1 **Selection on an antagonistic behavioral trait can drive rapid genital coevolution**

2 **in the burying beetle, *Nicrophorus vespilloides*.**

3 Paul E. Hopwood[1]*, Megan L. Head[1,2]*, Eleanor J. Jordan[1], Mauricio J. Carter[1,4],

4 Emma Davey[1], Allen J. Moore[1,3] & Nick J. Royle[1]∓

5 *1. Centre for Ecology and Conservation, College of Life and Environmental*
6 *Sciences, University of Exeter, Cornwall Campus, Penryn, TR10 9FE, UK*

7 *2. Division of Evolution, Ecology and Genetics, Research School of Biology,*
8 *Australian National University, Acton, ACT, 0200*

9 *3. Department of Genetics, University of Georgia, Athens, Georgia, 30602, USA*

10 *4. Centro Nacional del Medio Ambiente. Fundación de la Universidad de Chile.*
11 *Av. Larrain 9975, La Reina, Santiago, Chile.*

12 ***These authors contributed equally to the study**

13 **∓Author for correspondence**

14 N.J.Royle@exeter.ac.uk

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

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 (N50º11'47''E5º7'23'') 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

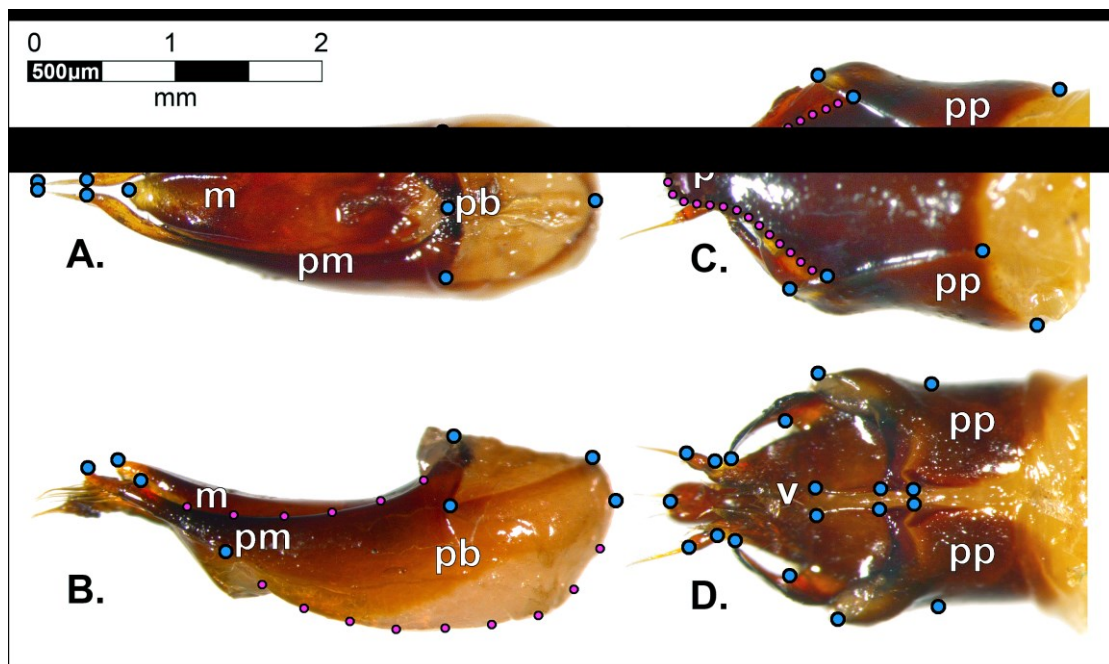152 reared as outlined above for stock beetles.

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

7

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.
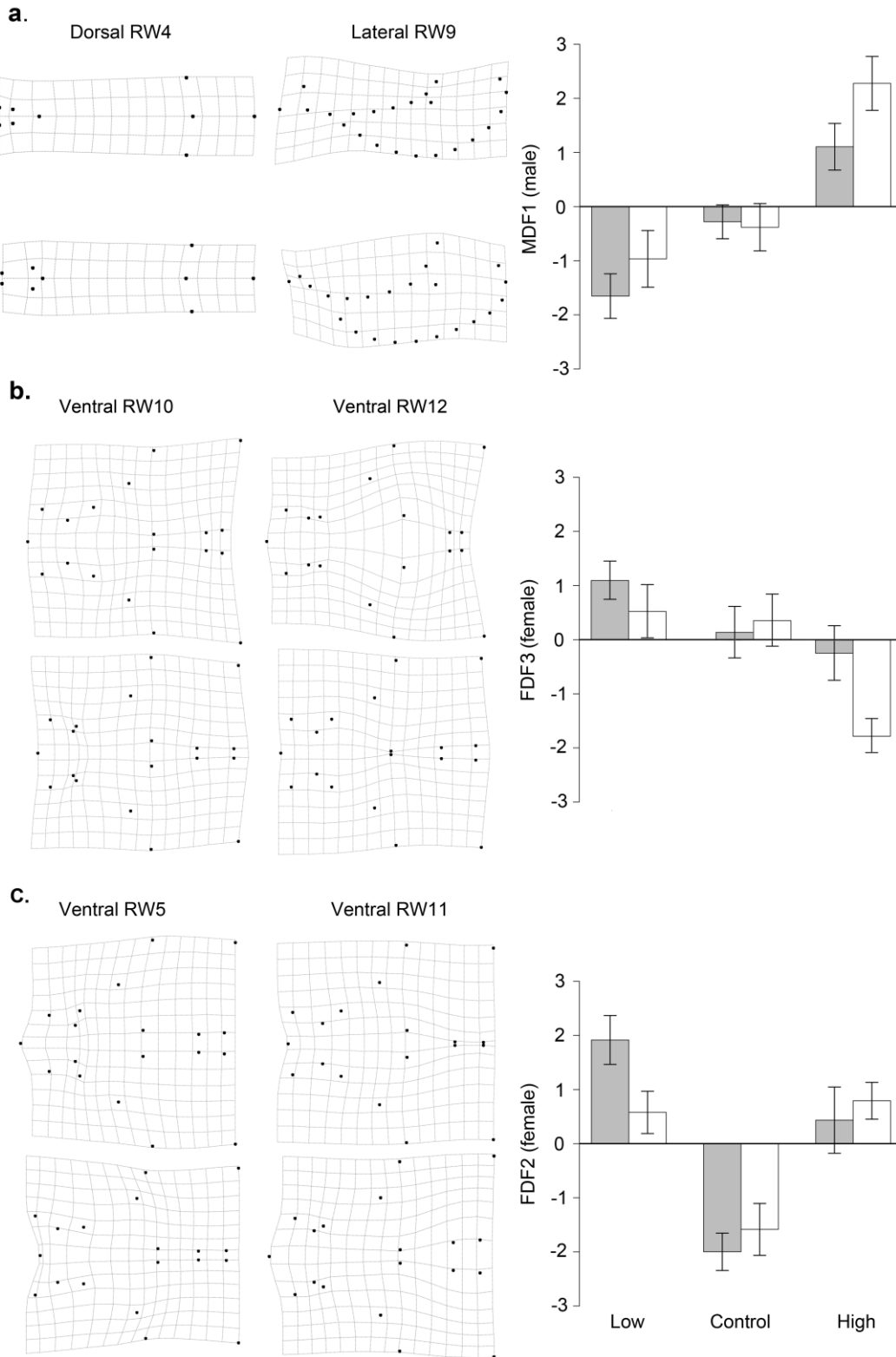
248

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 =

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 (± 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*
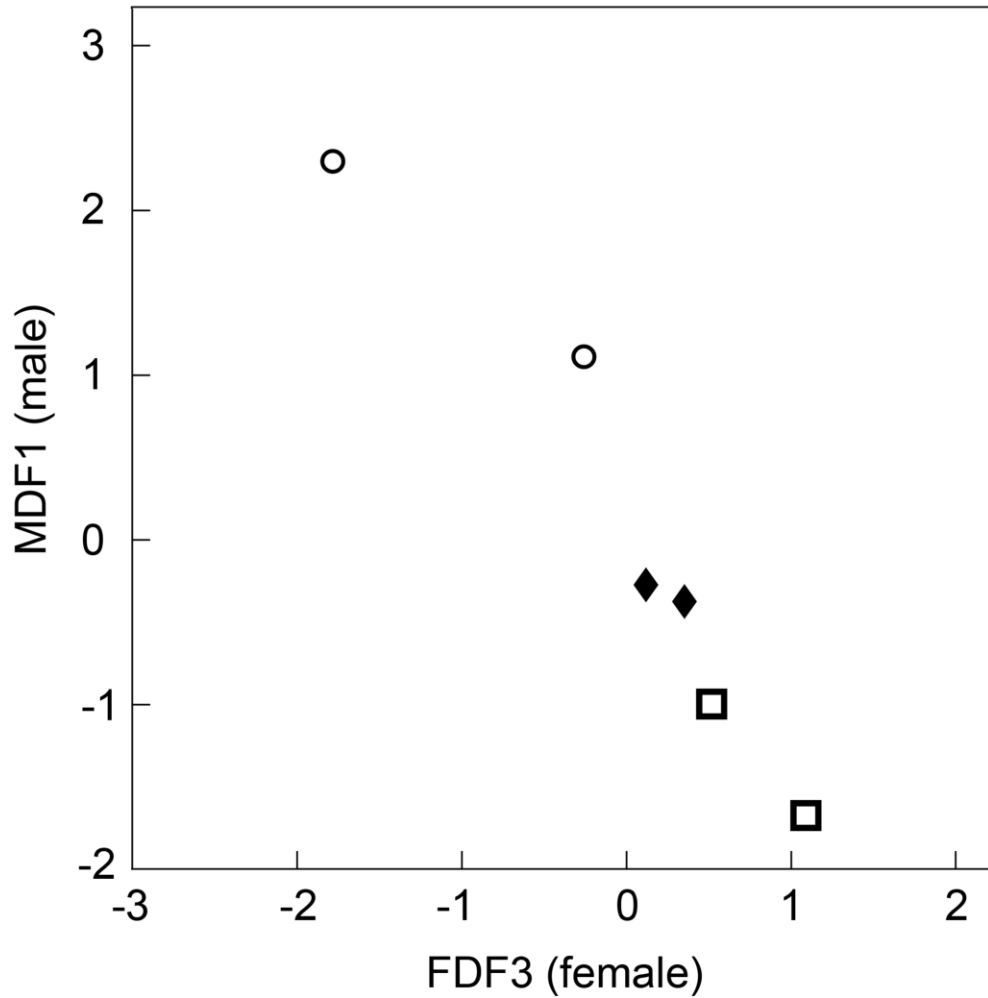
293   *genitalia?*

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

14

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_{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

Figure 3: Coevolution of male and female genital shape. Plot shows relationship

between male discriminant function 1 (MDF1, *y* axis), and female discriminant

function 3 (FDF3, *x* axis). Open circles = lines selected for high repeated mating rate;

open squares = lines selected for low repeated mating rate; solid diamonds = controls.

Shape differences for the relative warps that the discriminant functions represent are

shown in figure 2a and 2b.

335

Discussion

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. Crudgington 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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