyana natural population were selected for their absolute rear leg sizes and mated
420 with random females to initiate the successive sib-sib crosses. After fifteen generations of
421 sib-sib inbreeding, two populations selected for extreme phenotypes were amplified over
422 two generations before phenotyping.

423

424 *Condition-dependence experiment*

425 First instar nymphs were collected just after hatching and individuals were reared attributed
426 in either poor or rich nutritional condition. In the poor condition, hundred first instar
427 nymphs of the long-legged inbred line were fed everyday with ten crickets during the first
428 two nymphal instars, followed by only three cricket legs until adulthood. In the rich
429 condition fifty individuals of the same line were fed with ten crickets, changed everyday,
430 over their entire nymphal development until adulthood. In a second experiment we tested
431 the effect of condition in an independent set of individuals from the lab population. This
432 experiment was performed on three replicates per condition, with fifty individuals per
433 condition. Replicates were then pooled for the analysis. We started the poor condition by
434 feeding the first two nymphal instars with height crickets everyday and then switched to one
435 small cricket every two days until they reached adulthood. Individuals from the rich
436 condition were fed during their entire nymphal development with eight crickets everyday.

437

438 *Microsatellite development*

439 DNA from *M. longipes* was extracted from ten male and female individuals from the lab
440 population. Insects were first frozen in liquid nitrogen before DNA extraction with the
441 Genomic DNA Buffer Set kit from Qiagen. We used 12µg of DNA for sequencing on an Ion-
442 Torrent Sequencer machine (Sequencing Platform IGFL, Lyon, France) generating 3.7M
443 reads with median size of 317 bp.

444 We used the program Exact Tandem Repeat Analyzer 1.0 (available from
445 <ftp://ftp.akdeniz.edu.tr/>) in order to identify reads containing microsatellite repeats [53].
446 The software also provided primers for microsatellite amplification (Supplementary table 6).
447 Forty-seven markers of various tandem repeats were tested by PCR and forty of them were

448 successfully amplified from an aliquot of the genomic extraction used for the Ion-Torrent
449 sequencing (Supplementary table 7). We also tested the same pairs of primers on DNA
450 extraction from single individuals of the stock population (Supplementary table 7), as
451 described in [54].

452

453 In order to identify polymorphic microsatellites, we first performed PCRs of these forty
454 markers on fifteen individuals from the lab population and sent these samples for
455 genotyping (Genoscreen, Lille, France). We considered polymorphic, any marker that was
456 showing at least two genotypes in at least two individuals. Nine polymorphic reliable
457 markers could be identified, among which one of them (microsatellite 21, see
458 Supplementary table 6) was showing a polymorphism between our two lines of interest. This
459 microsatellite was genotyped in seven males and seven females from each line to confirm
460 the polymorphism prior to the paternity test experiment.

461

462 *Paternity test*

463 To assess the fertilization success of long and short-legged males, we collected six males
464 from both the short- and the long-legged inbred lines, and put them together in an artificial
465 puddle with twelve females from the long-legged inbred line. We conducted two
466 treatments, each with four replicates, where we provided twenty or three floaters in the
467 puddle to create conditions with abundant and limiting egg-laying sites, respectively. On day
468 3, the parents were collected, their DNA extracted and the microsatellite of interest
469 amplified for genotyping. We then isolated the floaters and genotyped the nymphs that
470 hatched from the floaters and those that hatched from the mud after adults and floaters
471 were removed.

472

473 **References:**

- 474 1. Darwin C. On the origin of species by means of natural selection, or, The
475 preservation of favoured races in the struggle for life. London: J. Murray; 1859. ix, [1],
476 502, 32, [1] fold. leaf of plates (32 at end advertisements) p.
- 477 2. Hall BH&BK. Variation: A Central Concept in Biology. Elsevier Academic Press
478 ed2005.
- 479 3. Andersson MB. Sexual selection. Princeton, N.J.: Princeton University Press; 1994.
480 xx, 599 p. p.
- 481 4. Darwin C. The descent of man, and selection in relation to sex. London,: J. Murray;
482 1871. 2 v. p.

- 483 5. McCullough EL, Ledger KJ, O'Brien DM, Emlen DJ. Variation in the allometry of
484 exaggerated rhinoceros beetle horns. *Anim Behav.* 2015;109:133-40. doi:
485 10.1016/j.anbehav.2015.08.013. PubMed PMID: WOS:000363268300017.
- 486 6. Emlen DJ. Reproductive contests and the evolution of extreme weaponry. In:
487 David M. Shuker LWS, editor. *The evolution of insect mating systems.* Oxford University
488 Press ed2014.
- 489 7. Zahavi A. Mate Selection - Selection for a Handicap. *J Theor Biol.* 1975;53(1):205-
490 14. doi: Doi 10.1016/0022-5193(75)90111-3. PubMed PMID: WOS:A1975AR81900012.
- 491 8. Emlen DJ, Warren IA, Johns A, Dworkin I, Lavine LC. A mechanism of extreme
492 growth and reliable signaling in sexually selected ornaments and weapons. *Science.*
493 2012;337(6096):860-4. Epub 2012/07/28. doi: 10.1126/science.1224286. PubMed
494 PMID: 22837386.
- 495 9. Rowe L, Houle D. The lek paradox and the capture of genetic variance by
496 condition dependent traits. *P R Soc B.* 1996;263(1375):1415-21. doi: DOI
497 10.1098/rspb.1996.0207. PubMed PMID: WOS:A1996VQ62200022.
- 498 10. Andersson M. Female Choice Selects for Extreme Tail Length in a Widowbird.
499 *Nature.* 1982;299(5886):818-20. doi: DOI 10.1038/299818a0. PubMed PMID:
500 WOS:A1982PM85200043.
- 501 11. Emlen DJ. The Evolution of Animal Weapons. *Annu Rev Ecol Evol S.* 2008;39:387-
502 413. doi: 10.1146/annurev.ecolsys.39.110707.173502. PubMed PMID:
503 WOS:000261725500019.
- 504 12. O'Brien DM, Katsuki M, Emlen DJ. Selection on an extreme weapon in the frog-
505 legged leaf beetle (*Sagra femorata*). *Evolution.* 2017;71(11):2584-98. doi:
506 10.1111/evo.13336. PubMed PMID: 28841226.
- 507 13. Miller CW. Sexual selection: Male-male competition. In: Losos JB, editor. *The*
508 *Princeton Guide to Evolution.* Princeton: Princeton University Press ed2013.
- 509 14. Miller CW, Emlen DJ. Dynamic effects of oviposition site on offspring sexually-
510 selected traits and scaling relationships. *Evol Ecol.* 2010;24(2):375-90. doi:
511 10.1007/s10682-009-9312-6. PubMed PMID: WOS:000274109600008.
- 512 15. Kirkpatrick M, Ryan MJ. The Evolution of Mating Preferences and the Paradox of
513 the Lek. *Nature.* 1991;350(6313):33-8. doi: DOI 10.1038/350033a0. PubMed PMID:
514 WOS:A1991FA69300054.
- 515 16. Fisher RA. *The genetical theory of natural selection.* Oxford,; The Clarendon
516 Press; 1930. xiv, 272 p. p.
- 517 17. Houle D. Comparing Evolvability and Variability of Quantitative Traits. *Genetics.*
518 1992;130(1):195-204. PubMed PMID: ISI:A1992GX54300018.
- 519 18. Radwan J. Maintenance of genetic variation in sexual ornaments: a review of the
520 mechanisms. *Genetica.* 2008;134(1):113-27. doi: 10.1007/s10709-007-9203-0. PubMed
521 PMID: WOS:000258371900012.
- 522 19. Pomiankowski A. Sexual Selection - the Handicap Principle Does Work
523 Sometimes. *Proc R Soc Ser B-Bio.* 1987;231(1262):123-45. doi: DOI
524 10.1098/rspb.1987.0038. PubMed PMID: WOS:A1987J032400008.
- 525 20. Grafen A. Sexual Selection Unhandicapped by the Fisher Process. *J Theor Biol.*
526 1990;144(4):473-516. doi: Doi 10.1016/S0022-5193(05)80087-6. PubMed PMID:
527 WOS:A1990DL42100003.
- 528 21. Pomiankowski A, Moller AP. A Resolution of the Lek Paradox. *P R Soc B.*
529 1995;260(1357):21-9. doi: DOI 10.1098/rspb.1995.0054. PubMed PMID:
530 WOS:A1995QX30200004.

- 531 22. Miller CW, Svensson EI. Sexual selection in complex environments. *Annu Rev*
532 *Entomol.* 2014;59:427-45. doi: 10.1146/annurev-ento-011613-162044. PubMed PMID:
533 24160419.
- 534 23. Cornwallis CK, Uller T. Towards an evolutionary ecology of sexual traits. *Trends*
535 *Ecol Evol.* 2010;25(3):145-52. doi: 10.1016/j.tree.2009.09.008. PubMed PMID:
536 19853321.
- 537 24. Robinson MR, Pilkington JG, Clutton-Brock TH, Pemberton JM, Kruuk LEB.
538 Environmental heterogeneity generates fluctuating selection on a secondary sexual trait.
539 *Curr Biol.* 2008;18(10):751-7. doi: 10.1016/j.cub.2008.04.059. PubMed PMID:
540 WOS:000256047600028.
- 541 25. Gosden TP, Svensson EI. Spatial and temporal dynamics in a sexual selection
542 mosaic. *Evolution.* 2008;62(4):845-56. doi: 10.1111/j.1558-5646.2008.00323.x.
543 PubMed PMID: WOS:000254640900010.
- 544 26. Johnston SE, Gratten J, Berenos C, Pilkington JG, Clutton-Brock TH, Pemberton JM,
545 et al. Life history trade-offs at a single locus maintain sexually selected genetic variation.
546 *Nature.* 2013;502(7469):93-+. doi: 10.1038/nature12489. PubMed PMID:
547 WOS:000325106000037.
- 548 27. Andersen NM. The semiaquatic bugs (Hemiptera: Gerromorpha). Klampenborg,
549 Denmark.: Scandinavian Science Press LTD.; 1982.
- 550 28. Baker RH, Wilkinson GS. Phylogenetic analysis of sexual dimorphism and eye-
551 span allometry in stalk-eyed flies (Diopsidae). *Evolution.* 2001;55(7):1373-85. PubMed
552 PMID: WOS:000170699300010.
- 553 29. Arnqvist G, Rowe L. Sexual conflict. Princeton, N.J.: Princeton University Press;
554 2005. xii, 330 p. p.
- 555 30. Da Motta FS, Moreira FFF, Crumiere AJJ, Santos ME, Khila A. A new species of
556 *Rhagovelia* Mayr, 1865 (Hemiptera: Heteroptera: Veliidae) from French Guiana, with
557 new records of Gerromorpha from the country. *Zootaxa.* 2018;4433(3):520-30. doi:
558 10.11646/zootaxa.4433.3.7. PubMed PMID: WOS:000435433900007.
- 559 31. Taylor JS, McPherson EJ. Morphological Variation and Polyvoltinism of *Microvelia*
560 *pulchella* (Heteroptera: Veliidae) in Southern Illinois, USA. *Acta Societatis Zoologicae*
561 *Bohemicae.* 1999.
- 562 32. Lanigan AC, Hyslop EJ. The aquatic and semiaquatic Hemiptera of Jamaica. *J*
563 *Freshwater Ecol.* 2011;26(2):295-7. doi: 10.1080/02705060.2011.559335. PubMed
564 PMID: WOS:000292949200016.
- 565 33. Kokko H, Rankin DJ. Lonely hearts or sex in the city? Density-dependent effects in
566 mating systems. *Philos Trans R Soc Lond B Biol Sci.* 2006;361(1466):319-34. doi:
567 10.1098/rstb.2005.1784. PubMed PMID: 16612890; PubMed Central PMCID:
568 PMC1569612.
- 569 34. Simmons LW. Sperm competition. In: Shuker DM, Simmons LW, editors. *The*
570 *evolution of insect mating systems.* Oxford University Press ed2014.
- 571 35. Arnqvist G. Mate Guarding and Sperm Displacement in the Water Strider *Gerris-*
572 *Lateralis* Schumm (Heteroptera, Gerridae). *Freshwater Biol.* 1988;19(2):269-74. doi:
573 DOI 10.1111/j.1365-2427.1988.tb00347.x. PubMed PMID: WOS:A1988M736900011.
- 574 36. Arnqvist G, Danielsson I. Copulatory behavior, genital morphology, and male
575 fertilization success in water striders. *Evolution.* 1999;53(1):147-56. doi: Doi
576 10.2307/2640927. PubMed PMID: WOS:000078927800013.
- 577 37. Arnqvist G. The evolution of water strider mating systems: causes and
578 consequences of sexual conflicts. In: Crespi B, editor. *The Evolution of Mating Systems in*
579 *Insects and Arachnids.* Cambridge University Press ed1997.

- 580 38. Devost E, Turgeon J. The combined effects of pre- and post-copulatory processes
581 are masking sexual conflict over mating rate in *Gerris buenoi*. *J Evolution Biol.*
582 2016;29(1):167-77. doi: 10.1111/jeb.12772. PubMed PMID: WOS:000368074900013.
- 583 39. Simmons LW. *Sperm Competition and Its Evolutionary Consequences in the*
584 *Insects*. Princeton University Press, Princeton, NJ ed2001.
- 585 40. Hunt BG, Ometto L, Wurm Y, Shoemaker D, Yi SV, Keller L, et al. Relaxed selection
586 is a precursor to the evolution of phenotypic plasticity. *P Natl Acad Sci USA.*
587 2011;108(38):15936-41. doi: 10.1073/pnas.1104825108. PubMed PMID:
588 WOS:000295030000056.
- 589 41. Lahti DC, Johnson NA, Ajie BC, Otto SP, Hendry AP, Blumstein DT, et al. Relaxed
590 selection in the wild. *Trends Ecol Evol.* 2009;24(9):487-96. doi:
591 10.1016/j.tree.2009.03.010. PubMed PMID: WOS:000270016800009.
- 592 42. Toubiana W, Khila A. The benefits of expanding studies of trait exaggeration to
593 hemimetabolous insects and beyond morphology. *Curr Opin Genet Dev.* 2016;39:14-20.
594 doi: 10.1016/j.gde.2016.05.015. PubMed PMID: WOS:000388922900004.
- 595 43. Moczek AP. Integrating micro- and macroevolution of development through the
596 study of horned beetles. *Heredity.* 2006;97(3):168-78. Epub 2006/07/13. doi:
597 10.1038/sj.hdy.6800871. PubMed PMID: 16835592.
- 598 44. Bonduriansky R. Condition dependence of developmental stability in the sexually
599 dimorphic fly *Telostylinus angusticollis* (Diptera: Neriidae). *J Evol Biol.* 2009;22(4):861-
600 72. Epub 2009/02/18. doi: JEB1686 [pii]
601 10.1111/j.1420-9101.2009.01686.x. PubMed PMID: 19220651.
- 602 45. Chapman T, Pomiankowski A, Fowler K. Stalk-eyed flies. *Curr Biol.*
603 2005;15(14):R533-R5. doi: DOI 10.1016/j.cub.2005.07.015. PubMed PMID:
604 WOS:000230903900006.
- 605 46. Armisen D, Refki PN, Crumiere AJJ, Viala S, Toubiana W, Khila A. Predator strike
606 shapes antipredator phenotype through new genetic interactions in water striders.
607 *Nature Communications.* 2015;6. doi: Artn 8153
608 10.1038/Ncomms9153. PubMed PMID: WOS:000363016300002.
- 609 47. Hayashi K. Alternative Mating Strategies in the Water Strider *Gerris-Elongatus*
610 (Heteroptera, Gerridae). *Behav Ecol Sociobiol.* 1985;16(4):301-6. doi: Doi
611 10.1007/Bf00295542. PubMed PMID: WOS:A1985AHR1900002.
- 612 48. Wilcox RS. Ripple Communication in Aquatic and Semiaquatic Insects. *Ecoscience.*
613 1995;2(2):109-15. doi: Doi 10.1080/11956860.1995.11682274. PubMed PMID:
614 WOS:A1995TF50800001.
- 615 49. Wilcox RS. Communication by Surface-Waves - Mating Behavior of a Water
616 Strider (Gerridae). *J Comp Physiol.* 1972;80(3):255-&. doi: Doi 10.1007/Bf00694840.
617 PubMed PMID: WOS:A1972N753600003.
- 618 50. Warton DI, Duursma RA, Falster DS, Taskinen S. smatr 3-an R package for
619 estimation and inference about allometric lines. *Methods Ecol Evol.* 2012;3(2):257-9.
620 doi: 10.1111/j.2041-210X.2011.00153.x. PubMed PMID: WOS:000302538500005.
- 621 51. Crumiere AJJ, Santos ME, Semon M, Armisen D, Moreira FFF, Khila A. Diversity in
622 Morphology and Locomotory Behavior Is Associated with Niche Expansion in the Semi-
623 aquatic Bugs. *Curr Biol.* 2016;26(24):3336-42. doi: 10.1016/j.cub.2016.09.061. PubMed
624 PMID: WOS:000390666200027.
- 625 52. Crumiere AJJ, Santos ME, Semon M, Armisen D, Moreira FFF, Khila A. Diversity in
626 Morphology and Locomotory Behavior Is Associated with Niche Expansion in the Semi-
627 aquatic Bugs. *Curr Biol.* 2016;26(24):3336-42. PubMed PMID: Medline:27939311.

- 628 53. Karaca M, Bilgen M, Onus AN, Ince AG, Elmasulu SY. Exact Tandem Repeats
629 Analyzer (E-TRA): A new program for DNA sequence mining. *J Genet.* 2005;84(1):49-54.
630 doi: Doi 10.1007/Bf02715889. PubMed PMID: WOS:000228875600008.
631 54. Gloor GB, Preston CR, Johnsonschlitz DM, Nassif NA, Phillis RW, Benz WK, et al.
632 Type-I Repressors of P-Element Mobility. *Genetics.* 1993;135(1):81-95. PubMed PMID:
633 WOS:A1993LW03300009.
634
635
636

637 **Figures:**

638

639

640

641

642

643

644

645

646

647

648

649

650

651

652

653

654

655

656

657

658

659

660 **Figure 1:** Diversity in leg sexual dimorphism and mating behaviours in *Microvelia*. A)

661 Phylogenetic relationships between five *Microvelia* species using Maximum Likelihood and

662 Bayesian analyses. Support values obtained after Bayesian posterior probabilities and 1000

663 bootstrap replicates, respectively, are shown for all branches. Pictures of males (right) and

664 females (left) illustrate divergence in sexual dimorphism in the five *Microvelia* species. Scale

665 bar represents 1mm. B) Scaling relationships of log-transformed data between rear legs and

666 body lengths were estimated in males (blue) and females (red) of the five *Microvelia* species

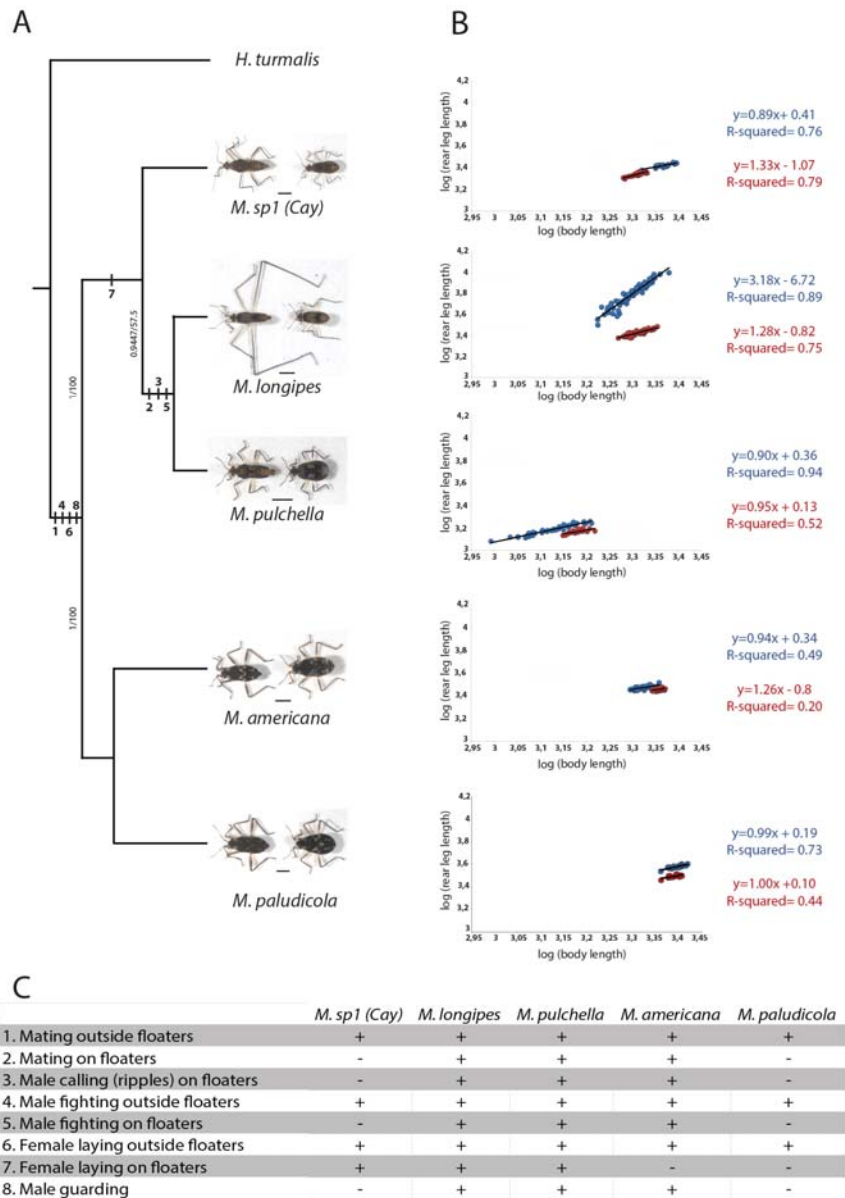
667 using Major Axis regressions. The equations and fitting (R-squared) of the linear regressions

668 in males and females were indicated using the same colour codes. C) Behavioural characters

669 describing the mating system of the five *Microvelia* species. These characters were mapped

670 onto the phylogeny based upon the parsimony criterion.

671



672

673

674

675

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694

695

696

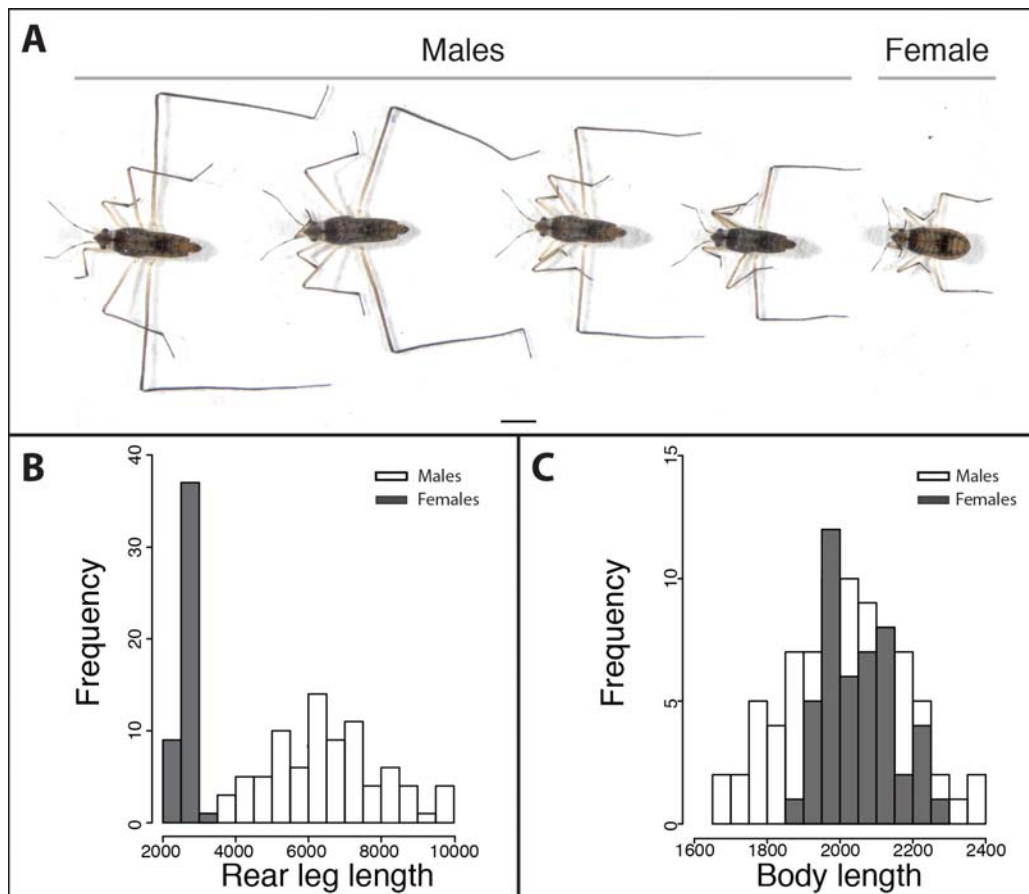
697

698

699

700 **Figure 2:** Phenotypic variation of rear leg exaggeration and body length in *M. longipes*. A)
701 Phenotypic variation of rear leg length in males and in a female. (B) Rear leg length and (C)
702 body length distributions of males (white) and females (grey) from a natural population
703 collected in French Guyana.

704



705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735

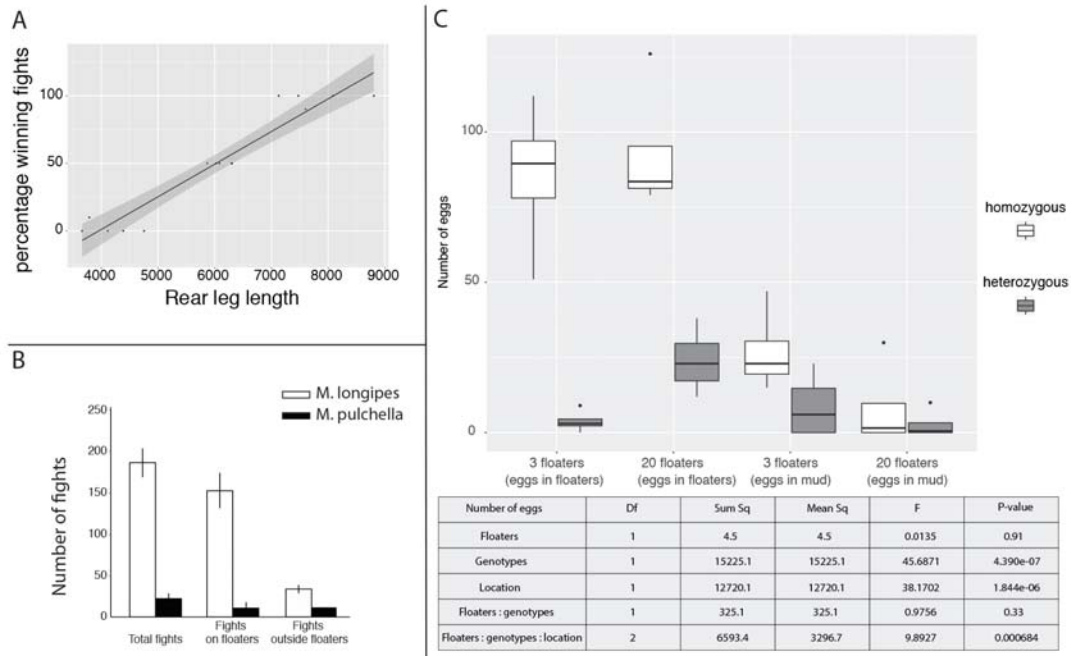


Figure 3: Selective pressures and reproductive fitness of leg exaggeration in *M. longipes* males. A) Relationships between fighting outcome and male rear leg length. Winners correspond to males keeping the access to the egg-laying sites after the fights. Solid line represents a fitted linear regression model, B) Frequency of fights between *M. longipes* and *M. pulchella* on both floaters and outside floaters, C) Fertilization success of large and small males and the contribution of egg-laying sites. Heterozygous eggs result from the siring of short-legged males (short-legs selected line) and females (long-legs selected line). Homozygous eggs result from the siring of long-legged males (long-legs selected line) and females (long-legs selected line). The table summarizes an ANOVA test recapitulating the influence of the floaters and the egg-laying locations on the egg genotypes.

736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756

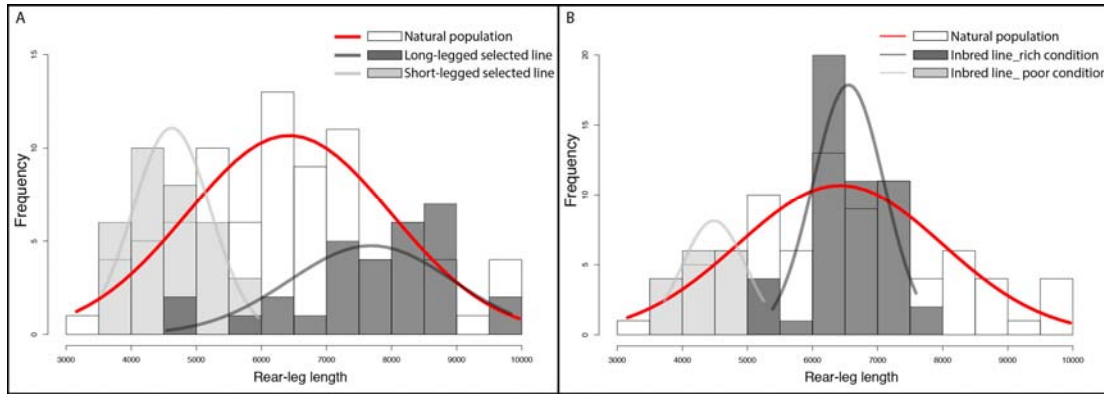


Figure 4: Environmental and genetic contributions to rear leg length variation in males *M. longipes*. A) Rear leg length distributions of adult males from natural population (white) and from an inbred line that developed under poor (light grey) and rich (dark grey) conditions. B) Rear leg length distributions of adult males from natural population (white) and from two inbred lines that were selected for short (light grey) or long (dark grey) rear legs under rich condition. Normal curves were fitted to each distribution after testing for normality of each condition.

757 **Supplementary figure 1:** Comparative morphology of the legs across *Microvelia* species
758 sample in figure 1. Note the presence of grasping traits on male legs in *Microvelia*
759 *americana*, *Microvelia paludicola* and *Microvelia sp.* These traits are absent in *Microvelia*
760 *longipes* and *Microvelia pulchella*.

761

762 **Supplementary figure 2:** Schematic summary representation of the mating systems in the
763 five *Microvelia* species

764

765 **Supplementary figure 3:** *M. longipes* natural habitat. Top panel: Example of rain-filled
766 puddle in French Guyana in Crique Patate near Cayenne where *M. longipes* population was
767 collected. Middle panel: Zoom on the floating substrates deposited on the water surface of
768 the puddle. Bottom panel: Example of floater full of *M. longipes* eggs. Scale bar represents
769 5mm.

770

771 **Supplementary figure 4:** Fluctuating environment. Pictures of a rain-filled puddle, over a
772 five-day period, in Rio de Janeiro where a *M. longipes* natural population was found.

773

774 **Supplementary figure 5:** Artificial selection effects on body-leg scaling relationships. Static
775 allometry on log-transformed data between rear leg and body lengths for adult individuals
776 selected for long (black) and short-legged (grey) males.

777

778 **Supplementary figure 6:** Nutrition effects on body-leg scaling relationships in the long-
779 legged inbred line. Static allometry on log-transformed data between rear leg and body
780 lengths for inbred adult individuals fed on rich (black) and poor (grey) diets.

781

782 **Supplementary figure 7:** Nutrition effects on body-leg scaling relationships in the lab
783 population. Static allometry on log-transformed data between rear leg and body lengths for
784 unselected adult individuals fed on rich (black) and poor (grey) diets.

785

786 **Supplementary table 1:** Morphometric data and associated statistical tests. Summary
787 table of the adult measurements and statistical tests for all *Microvelia* species.

788

789 **Supplementary table 2:** normality tests: Tests for normal distribution in all *M. longipes*
790 conditions. Values of Shapiro tests and associated p-values for each *M. longipes* adult
791 population reared in different condition.

792

793 **Supplementary table 3:** Fight frequency between *M. longipes* and *M. pulchella* males.
794 Summary table of the number of fights in *M. longipes* and *M. pulchella* males in different
795 conditions for a period of one hour. Below are the associated statistical tests for differences
796 in fight frequency.

797

798 **Supplementary table 4:** Count and genotype of the total number of eggs laid by females in
799 each condition.

800

801 **Supplementary table 5:** Statistical tests associated with differences in leg length and scaling
802 relationships between artificially selected and nutritionally manipulated *M. longipes*
803 populations.

804

805 **Supplementary table 6:** Table containing the primer sequences, the full sequence, the
806 motif and the length of each tested microsatellite.

807

808 **Supplementary table 7:** Table PCR protocols for microsatellite amplifications and single
809 individual genotyping.

810

811 **Supplementary video 1:** *M. longipes* male vibrations in slow motion.

812

813 **Supplementary video 2:** *M. longipes* mating system.

814

815

816

817

818

819

820

821

822

823

824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841