

1 **Selection on an antagonistic behavioral trait can drive rapid genital coevolution**
2 **in the burying beetle, *Nicrophorus vespilloides*.**

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16 **Running head:** Genital coevolution via sexual conflict.

17 **Keywords:** Sexual conflict, genital morphology, sexually antagonistic coevolution,
18 sexual selection, burying beetle, artificial selection, repeated mating

19 **Data accessibility**

20 The data will be deposited in Dryad, and will be freely available.

21

22 **Abstract**

23 Male and female genital morphology varies widely across many taxa, and even
24 among populations. Disentangling potential sources of selection on genital
25 morphology is problematic because each sex is predicted to respond to adaptations in
26 the other due to reproductive conflicts of interest. To test how variation in this sexual
27 conflict trait relates to variation in genital morphology we used our previously
28 developed artificial selection lines for high and low repeated mating rates. We
29 selected for high and low repeated mating rates using monogamous pairings to
30 eliminate contemporaneous female choice and male-male competition. Male and
31 female genital shape responded rapidly to selection on repeated mating rate. High and
32 low mating rate lines diverged from control lines after only 10 generations of
33 selection. We also detected significant patterns of male and female genital shape
34 coevolution among selection regimes. We argue that because our selection lines differ
35 in sexual conflict, these results support the hypothesis that sexually antagonistic
36 coevolution can drive the rapid divergence of genital morphology. The greatest
37 divergence in morphology corresponded with lines in which the resolution of sexual
38 conflict over mating rate was biased in favor of male interests.

39

40 **Introduction**

41 Genital morphology is often disproportionately diverse compared to other
42 morphological traits even among closely related species (Eberhard 1985; Hosken and
43 Stockley 2004; Arnqvist and Rowe 2005; Simmons 2014). Several evolutionary
44 mechanisms have been hypothesized to account for genital divergence (Arnqvist
45 1998; Hosken and Stockley 2004; Eberhard 2010) but recent theoretical and empirical
46 work supports sexual selection as the key driver of genital diversification. Cryptic

47 female choice could drive genital evolution if female genital traits facilitate biasing of
48 paternity towards ‘preferred’ males (e.g. Briceño and Eberhard 2009). Alternatively,
49 selection may act on male genital traits associated with competition for fertilization
50 success (Arnqvist 1997). A well-known example of the latter scenario is retrorse hairs
51 on intromittent organs of male damselflies that remove rivals’ sperm from pre-mated
52 females’ sperm storage structures (Waage 1979). However, genital traits
53 predominantly selected to benefit individuals of one sex are likely to have
54 implications for individuals of the other sex due to intersexual conflicts of interest
55 (Parker 1979; Kokko and Jennions 2014; Parker 2014). For example, in seed beetles
56 male genital spines may reduce the chance of an individual male being dislodged
57 during intromission thus enhancing his relative mating success. However, as a side
58 effect the female genital tract suffers damage from matings (Rönn et al. 2007). This
59 type of conflict generates the potential for selection for female defensive counter-
60 adaptations that mitigate costs, leading to sexually antagonistic coevolution (Arnqvist
61 and Rowe 2005). Mating with males that are successful by virtue of adaptations that
62 circumvent female defensive counter-adaptations can still provide indirect benefits for
63 females via their own successful sons (Kokko 2005; Kokko and Jennions 2014).
64 Thus, reproductive fitness for each sex potentially involves conflict between the
65 sexes, the extent of which might vary with regard to which sex is subjected to the
66 strongest selection for counter-responses (Holland and Rice 1998; Gavrilets et al.
67 2001; Hosken and Stockley 2004; Arnqvist and Rowe 2005; Kokko and Jennions
68 2014).

69 Quantitative genetic studies have demonstrated a genetic basis that could underlie
70 patterns of genital coevolution as one sex responds to the adaptations of the other
71 (Sasabe et al. 2010; Simmons and Garcia-Gonzalez 2011; Evans et al. 2013).

72 Furthermore, patterns of coevolution between male and female genital structures have
73 recently been found among closely related species at the phylogenetic level (Yassin
74 and Orgogozo 2013; Burns and Shultz 2015). Under sexually antagonistic coevolution
75 the sex currently having the ‘upper hand’ may change through time and different
76 mechanisms of sexual selection may be acting on alternate traits in each sex during
77 different copulatory phases (Kokko and Jennions 2014; Parker 2014). This makes
78 establishing clear mechanisms of evolutionary cause and effect problematic even in
79 the few experimental studies that have looked at patterns of genital coevolution
80 between males and females (Evans et al. 2011; Simmons and Garcia-Gonzalez 2011;
81 Evans et al. 2013; Yassin and Orgogozo 2013). This is because the functional
82 relationship between variation in genital morphology and fertilization success (were
83 they known) are interdependent even though the interests of males and females are
84 never perfectly aligned (Arnqvist 1997; Eberhard 2004; Arnqvist and Rowe 2005;
85 Simmons 2014).

86 In this study we test how sexual conflict might influence the evolution of male and
87 female genitalia in *Nicrophorus vespilloides* using our existing artificial selection
88 lines selected for either high, control or low repeated mating rates. In these lines the
89 effects of cryptic female choice were controlled by excluding the effects of sperm
90 competition. Using these lines we have previously shown that there is sexual conflict
91 over repeated mating rate, with high repeated mating rates being more costly for
92 females than low rates of repeated mating (Head et al. 2014). For males however,
93 high repeated mating is beneficial as a paternity protection mechanism (Müller and
94 Eggert 1989; House et al. 2008). Our selection lines represent two scenarios in which
95 either one sex or the other appears to be favored (i.e. females suffering minimal
96 harassment by males in low lines versus females facing repeated mating attempts

97 from persistent males in high lines). Our aims, by directly manipulating a conflict
98 trait, were both to test whether male and female genital morphology would coevolve
99 and also identify morphological structural variation upon which selection may act.

100

101 **Methods**

102 *Origin and Maintenance of burying beetles*

103 Our stock population of *N. vespilloides* was established from 90 males and 90 females
104 collected from Devichoys Wood, Cornwall, UK (N50°11'47''E5°7'23'') in July 2010
105 (for a brief summary of burying beetles as a model system see Royle et al. 2013). Full
106 details of stock maintenance are given in Head et al. (2012). Briefly, we maintained
107 the stock by breeding 50-60 pairs per generation. Each generation males and females
108 were randomly paired for breeding, whilst avoiding brother-sister and first cousin
109 matings. Additionally, beetles never contributed more than one brood to the following
110 generation. To breed, each pair of virgin male and female beetles were placed in
111 individual breeding chambers (17 x 12 x 6cm) with 2 cm of moist soil and a 15-25g
112 mouse carcass (Livefoods Direct Ltd, Sheffield, UK). Once larvae dispersed from the
113 mouse carcass they were removed from the breeding chamber and placed in
114 individual rearing containers (7 x 7 x 4cm). After eclosion, beetles were sexed and fed
115 2 decapitated mealworms twice a week until they reached sexual maturity (~14 days
116 post eclosion). All rearing was conducted in a constant temperature room at 21±1°C
117 with a 16L:8D light regime.

118 *Selection regime*

119 Full details of our artificial selection regime are given in Head et al. (2014). In brief,
120 we established and maintained two replicates of each line and maintained all lines at
121 the same population size (we always avoid brother-sister and first cousin
122 combinations). In each of 10 generations of selection males and females were mated
123 monogamously controlling for mating competition and mate choice in both sexes.
124 Using geometric morphometric analysis we tested whether male and female genital
125 shape evolved in response to selection on repeated mating rate and if so whether the
126 change in male and female genital shape resulting from selection on repeated mating
127 rate was correlated. Given that we used monogamous pairings to eliminate potential
128 effects of cryptic female choice and sperm competition, changes in genital
129 morphology that were correlated with selection on mating rate or coevolution of male
130 and female genital morphology provides evidence that sexually antagonistic
131 coevolution is capable of altering genital morphology. Our F0 generation was derived
132 from randomly paired 107 males and females (avoiding brother-sister and first cousin
133 matings) and mating rate was recorded (number of times mating occurred in 1 hr),
134 before being allowed to breed. Offspring from families with the top ~30% (33
135 families) and the bottom ~30% (34 families) values of parental mating rate were
136 allocated to the High (H) and Low (L) mating regimes respectively. The Control (C)
137 lines (30 families) were derived from randomly selected pairs, independent of mating
138 rate (i.e. drawn from the whole pool of 107 pairs). All larvae were kept from breeding
139 attempts meaning that each of the three different regimes consisted of ~800-1000
140 individuals.

141 In the F1 generation we split each selection regime into 2 different replicates to create
142 a total of 6 lines (i.e. H1, H2, C1, C2, L1, L2), which allows us to control for drift.
143 The replicates were created by randomly allocating males and females to pairs, with

144 half (82 pairs) randomly allocated to replicate 1 and the other half (82 pairs) allocated
145 to replicate 2 within each selection regime. Once the replicates were set up the top (H
146 lines), bottom (L lines) or a random selection of 35 families was chosen to contribute
147 to the next generation (~800-1000 individuals per line). In the subsequent, F2
148 generation, and beyond, mating rate was measured for 100 randomly paired males and
149 females (avoiding brother-sister matings) in each of the six lines and the top (H lines),
150 bottom (L lines) or random 20-25 families chosen (i.e. a population size of ~400-500
151 individuals per line per generation). Beetles within these selection lines were bred and
152 reared as outlined above for stock beetles.

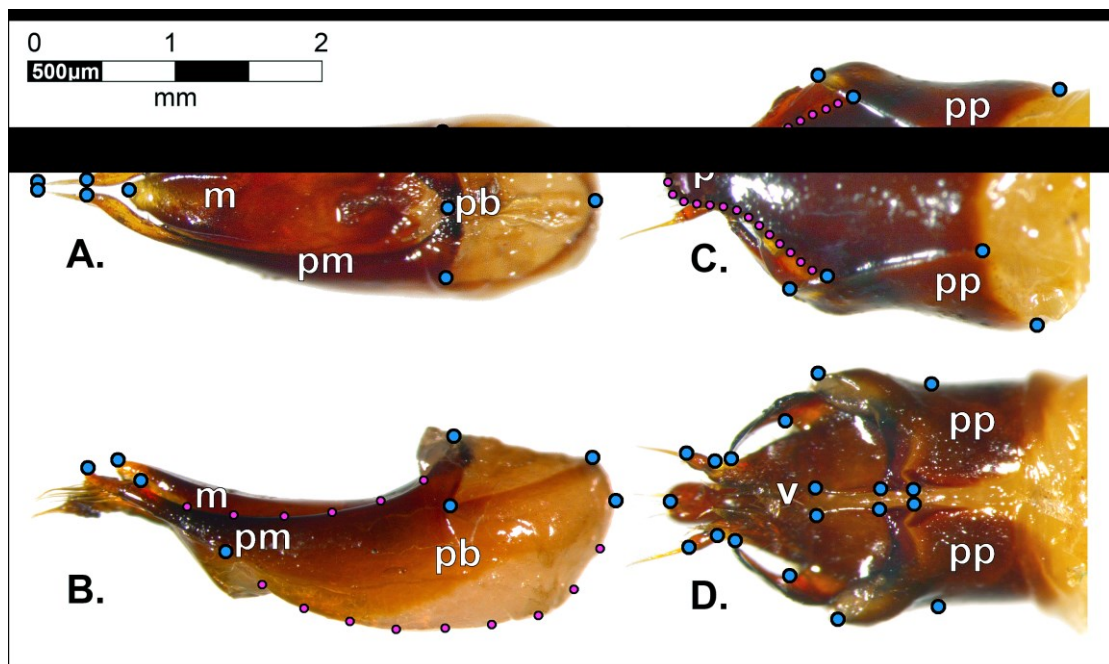
153 *Experimental design*

154 To investigate how selection on repeated mating rate influences the evolution of male
155 and female genitalia we conducted geometric morphometric shape analysis of a
156 sample of male and female beetles (16- 20 beetles of each sex from each line) from
157 the tenth generation of selection of each of the 6 selection lines described above.

158 Genitalia were dissected from sexually mature, virgin male and female beetles that
159 had been euthanized and stored in a -20°C freezer (~ 6 months prior).

160 Prior to dissection beetles were removed from the freezer, allowed to defrost and their
161 mass was recorded (to 0.001g, using an Ohaus, Explorer microbalance). Once beetles
162 had thawed we dissected male and female genitalia. Dissections were performed on
163 wax filled petri dishes with a pair of fine forceps and micro-scissors under a
164 dissecting microscope (Leica M125). For both males and females, the posterior
165 abdominal segment (which houses the genitalia) was separated from the rest of the
166 beetle. This was achieved by making an incision in the cuticle just above the required
167 segment and cutting along the sides of the cuticle so that the final segment could

168 gently be pulled out and placed in a clear petri dish. For males, the aedeagus was then
 169 removed by gently pulling away the tergites, pygidium and remaining membranous
 170 tissue. The parameres and aedeagus were left intact, mounted onto a glass slide using
 171 petroleum jelly and photographed immediately. Care was taken to position genitalia
 172 in the same plane in all photos. The female genitalia was removed and mounted in a
 173 similar way. We photographed mounted male and female genitalia using a Leica
 174 M125 microscope with mounted camera that conveyed images to a PC. Digital
 175 images were processed using Image J. For males, we photographed the lateral and
 176 ventral view of the genitalia, while for females we photographed the dorsal and
 177 ventral view (Fig. 1).



178

179 **Figure 1:** Micrographs of *N. vespilloides* genitalia showing positioning of fixed
 180 landmarks (blue points) and semi- landmarks (magenta points): male (A: dorsal view
 181 & B: left lateral view) and female (C: dorsal view & D: ventral view). Lower case
 182 letters indicate genital structures: median lobe (*m*); parameres (*pm*); phallobase (*pb*);
 183 paraproct (*pp*); proctiger (*p*); vulva (*v*).

184 *Morphometric analysis*

185 In order to quantify variation in the shape and size of the genitalia we used geometric
186 morphometric analysis (Adams et al. 2004). Landmarks for all images were digitized
187 (using software tpsDig version 2.12; 25) and are given in figure 1. To conduct
188 geometric morphometric analysis we followed the methods outlined in Zelditch et al.
189 (2012) for images with bilateral symmetry and, when appropriate, semi-landmarks,
190 (using software tpsRelw version 1.46; (Rohlf 2008)) and morphoJ software
191 (http://www.flywings.org.uk/MorphoJ_page.htm).

192 Landmarks to be digitized were chosen based on their ease and reliability of
193 placement while semi-landmarks were used on curved structures with no insertion
194 points. All dissections and photography were performed by one person (E. Jordan)
195 blind with respect to the selection regime from which beetles came. Landmark
196 digitization was similarly performed by one person (M. Head) who was blind to
197 selection regime. Collecting data in this way was intended to minimize measurement
198 error and prevent observer bias. Once the landmarks had been digitized and
199 superimposed, we obtained relative warps (RW) from each of the images (using
200 software tpsRelw version 1.46; (Rohlf 2008)). This program uses Procrustes methods
201 to standardize each set of images to a common size, as well as center and align the
202 landmarks so that differences in size and 2-dimensional positioning of the genitalia do
203 not contribute to shape differences between images. The tpsRelw software then
204 calculates a consensus configuration from the standardized coordinates and compares
205 each set of coordinates to the consensus configuration using thin-plate spline analysis
206 (Bookstein 1991). The method deforms each set of coordinates toward the consensus
207 configuration, producing a unique set of energy values called ‘partial warps’. The

208 principal components of these partial warps, called ‘relative warps’, summarize the
209 major trends of shape variation in the set of images (Rohlf 1999). We conducted a
210 single shape analysis for each image type. This means that individuals from different
211 selection lines were all scored (for each image type) along the same axes of shape
212 variation.

213 *Data analysis*

214 To investigate whether selection on repeated mating rate influenced the evolution of
215 male and/or female genitalia we first conducted a discriminant function analysis
216 (DFA) on the relative warps obtained from the geometric morphometric analyses
217 detailed above. We conducted DFA for males and females separately. For each sex
218 we included all relative warps that explained up to 99% of the shape variation in each
219 of the two images for that sex. For females, this included relative warps 1 - 15 for the
220 ventral view, and relative warps 1 - 12 for the dorsal view. For males, this included
221 relative warps 1 - 15 of the lateral view and relative warps 1 - 7 of the dorsal view.
222 Selection line was used as the grouping variable for both male and female analyses.
223 Thus the first discriminant function gives a score representing the weighted linear
224 combination of relative warps that best discriminates between selection lines, while
225 the second discriminant function gives a score that best discriminates between
226 selection lines based on the remaining shape variation described by the relative warps,
227 and likewise for subsequent discriminant functions.

228 Using the discriminant function scores resulting from this analysis we then looked to
229 see whether there were any consistent differences in male and female genital shape
230 associated with selection regime. To do this we conducted univariate nested ANOVA,
231 for both males and females, on each of the five discriminate functions. In these

232 analyses selection line was nested within selection regime as a random factor. We
233 also conducted analyses using MCMCglmm that allows multivariate analysis with
234 nested designs. This analysis (supplementary material, tables S1.1 & S1.2) gave
235 qualitatively similar results to our univariate analyses and so for ease of presentation
236 and interpretation we present only the univariate analyses in this manuscript.

237 After determining whether male and female genitalia differed depending on selection
238 regime we then looked to see if male and female genitalia had coevolved i.e. whether
239 shape variation in male genitalia was correlated with shape variation in female
240 genitalia. To do this we performed bivariate correlations on line means of the first
241 three discriminant functions describing shape variation in male genitalia and the first
242 three discriminant functions describing shape variation of female genitalia. This
243 resulted in a total of 9 correlations. We corrected for the use of multiple tests using
244 the false discovery rate in the LBE 1.22 software package in R (Dalmasso et al. 2005;
245 R Development Core Team 2014). The presence of significant correlations between
246 line means of the discriminant functions describing among line variation in male and
247 female genital shape is consistent with evidence for correlated evolution of these traits.

248

249 **Results**

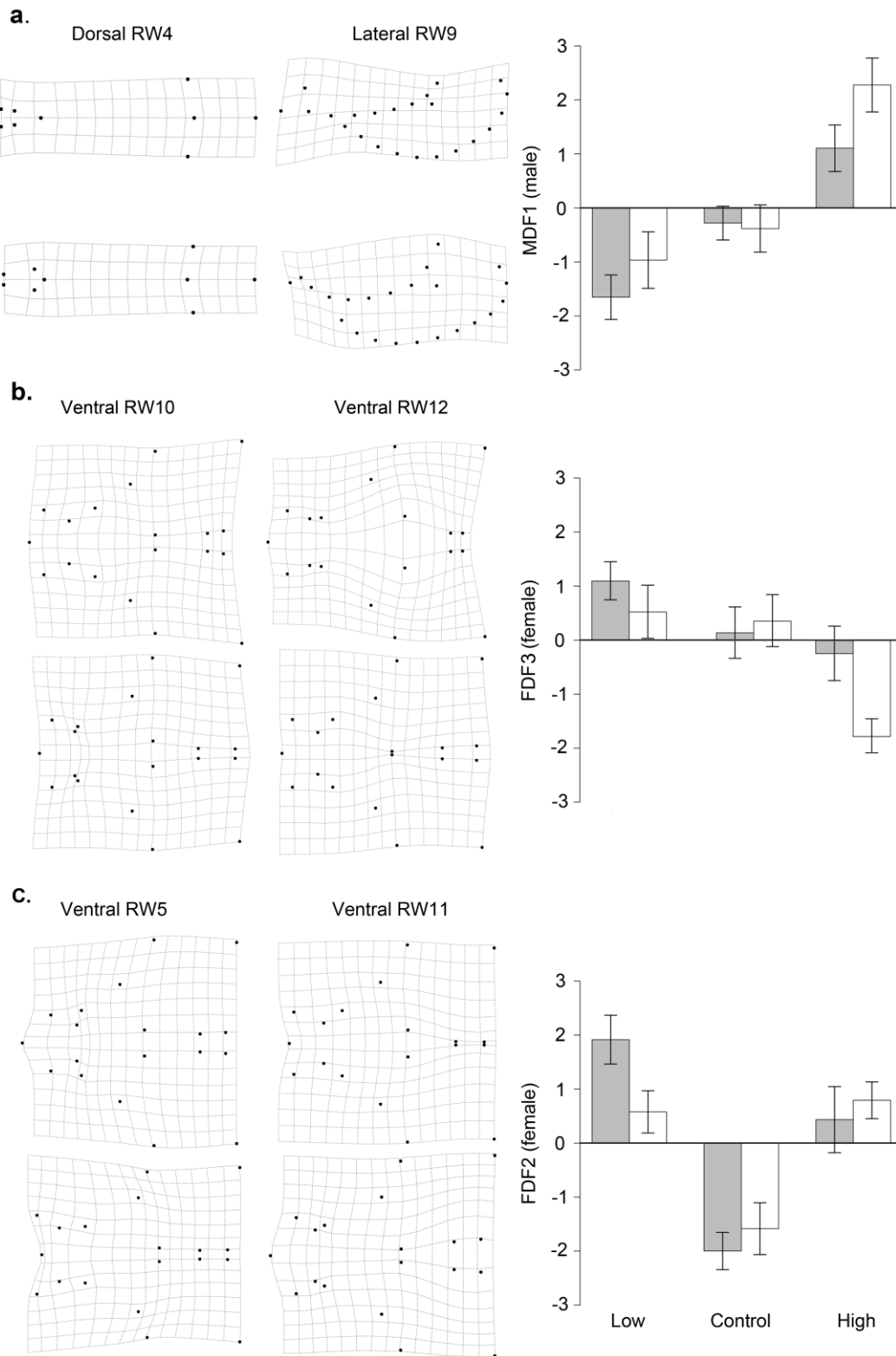
250 *Does selection on repeated mating rate lead to changes in the shape of male*
251 *genitalia?*

252 The canonical discriminant function analysis identified five axes of shape variation in
253 male genitalia. The first axis (MDF1) explained 38.8% of male genital shape variation
254 between selection lines, and describes variation in how far the parameres extend past

255 the median lobe, length of the terminal paramere setae (dorsal relative warp 4, Fig.
256 2a) as well as curvature of the parameres (lateral relative warp 9, Fig. 2a). Individuals
257 with high MDF1 scores had long straight parameres with short setae. The second axis
258 (MDF2) explained 28.2% of male genital shape variation between selection lines and
259 describes variation in the distance between the terminal tips of the parameres (i.e.,
260 their “openness”, dorsal relative warp 1) and the curvature of the overall structure
261 including parameres and phallobase (lateral relative warp 2). Individuals with high
262 MDF2 scores had highly curved structures with widely set parameres. The third axis
263 (MDF3) explained 17.6% of male genital shape variation between selection lines and
264 describes variation in the relative positioning of the terminal ends of the parameres
265 and the terminal ends of the setae (dorsal relative warp 6) as well as curvature of the
266 whole structure (lateral relative warp 2). Individuals with high MDF3 scores had
267 narrowly set parameres with outwardly pointing setae and low curvature of the
268 parameres and phallobase. The remaining two discriminant functions each explained
269 less than 10% of the variation in genital shape and so are not considered further.
270 Relative warps and how they contribute to each discriminant function are given in the
271 supplementary material (Table S2.1).

272 Of these three discriminant functions MDF1 differed among selection regimes:
273 selection on high and low repeated mating rate caused divergent evolution of male
274 genital shape with males from lines selected for high repeated mating rates having
275 shorter setae and parameres that extended further past the median lobe than control
276 lines, while males from lines selected for low repeated mating rate had longer setae
277 and parameres that did not extend as far past the median lobe than control lines
278 ($F_{2,2,998} = 15.151$, $p = 0.027$, Fig. 2a). MDF2 and MDF3 did not differ among
279 selection regimes (MDF2 - $F_{2,3,001} = 2.990$, $p = 0.193$; MDF3 - $F_{2,2,998} = 0.126$, $p =$

280 0.886).



281

282 **Figure 2:** Morphological responses among lines selected for mating rate in **a).** male setae

283 length, and paramere extension relative to median lobe; **b**). female width of vulval claws and
284 claw extension relative to the vulva; **c**). female vulval claw shape relative to the length of the
285 vulva. Bar charts (right) show selection line means (\pm CI) of discriminant functions. Solid
286 grey bars denote the first replicate and open bars the second replicate of each treatment.
287 Extreme positive (top left) and negative (bottom left) values of relative warps comprising
288 discriminant functions are graphically represented by thin-plate splines, i.e., dorsal relative
289 warp 4 and lateral relative warp 9 (MDF1, males); ventral relative warps 10 and 12 (FDF3,
290 females) and ventral relative warps 5 and 11 (FDF2, females).

291

292 *Does selection on repeated mating rate lead to changes in the shape of female*
293 *genitalia?*

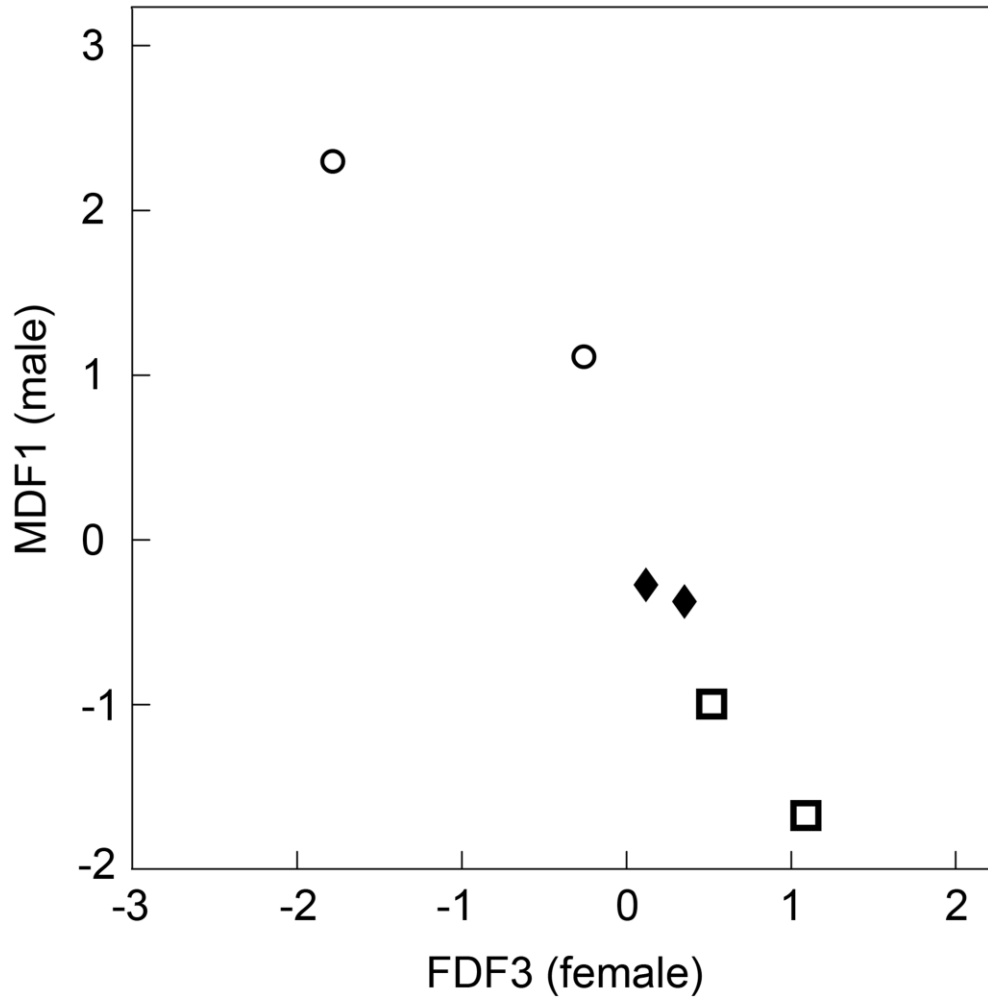
294 The canonical discriminant function analysis identified five axes of shape variation in
295 female genitalia. The first axis (FDF1) explained 45.0% of female genital shape
296 variation between selection lines, and describes the width of the vulval opening, width
297 of the base (ventral relative warp 4) as well as the extension of the base collar up the
298 vulval claw and the extension of the proctiger past the vulval lobes (ventral relative
299 warp 2). Individuals with high FDF1 scores had wider vulval openings, wider bases,
300 greater proctiger and collar extension. The second axis (FDF2) explained 27.5% of
301 female genital shape variation between selection lines and describes variation in the
302 shape of the vulval claw (ventral relative warp 11 and 5, Fig. 2c) and the length of the
303 vulva (ventral relative warp 5, Fig. 2c). Individuals with high FDF2 scores had short
304 vulvas and shorter thicker claws. The third axis (FDF3) explained 11.7% of female
305 genital shape variation between selection lines and describes variation in how far the
306 vulval claws extend up the vulva (ventral relative warp 10, Fig. 2b) and the openness
307 of the claw base (ventral relative warp 12, Fig. 2b). Individuals that had high values

308 of FDF3 had narrow-set claws that extend further up the vulva. The remaining two
309 discriminant functions each explained less than 10% of the variation in genital shape
310 and so are not considered further. Relative warps and how they contribute to each
311 discriminant function are given in the supplementary material (Table S2.2).

312 Of these three discriminant functions FDF2 was statistically significantly different
313 among selection regimes: selection on both high and low repeated mating rate led to
314 female genitals having shorter vulvas and shorter thicker claws than females from
315 control lines ($F_{2,2,948} = 15.117$, $p = 0.028$, Fig. 2c.). FDF1 and FDF3 were not
316 significantly different among selection regimes (FDF1 - $F_{2,3,002} = 0.027$, $p = 0.974$;
317 FDF3 - $F_{2,3,007} = 3.841$, $p = 0.149$).

318 *Are changes in genital shape of males and females correlated?*

319 Of the nine tests examining the relationship between line variation in male genital
320 shape and line variation in female genital shape only MDF1 and FDF3 showed a
321 statistically significant correlation ($r = -0.965$, $p = 0.002$, Fig. 3), which remained
322 statistically significant after controlling for multiple tests ($p_{\text{FDR}} = 0.018$). This
323 relationship shows that selection lines that evolve to have males with long straight
324 parameres and short setae also evolve to have females that have narrow-set claws that
325 extend further up (alongside) the vulva. Both male and female genital shape along
326 these axes have diverged from the control lines with the divergence significant for
327 males but not for females (see above).



328

329 **Figure 3:** Coevolution of male and female genital shape. Plot shows relationship
 330 between male discriminant function 1 (MDF1, y axis), and female discriminant
 331 function 3 (FDF3, x axis). Open circles = lines selected for high repeated mating rate;
 332 open squares = lines selected for low repeated mating rate; solid diamonds = controls.
 333 Shape differences for the relative warps that the discriminant functions represent are
 334 shown in figure 2a and 2b.

335

336 Discussion

337 Genital morphology evolved in *N. vespilloides* when we selected for high and low

338 repeated mating rate, and this evolution was rapid given both male and female genital
339 morphology evolved after only 10 generations of selection. This evolution occurred
340 under enforced monogamy that removed the potential for mate choice and male-male
341 competition. Males in lines selected for high repeated mating rates had shorter setae,
342 and parameres that extended further past the median lobe than did males in control
343 lines, while males from low lines had longer setae, and parameres that did not extend
344 as far past the median lobe (Fig. 2a). In both high and low lines female genitals had
345 shorter vulvas and shorter thicker claws than those of females in control lines (Fig.
346 2c). Male and female genitals coevolved among selection lines: lines with males that
347 evolved long straight parameres and short setae had females that evolved narrow-set
348 claws extending further up (alongside) the vulva.

349

350 Thus far the best support for a significant role of sexual conflict in the coevolution of
351 genital morphology comes from recent studies of guppies, *Poecilia reticulata* (Evans
352 et al. 2011; Evans et al. 2013) and comparative studies of seed beetles. (Rönn et al.
353 2007), and water striders (Arnqvist and Rowe 2002; Perry and Rowe 2012). In
354 leiobunine harvestmen (Opiliones) the coevolution of male and female genital
355 structures appears to be influenced by eco-evolutionary feedbacks related to resource
356 availability (Burns and Shultz 2015). These studies provide strong support for the role
357 of sexually antagonistic coevolution in producing patterns of genital divergence
358 across species and populations but also highlight the potential dynamic relationships
359 among the mechanisms of selection responsible. Here we showed that directly
360 manipulating a known mating conflict trait leads to rapid genital coevolution. The
361 selection regime used here produces lines in which resolution of conflict between

362 males and females is biased towards one sex or the other. The conclusion follows that
363 there are likely to be functional correlations associated with the axes of evolved
364 genital morphological structures that are important in controlling mating rates and
365 maintaining a 'balance of power' between the sexes. This possibility could potentially
366 be examined in the future by reversing the direction of selection within lines with the
367 prediction that the change in genital morphology would also be reversed. Although it
368 is beyond the scope of the present study on its own to identify the specific
369 mechanisms of selection that led to this pattern (for example we cannot categorically
370 dismiss the possibility that we may have exposed a genetic linkage whose origin lies
371 in cryptic female choice or elsewhere) our results strongly suggest that genital
372 morphology can respond to selection that influences the resolution of sexual
373 antagonism. Combining the phylogenetic approach of Burns and Shultz (2015) with
374 our approach may be a powerful way of resolving interactions between mechanisms
375 of selection.

376 There is still a puzzle in that the direction of the female response to selection in
377 (FDF2) was the same in both high and low lines (Fig. 2c). One possibility is that the
378 female response seen in this study is a correlated response to male genital evolution.
379 If this were the case the direction of the response is expected to be more predictable in
380 males, and also stronger, than that in females. For example, in a recent study that
381 directly tested the evolutionary response in male and female genitalia to changes in
382 sexual conflict, Cayetano et al. (2011) found that while male genitalia evolved rapidly
383 and predictably, female genitalia did not respond. Our results, show a relative weak
384 response in female morphology compared to males and also apparent differences
385 between males and females in the extent of divergence from control lines along the
386 correlated axes (i.e., divergence was stronger for males than in females). This is

387 broadly consistent with the view that female genital morphology evolved as a result of
388 intersexual genetic correlation or even genetic hitchhiking. However, this view does
389 not provide a complete picture. Because male and female genitals differ it is difficult
390 to evaluate functional significance based on the extent of divergence in each sex.
391 Moreover, evolution of female genital traits may be subject to constraints due to
392 multiple functions (e.g., egg laying), which may limit the ability of females to respond
393 to selection on male traits.

394 The pattern of divergence in the correlated axes of at least some aspects of male and
395 female genital shape followed the direction of artificial selection on repeated mating
396 rate, with high lines at one end of the relationship, low lines at the other and controls
397 in between (Fig. 3). The magnitude of genital divergence among selection lines
398 mirrors the response of repeated mating rate with high lines diverging further from
399 control lines than low lines (See supplementary material Fig. S3, and see Carter et al.
400 2015 supplementary material). This, and the striking mirror image of the male and
401 female correlated response (i.e. Fig. 2a and Fig. 2b) indicates that the sexes have
402 responded one to the other. We argue that this supports sexually antagonistic
403 coevolution because of the difference in sexual conflict in our lines and because our
404 experimental selection regime limited the opportunity for inter- and intrasexual
405 selection, and thus cryptic female choice. In *N. vespilloides*, repeated mating provides
406 direct fitness benefits for males (Bartlett 1988; Müller and Eggert 1989; Müller et al.
407 2007). However, an increase in mating rate apparently reduces maternal care, leading
408 to fecundity costs to females both when increased mating frequency is the result of
409 artificial selection (Head et al. 2014) and when females are mated more as a result of
410 males responding to increased threats to their paternity (Hopwood et al. 2015).
411 Repeated mating rate appears to be primarily under male control leading to the

412 evolution of ‘persistent males’ and ‘resistant females’ under sexually antagonistic
413 coevolution (Head et al. 2014).

414 We observed female behavioral resistance consisting of wrestling, kicking and curling
415 the abdomen away from the male (see also Head et al. 2014) but the measure of
416 repeated mating on which we based selection was successful copulations. Females in
417 nature might employ selective resistance to hinder penetration by non-preferred males
418 (Blanckenhorn et al. 2000; Eberhard 2002) theoretically limiting direct costs from
419 excessive mating while still gaining indirect benefits from a successfully coercive
420 male (Kokko et al. 2003; Kokko 2005). Commonly observed resistance behaviors in
421 insects such as running away or kicking can be generally effective against a suite of
422 different male genital adaptations and thus shared across taxa (e.g. Crudginton and
423 Siva-Jothy 2000; Blanckenhorn et al. 2002; Perry et al. 2009). Longer parameres
424 might facilitate successful insertion and anchorage of male genitalia perhaps affecting
425 mating rate when males struggle against female resistance but the relationship
426 between genital structures and how they affect mating rate and/or mating success is
427 not known at present but may be testable in future experiments (e.g. Hotzy et al.
428 2012; Dougherty et al. 2015). Because we eliminated female choice and sperm
429 competition, coevolution could have occurred because genital morphology shares a
430 similar developmental basis in both sexes. Increased mating rate can in itself be costly
431 to females independent of the phenotype of the male (e.g. Priest et al. 2008). In such
432 cases genital morphology could be selectively neutral in either one sex or the other
433 (e.g., females that employ behavioral resistance against male genital adaptations or
434 males that increase mating rate against female genital adaptations) with genital
435 coevolution driven indirectly in the other sex through pleiotropy. Nevertheless, our
436 selection lines still represent the pattern of a ‘high line’ male advantage and ‘low line’

437 female advantage.

438 **Conclusions**

439 Our experimental evidence suggests that sexual conflict can result in the rapid
440 coevolution of male and female genitalia. Genital morphology of lines selected for
441 high and low repeated mating rate diverged from controls after 10 generations of
442 selection. The greatest divergence in morphology corresponded with lines in which
443 the resolution of sexual conflict over mating rate was biased in favor of male interests.
444 Future studies are needed to further understand the relative influences of different
445 mechanisms of selection by including the eco-evolutionary context and functional
446 payoffs associated with genital morphological adaptations. Achieving these goals will
447 be an important next step towards better understanding of selective processes
448 underlying the maintenance of sexually dimorphic traits in general.

449 **Competing interests:**

450 We have no competing interests.

451 **Author contributions:**

452 MLH; EJJ; MJC & ED collected and analyzed the data. All authors designed the
453 experiments, were involved in interpreting the data and co-wrote the manuscript.

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