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1 A LINK BETWEEN HERITABLE PARASITE RESISTANCE AND MATE CHOICE IN  
2 DUNG BEETLES

3

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22

23 ABSTRACT

24 Parasites play a central role in the adaptiveness of sexual reproduction. Sexual selection  
25 theory suggests a role for parasite resistance in the context of mate choice, but the evidence is  
26 mixed. The parasite-mediated sexual selection (PMSS) hypothesis derives a number of  
27 predictions, among which that resistance to parasites is heritable, and that female choice  
28 favours parasite resistance genes in males. Here we tested the PMSS hypothesis using the  
29 dung beetle *Onthophagus taurus*, a species that can be heavily parasitised by *Macrocheles*  
30 *merdarius* mites, which are known to affect adult survival. We investigated the heritability of  
31 resistance to *M. merdarius*, as well as whether female *O. taurus* impose a mating bias against  
32 males susceptible to mite infestation. Female choice for parasite resistance is difficult to  
33 disentangle from the possibility that females are simply choosing less parasitised males due  
34 to naturally selected benefits of avoiding contracting those parasites. This is especially likely  
35 for ectoparasites, such as mites. We tackled this problem by performing a mate choice trial  
36 first, and then measuring a male's resistance to mite infestation. Resistance to mite  
37 infestation exhibited significant levels of additive genetic variance. Although we found no  
38 relationship between mating success and parasite resistance, males with greater resistance to  
39 infestation mated for longer. If females control copula duration, given that short copulations  
40 often result in mating failure, female choice could act on parasite resistance.

41

## 42 INTRODUCTION

43 Parasitism is a predominant lifestyle, and most interspecies interactions are parasitic in  
44 nature. Host species typically interact with a multitude of parasites through their life cycle, a  
45 good proportion of which are species specific (Combes, 2001; Price, 1980). Given the  
46 importance of parasitism, it is no surprise that these interactions have been implicated in our  
47 understanding of a myriad of evolutionary processes (Price, 1980), including mechanisms of  
48 sexual selection (Andersson, 1994). Hamilton and Zuk (1982) proposed that parasites,  
49 through their effects on male hosts, could provide the variability in condition and expression  
50 of sexual ornaments necessary to maintain female choice for brightly ornamented males in  
51 birds. Hamilton and Zuk's (1982) parasite-mediated sexual selection (PMSS) hypothesis has  
52 been highly influential, and received much attention and support from a variety of taxa  
53 ranging from insects (Rantala and Kortet, 2003; Siva-Jothy, 2000) to vertebrates (Hamilton  
54 and Poulin, 1997; Møller, 1990a). Nonetheless, criticism has also arisen from the fact that  
55 most of the support for the PMSS hypothesis, including the data used in the original study,  
56 had been based on phenotypic correlations between levels of parasitism and the expression  
57 of male ornaments (Balenger and Zuk, 2014; Clayton, 1991). Even in studies that  
58 manipulated levels of parasitism experimentally (see for example Worden et al., 2000), an  
59 effect of parasites on the sexual ornaments or attractiveness of males is just one of the  
60 assumptions of the PMSS hypothesis. Testing its exclusive predictions has been extremely  
61 difficult, especially due to the genetic basis of parasite resistance that is necessary for  
62 parasites to mediate sexual selection (Balenger and Zuk, 2014).

63 The PMSS hypothesis is essentially a 'good genes' model of sexual selection (Andersson,  
64 1994), because it suggests that females who choose parasite resistant males gain an indirect  
65 genetic benefit via the inheritance of parasite resistance in their offspring. Therefore, it is  
66 crucial that parasite resistance is heritable, and that mate choice favours parasite resistance  
67 per se, not only lower levels of parasitism in males. Even though parasite resistance is

68 expected to correlate with levels of parasitism, there is a subtle but important difference  
69 between the two. If females merely choose males with fewer parasites, they might be  
70 protecting themselves, and potentially their offspring, from direct parasite transmission,  
71 which is especially likely in the case of ectoparasites and sexually transmitted parasites  
72 (Knell and Webberley, 2004; Lehmann, 1993). Alternatively, females might be choosing males  
73 that are more likely to provide resources and/or paternal care. These two mechanisms,  
74 respectively named the 'avoidance hypothesis' (Borgia and Collis, 1990) and the 'resource  
75 provisioning hypothesis' (Hamilton, 1990), both constitute natural selection based alternatives  
76 to PMSS. It is certainly true that PMSS and its natural selection alternatives are not mutually  
77 exclusive, and likely to act simultaneously in a system. But the important point is that  
78 demonstrating mate choice for the least parasitised males, or males with better ornaments, is  
79 insufficient to conclusively support PMSS.

80         Here we endeavoured to test the PMSS hypothesis, asking whether resistance to  
81 parasites harbors additive genetic variance, and whether males successful in mate choice  
82 trials were less likely to become infected with parasites when later challenged. As our model  
83 system we looked at the relationship between dung beetles and the mites they often carry  
84 (Hanski and Cambefort, 1991; Simmons and Ridsdill-Smith, 2011). Even though some of  
85 these interactions seem to be mostly phoretic (Ridsdill-Smith and Hall, 1984), rather than  
86 parasitic, we focused on the relationship between *Onthophagus taurus* and the mite  
87 *Macrocheles merdarius*, a species known to negatively affect the survival of the congeneric *O.*  
88 *binodis* dung beetle (Kotiaho and Simmons, 2001). We firstly investigated the heritability of  
89 *O. taurus* resistance to *M. merdarius* with a quantitative genetics experiment coupled with a  
90 mite exposure trial. In a completely separate experiment, we then measured the  
91 attractiveness of *O. taurus* males to females, and later submitted these same males to a mite  
92 exposure trial similar to the one we used to estimate the heritability of parasite resistance.

93

94 MATERIALS AND METHODS

95 Heritability of parasite resistance

96 We collected adult *Onthophagus taurus* dung beetles from cow dung in Margaret River  
97 (Western Australia), maintained and bred them in the laboratory following the protocol  
98 described by Buzatto et al. (2015). Using laboratory-reared unmated beetles, we established a  
99 half-sibling breeding design (Falconer and Mackay, 1996) starting with 33 males, each paired  
100 with three females, resulting in 99 full-sibling families, and 33 paternal half-sibling families.  
101 We kept the mating groups in separate plastic boxes (7 x 7 x 5 cm) filled with moist sand for  
102 five days, and provided them with ad libitum fresh dung. After the mating period, we  
103 established females individually in breeding chambers (PVC piping: 25 cm long and 6 cm  
104 diameter) for seven days. These chambers were three-quarters filled with moist sand and 250  
105 mL of cow dung was added to each of them. We then sieved the soil from the chambers and  
106 incubated the retrieved brood masses in moist sand for approximately 3 weeks. The  
107 emerging adult males were housed individually in plastic boxes (7 x 7 x 5 cm) filled with  
108 moist sand and provided with a teaspoon of fresh dung. This sand was previously infested  
109 with *Macrocheles merdarius* mites that had been brushed off beetles from a non-experimental  
110 population. This sand had also been homogenised before assigning it to the beetles. The final  
111 dataset contained 563 male beetles (average of 5.7/family, ranging from 1-13/family). We  
112 also measured the pronotum width of all males with digital calipers, to the nearest 0.01 mm.

113 We considered the number of mites found on males after 24 hours as the intensity of  
114 parasitism, which is indicative of their parasite resistance. In addition to the standard plastic  
115 boxes and the same amount of homogenised moist sand and fresh dung given to each male,  
116 all individuals were also kept in the same experimental room with the same light cycle,  
117 temperature and humidity. Therefore, by exclusion, the only factors that could have played a  
118 role in explaining nonrandom variation in the levels of mite infestation between individuals  
119 would be intrinsic traits of the hosts themselves — which we collectively called ‘parasite

120 resistance'. Importantly, we refrain from speculating on the mechanisms underlying  
121 resistance, which can be immunological, behavioral (actively removing mites from the  
122 cuticle, for example) or a combination of these two types of processes.

123 We estimated the heritability for resistance using a univariate animal model (Wilson et  
124 al., 2010) with a Poisson error distribution, fitting pronotum width as a fixed effect (to  
125 control for body size effects) and the additive genetic effect of each individual, as well as the  
126 identity of their mother (to control for maternal effects), as random effects. The heritability of  
127 pronotum width was estimated in the same way, but without any covariates. We fit these  
128 models using restricted maximum likelihood as implemented in ASReml-R (Butler et al.,  
129 2009), and measured heritability ( $h^2$ ) as the proportion of total phenotypic variance (VP) due  
130 to the additive genetic effect of individuals (VA):  $h^2 = VA/VP$ , where VP includes VA and the  
131 residual variance (VR). We also calculated the coefficients of additive genetic variation (CVA;  
132 expressed without a 100 multiplier), and their standard errors (following equation (5) in  
133 Garcia-Gonzalez et al., 2012). Finally, we repeated this analysis after removing males who  
134 had no mites (also ran as a Poisson animal model, but with no zeros) and later using all  
135 males but focusing on the dichotomous response of infested (1) versus resistant (0) males  
136 (ran as a binomial animal model), which is a different measure of parasite resistance that  
137 focuses on the prevalence of parasitism.

138

139 Mate choice trial

140 For the female choice trial, we again collected *O. taurus* from the field and maintained  
141 individuals in 28°C and 12:12 hours of light/dark photoperiod, providing them with ad  
142 libitum fresh cow dung that was renewed weekly. Since wild caught individuals probably  
143 contained a mixture of recently emerged (and therefore not sexually mature) and already  
144 mated individuals, we kept males and females in single sex populations for at least 2 weeks  
145 before the trial, ensuring that males were not sperm depleted and that all individuals were

146 sexually mature. Most individuals in this sample of *O. taurus* harboured a small number of  
147 *Macrocheles merdarius* mites, which we brushed off all beetles and kept in 1L plastic  
148 containers with moist sand and a teaspoon of fresh cow dung. Importantly, however, we  
149 avoided using any males who harboured a high parasite load (more than ~50 mites).

150 Mating trials were conducted in artificial arenas made of transparent plastic boxes (6  
151 cm tall, 3.5 cm wide, 1.2 cm depth), half-filled with Plaster of Paris to generate a narrow  
152 tunnel at the top, which was moistened with a solution of dung and water. For each trial, we  
153 placed a randomly selected male and female in the mating arena, excluding the possibility of  
154 competition between the males. No individual was used more than once. Importantly, all  
155 mites previously present on the beetles had been removed before the trial, since we wanted  
156 to evaluate female preference for parasite resistance, and not female aversion to parasites  
157 currently present on their partners (Jaenike, 1988; Zuk et al., 1990). Males court females in  
158 bouts, during which males tap females with their antennae and front legs, a behaviour that  
159 induces females to open their pygidium so that males can insert their endophallus (Kotiaho,  
160 2002; Werner and Simmons, 2008). We kept each pair together and observed them under red  
161 light for 60 minutes, scoring whether a male was courting or not (a bout) every two minutes.  
162 Once a male successfully mated with the female, we recorded the duration of the mating.  
163 Mating trials were performed in eight blocks ranging from 15 to 60 pairs per block, with each  
164 observer scoring a maximum of 15 pairs at a time.

165

166 Parasite resistance trial

167 After the one-hour mating trial, we housed males individually in transparent plastic boxes (7  
168 x 7 x 5 cm) half-filled with moist sand infested with *M. merdarius* mites and one teaspoon of  
169 fresh cow dung. This sand had been previously infested with mites that were brushed off the  
170 individuals used in the mating trial block. We again homogenised the sand before assigning  
171 it to the beetles. Therefore, each block differed in the number of mites, depending on how



172 many individuals (who could have anything from 1 to ~100 mites on them) contributed to  
173 the mite population for that block. We exposed males to mite infested sand for 24 hours,  
174 after which we froze them. The number of mites attached to each male was counted under a  
175 dissection microscope.

176

177 Relating parasite resistance to female choice

178 We removed males that did not court from the data set, since a female is unable to express  
179 mate choice unless the male attempts to mate with her. We calculated courtship rate as the  
180 number of courtship bouts per minute (as in McCullough and Simmons, 2016), from the start  
181 of courtship to the start of mating or to the end of the trial (when couples did not mate). We  
182 also standardized the number of mites across blocks by centering this variable's mean to zero  
183 and making its variance uniform by dividing the centered values by two times their standard  
184 deviation within each block. Next, we investigated the effect of a male's resistance to  
185 parasites on his mating success using generalized linear mixed effects models with a  
186 binomial error distribution, fit with maximum likelihood implemented in package lme4  
187 (Bates et al., 2013) in R version 3.5.0 (R Core Team, 2018). For model selection, we built a set  
188 of five candidate models with the possible combinations of the fixed effects of parasite  
189 resistance (measured as the standardized number of mites), courtship rate, and their  
190 interaction, and then compared these models on the basis of their AIC<sub>c</sub>. All models had the  
191 random effect of block. Finally, we checked our best model for overdispersion by comparing  
192 the sum of squared Pearson residuals to the model's residual degrees of freedom.

193 We then focused on the subset of males who successfully mated, in order to investigate  
194 the effect of parasite resistance on mating duration. For that we compared a general mixed  
195 effects model with the fixed effect of the (standardized) number of mites against a null  
196 model (both with the random effect of block) on the basis of their AIC<sub>c</sub>. We visually checked  
197 our best model for heteroscedasticity of residuals by plotting the standardized residuals

198 against fitted values. We also checked the best model for normality of residuals by testing  
199 their distribution (with Kolmogorov-Smirnov tests) against 1,000 normal distributions that  
200 were simulated with the same mean, standard deviation, and sample size of the actual  
201 residuals, and considering the residuals normally distributed if the tests were not significant  
202 >90% of the times.

203

## 204 RESULTS

### 205 Heritability of parasite resistance

206 We did not find significant heritability of pronotum width (Table 1), contrary to what has  
207 been reported previously for this species without taking maternal effects into account  
208 (Buzatto et al., 2015; Kotiaho et al., 2003). In comparison, resistance to infestation from *M.*  
209 *merdarius* mites was highly heritable (0.80) when calculated using the intensity of parasitism,  
210 and only about 20% weaker when males with no mites (zeros in the response variable) were  
211 removed from the sample (0.65; Table 1). Heritability was 0.39 when using a binomial model  
212 to compare infested and non-infested males (prevalence of parasitism), and with a  $CV_A$   
213 around one order of magnitude higher than the estimates using parasite intensity.

214

### 215 Female choice and parasite resistance

216 We observed a total of 203 couples, but in only 179 of them the males courted the female,  
217 leading to 41 successful copulations, which lasted from 10 to 172 seconds (mean =  $116 \pm SE$   
218 seconds). In the parasite exposure stage, 40 males were completely resistant to *M. merdarius*,  
219 whereas the remaining 128 males had from one to 74 mites (mean =  $9.6 \pm SE$  mites). The best  
220 minimal model to explain mating success in male *O. taurus* only contained the effect of  
221 courtship rate (Table 2), providing no support for a connection between mating success and  
222 parasite resistance. However, when looking at the subset of males that successfully mated,  
223 the most parsimonious model to explain mating duration contained the additive effect of

224 parasite resistance (Table 3). This was due to the fact that the intensity of infestation of *M.*  
225 *merdarius* mites was higher for males that achieved relatively short copulations (Figure 1).

226

## 227 DISCUSSION

228 Most of the literature on parasite-mediated sexual selection (Hamilton and Zuk, 1982) has  
229 focused on the following assumptions: (1) host fitness decreases with higher levels of  
230 parasitism; (2) expression of ornaments is negatively affected by parasitism; (3) resistance to  
231 parasites is heritable; (4) females preferentially mate with highly ornamented males; and (5)  
232 females preferentially mate with the least parasitised males. Assumptions 1 and 4 have  
233 substantial support from parasitology (Combes, 2001) and sexual selection literature  
234 (Andersson, 1994), respectively. Assumptions 2 and 5 have been tested before with direct  
235 reference to the PMSS hypothesis, also providing relatively strong support (Borgia and  
236 Collis, 1990; Clayton, 1991; Møller, 1990a, b; Zuk et al., 1990). Meanwhile, assumption 3 has  
237 received some empirical attention (see for example Boulinier et al., 1997; Mazé-Guilmo et al.,  
238 2014; Polak, 2003), but very little in the context of sexual selection (but see Møller, 1990a;  
239 Sorci et al., 1997), making our first result a valuable contribution to the body of evidence for  
240 PMSS.

241 We found that resistance to *Macrocheles merdarius* in *O. taurus* males was highly  
242 heritable (table 1), with our estimates using intensity of parasitism being significantly higher  
243 than that of body size, regardless of whether non-infested males were kept or removed from  
244 our analysis. Heritability was lower, but still moderate and significant, when looking at  
245 parasite prevalence (table 1). Our estimate of heritability (0.39) of resistance to mite  
246 infestation based on prevalence is much higher than the realized heritability ( $h^2 = 0.15$ ) for  
247 *Drosophila nigrospiracula* resistance measured as the prevalence of a congeneric  
248 haematophagous mite, *Macrocheles subbadius* (Polak, 2003). It is however important to  
249 emphasize that Polak (2003) estimated the realized heritability, which is calculated from the

250 actual response to selection (Falconer and Mackay, 1996), whereas in our study we used a  
251 breeding design to infer heritability based on a pedigree. A study of a natural population of  
252 the sea bird kittiwake and its ectoparasitic tick measured the heritability of parasite  
253 resistance using parasite load (intensity), and found a result much more comparable ( $h^2 =$   
254 0.72) to the one we report for parasite intensity ( $h^2$  between 0.65 and 0.80). But that study  
255 used field data and estimated heritability with a parent-offspring regression (Boulinier et al.,  
256 1997), which is once again a very different approach to ours. In a wild population of the  
257 freshwater fish *Leuciscus burdigalensis*, Mazé-Guilmo and collaborators (2014) used genetic  
258 data to build a pedigree and infer the heritability (using a similar animal model to ours and  
259 measuring intensity of parasitism) of approximately 0.18 for the fish's resistance to the  
260 ectoparasite *Tracheliastes polycolpus*. This study has a much more comparable approach to  
261 ours, showing that the heritability of *O. taurus* resistance to *M. merdarius* is very high  
262 compared to previous studies of different systems.

263 Similarly to the studies mentioned above, our estimates of parasite resistance rely on  
264 parasite prevalence and intensity, which probably results from a collection of *O. taurus* traits  
265 that we did not measure directly, including perhaps kairomones (Berson and Simmons, 2018;  
266 Simmons and Ridsdill-Smith, 2011) that are less attractive to parasites, active immunological  
267 responses, and/or the ability to actively brush mites off the cuticle, for example.  
268 Investigating the specific mechanisms underlying resistance was beyond the scope of the  
269 present study, but it is noteworthy that similar experimental exposure to *Machoches* mites  
270 in a closely related dung beetle species are known to reduce male lifespan (Kotiaho and  
271 Simmons, 2001), indicating that our measures of resistance are biologically relevant,  
272 regardless of the mechanisms responsible for them.

273 We tested whether female choice can be linked to parasite resistance directly, rather  
274 than indirectly via levels of parasitism. This prediction is exclusive to the PMSS hypothesis,  
275 and as far as we know it has not been directly tested to date. Our approach offered a unique

276 opportunity to test this prediction by measuring each male's attractiveness to females before  
277 they were experimentally exposed to parasitism. Contrary to our prediction, we found no  
278 effect of future resistance to parasites on mating success, even when controlling for courtship  
279 rate, which positively affected mating success (Table 2), consistently with previous studies in  
280 the species (Kotiaho, 2002; Kotiaho et al., 2001). This contrasts with the results of an artificial  
281 selection approach in *Drosophila melanogaster*, which found that increased resistance to a  
282 parasitoid was genetically correlated with higher mating success (Rolff and Kraaijeveld,  
283 2003).

284         When looking at mating duration, however, our result was consistent with the PMSS  
285 hypothesis, as we found a link between parasite resistance and mating duration (Table 3).  
286 This was true across most of our blocks, although the magnitude of the effect differed  
287 between blocks. This difference was expected a priori, since blocks also differed in the  
288 number of mites available to infest males, and we dealt with it analytically by standardizing  
289 the number of mites across blocks (see our methods). Nonetheless, it was very clear that  
290 lower levels of parasitism (indicating high resistance) were seen in the males that had mated  
291 for the longest. In theory, longer copulations could provide an opportunity for more sperm  
292 transfer (but see House and Simmons, 2003), and therefore be a proxy of fertilization success.  
293 Moreover, the short copulations of some of the males that subsequently became highly  
294 infested are atypical, being more than two SDs away from the mean copula duration typical  
295 for this species (Simmons and Kotiaho, 2002). It may be that males fail to deliver an ejaculate  
296 in these very short copulations, which may be indicative of mating failures (García-  
297 González, 2004; Greenway et al., 2015). If females control copula duration, then longer  
298 matings in our experiment would indicate female choice for males who later presented  
299 higher parasite resistance. The mechanism through which females detect parasite resistance  
300 in males is still unclear, but we speculate that male cuticular hydrocarbons, which are known  
301 to be under mate choice in the species (Berson and Simmons, 2018), might play a role.

302           On the one hand, the order of our experimental manipulations avoided the problem of  
303 confounding female choice for parasite resistance with female choice for least parasitised  
304 males. Whereas the latter is an important assumption of PMSS (assumption 5 mentioned  
305 above), only the former can be considered an exclusive prediction of this hypothesis, when  
306 contrasted with the alternative 'avoidance hypothesis' (Borgia and Collis, 1990) and 'resource  
307 provisioning hypothesis' (Hamilton, 1990), for example. On the other hand, our approach can  
308 be criticized on the basis of mating costs in insects: males that mated successfully and/or for  
309 longer might be weakened (McKean and Nunney, 2001), hampering their ability to resist  
310 parasitism. While this could have impacted our ability to detect a relationship between  
311 mating success and parasite resistance, when looking only at males that mated successfully,  
312 we showed that resistance to the ectoparasite *M. merdarius* was higher in males that mated  
313 for longer (Table 3). This result is consistent with PMSS, and the opposite of what would be  
314 predicted if mating duration had negatively affected parasite resistance.

315           Our results on mating duration should be interpreted with caution, since this variable  
316 did not affect fertilization success when females were mated to more than one male in this  
317 species (House and Simmons, 2003). Moreover, even though females must open their  
318 pygidium for copulation to start (Werner and Simmons, 2008), we have no evidence that the  
319 termination of copulation is under female control, which would be necessary for mating  
320 duration to reflect female choice. However, longer copulations should provide more  
321 opportunity for ejaculate transfer, and if that assumption holds true, our results reveal sexual  
322 selection for heritable parasite resistance in *O. taurus*. This is tentatively a crucial test of this  
323 exclusive prediction of parasite-mediated sexual selection (Hamilton and Zuk, 1982) that  
324 remains poorly investigated despite all the theoretical and empirical attention that PMSS has  
325 attracted in over three decades.

326           A potential shortcoming of our approach is that males used here were wild-caught,  
327 and already harboured varying levels of *M. merdarius* infestation. We minimized this

328 problem by avoiding using males that came from the field with high levels of infestation (see  
329 Materials and Methods), but there is still a chance that previous levels of parasitism caused a  
330 delayed effect on male mating ability. If such effect also caused the same males to be less  
331 capable of resisting a secondary infestation (our experimental one that followed the mating  
332 trials), then this could have caused a correlation between mating duration and experimental  
333 parasite load that was not a result of females assessing males' parasite resistance directly.  
334 This alternative explanation could be tested in the future by investigating whether a male's  
335 prior parasite load has long-term effects on his subsequent mating behaviour or parasite  
336 load. Alternatively, our experiment could be repeated with lab-reared adults with no  
337 previous history of parasite exposure at all, in order to completely avoid the shortcoming of  
338 our approach.

339 In conclusion, from a host-parasite coevolution perspective, our results support the  
340 hypothesis that sexual selection may act as an additional force driving coevolutionary cycles  
341 via boosting the adaptiveness of parasite resistance, which would then be under natural and  
342 sexual selection. From a sexual selection perspective, our results corroborate Hamilton and  
343 Zuk's idea that the co-adaptive cycles of host resistance and parasite virulence renew the  
344 genetic variation for fitness (Balenger and Zuk, 2014) that would otherwise be completely  
345 eroded due to strong mate choice (the 'Lek paradox'; Kirkpatrick and Ryan, 1991).

346

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356

357 Data accessibility: Analyses reported in this article can be reproduced using the data  
358 provided by Buzatto et al. (2019).



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466

467 Table 1: Descriptive statistics and quantitative genetics estimates (from a half-sibling breeding design analysed with an animal model) for body  
 468 size (pronotum width) and three different measures of parasite resistance (to the mite *Macrocheles merdarius*) in the dung beetle *Onthophagus*  
 469 *taurus*. Parasite resistance was measured as the number of mites counted on each male (ran as a Poisson model), the number of mites in the  
 470 infested males (removing males who had no mites; also ran as a Poisson model, but with no zeros) and with the dichotomous response of  
 471 infested (1) versus resistant (0) males (ran as a binomial model).

472

Trait	Descriptive statistics			Coefficients of Variation			Heritability				
	Mean	$\sigma$	N	VA	CV <sub>A</sub>	$\sigma$ CVA	H <sup>2</sup>	$\sigma$ H <sup>2</sup>	84%CI	P-value	
Pronotum width	5.37	0.01	563	0.004	0.01	0.01	0.08	0.19	-0.18	0.34	0.6727
Mite resistance											
Number of mites (all males)	11.40	0.73	563	4.09	0.18	0.01	0.80	0.01	0.79	0.82	< 0.0001
Number of mites (infested males)	16.01	0.93	401	1.82	0.08	0.01	0.65	0.02	0.62	0.67	< 0.0001
Infested vs resistant males (binomial)	0.71	0.02	563	0.63	1.11	0.23	0.39	0.10	0.25	0.52	< 0.0001

473

474 Table 2: Model selection for the effects of courtship rate and number of *Macrocheles merdarius*  
 475 mites on mating success in males of the dung beetle *Onthophagus taurus*, after a parasite  
 476 resistance trial following a mating trial (see methods for details).

N°	Fixed effects	k	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Weight	log-likelihood	Cumulative weight
1	Courtship rate	3	111.93	—	0.5652	-52.89	0.5652
2	Courtship rate + Number of mites	4	113.51	1.58	0.2570	-52.63	0.8222
3	Courtship rate + Number of mites + (Courtship rate X Number of mites)	5	114.25	2.31	0.1778	-51.93	1.0000
4	Null	2	185.39	73.46	< 0.001	-90.66	1.0000
5	Number of mites	3	186.16	74.22	< 0.001	-90.01	1.0000

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479

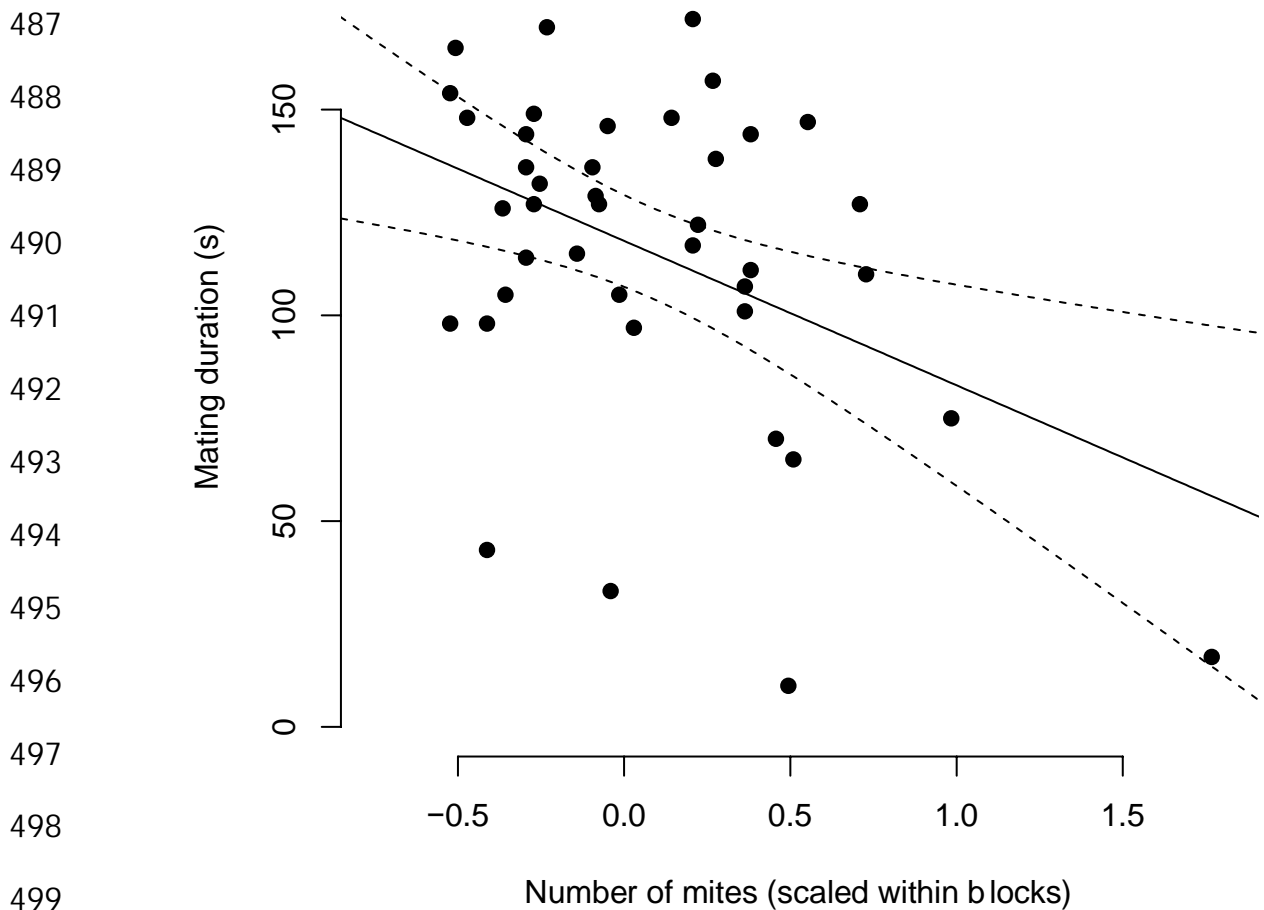
480 Table 3: Model selection for the effect of the number of *Macrocheles merdarius* mites on  
 481 mating duration in males of the dung beetle *Onthophagus taurus*, after a parasite resistance  
 482 trial following a mating trial (see methods for details). This analysis only includes males that  
 483 mated successfully during the mating trial.  
 484

N°	Fixed effects	k	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Weight	log-likelihood	Cumulative weight
1	Number of mites	3	416.16	—	0.9474	-204.08	0.9474
2	Null	2	422.27	5.78	0.0526	-208.14	1.0000

485

486





501 Figure 1: Males of the dung beetle *Onthophagus taurus* that mated for longer had higher  
 502 parasite resistance, as measured by the number of *Macrocheles merdarius* mites found on them  
 503 following an experimental exposure to these parasites. The solid line is based on the  
 504 prediction from a model equivalent to 1 in Table 3, but without random effects (the model  
 505 without random effects had lower  $AIC_c$  than the model with the random effect of block), and  
 506 the dashed lines represent the 95% confidence interval built by multiplying the standard  
 507 error of the predictions by 1.96. Males were exposed to mites after the mating trial where  
 508 mating duration was measured, and all males included in this analysis mated successfully.