

1 **Experimental evidence for the role of sexual selection in the evolution of cuticular**
2 **hydrocarbons in the dung beetle, *Onthophagus taurus***

3

4 **Running title:** Sexual selection and evolution of CHCs

5

6 **Authors:** Jacob D. Berson^{1*}, Francisco Garcia-Gonzalez^{1, 2} and Leigh W. Simmons¹

7

8 **Author Affiliations:** ¹Centre for Evolutionary Biology, School of Biological Sciences, The
9 University of Western Australia, Crawley, Western Australia 6009, Australia

10 ²Doñana Biological Station, Spanish Research Council CSIC, Sevilla, Spain

11

12 ***Corresponding Author:** Email: jacob.berson@uwa.edu.au; Phone: +61 8 6488 2923; Fax:

13 +61 8 6488 1029

14

15

16 **Abstract**

17 A role for sexual selection in the evolution of insect cuticular hydrocarbons (CHCs) is
18 suggested by observations of selection acting on male CHCs during female mate choice.
19 However, evidence that CHCs evolve in response to sexual selection is generally lacking, and
20 there is a need to extend our understanding beyond well-studied taxa. Experimental evolution
21 offers a powerful approach to investigate the effect of sexual selection on the evolution of
22 insect CHCs. We conducted such an experiment using the dung beetle, *Onthophagus taurus*.
23 After six, 12 and 21 generations of experimental evolution, we measured the CHCs of beetles
24 from three populations subject to sexual selection and three populations within which sexual
25 selection had been removed via enforced monogamy. We found that the male CHC profile
26 responded to the experimental removal of sexual selection. Conversely, the CHC profile of
27 females responded to the presence of sexual selection but not to its removal. These results show
28 that sexual selection can be an important mechanism affecting the evolution of insect CHCs,
29 and that male and female CHCs can evolve independently.

30

31 **Keywords:** Experimental evolution, sexual selection, CHC, sexual dimorphism, dung beetles

32

33

34 **Introduction**

35 Some of the most extravagant traits in the natural world are thought to have evolved in
36 response to sexual selection (Darwin, 1871, Andersson, 1994). There is now a wealth of
37 evidence across a diverse array of taxa that visual, auditory and olfactory sexual signals do
38 affect an individual's reproductive success and are subject to sexual selection (Rosenthal, 2017,
39 Zuk & Simmons, 2018). However, remarkably little work is available to show that sexual
40 selection is responsible for the divergence in sexual traits among contemporary populations
41 (Rodríguez et al., 2013, Svensson & Gosden, 2007). This lack of information can be at least
42 partly explained by the difficulty isolating the effects of natural and sexual selection on trait
43 divergence among natural populations. Experimental evolution offers a powerful tool to help
44 overcome this difficulty (Kawecki et al., 2012). Using this approach, sexual selection can be
45 manipulated whilst controlling natural selection, allowing for direct testing of the effect of
46 sexual selection on the divergence of sexual traits.

47 Sexual selection is expected to generate divergence in sexual traits both among
48 populations, and also between the sexes. In particular, Darwin (1871) argued that sex-
49 dependent evolution would result from the strength of sexual selection differing between the
50 sexes, most commonly being strong on males and weak or absent on females (Darwin, 1871).
51 In support of Darwin's theory, over a century of research has found associations between
52 sexually dimorphic traits and male mating success (Andersson, 1994, Andersson & Simmons,
53 2006, Fairbairn et al., 2007). However, sex-dependent evolution can also occur through natural
54 selection (Darwin, 1871, Lande, 1980, Slatkin, 1984, Shine, 1989, Cooper, 2010) and we
55 cannot assume that sexual selection represents the ultimate cause of the observed phenotypic
56 divergence between the sexes (Hedrick & Temeles, 1989). Isolating the effects of natural and
57 sexual selection is therefore necessary to understand the drivers of divergence both among
58 populations and between the sexes.

59 The cuticular hydrocarbons (CHCs) of insects were traditionally thought to be naturally
60 selected anti-desiccants, but they can also be subject to sexual selection through female choice
61 (Steiger & Stökl, 2014), and a signalling function could explain the complex blends of CHCs
62 commonly produced (Blomquist & Bagnères, 2010). However, recent work with field crickets,
63 *Teleogryllus oceanicus*, has found that despite evidence of sexual selection acting on the CHC
64 profiles of males and females via mate choice (Thomas & Simmons, 2009, Thomas &
65 Simmons, 2010), patterns of divergence among populations are more consistent with random
66 genetic drift than sexual selection (Pascoal et al., 2017). Evidence that sexual selection leads
67 to an evolutionary response in CHCs has been found using fruit flies from the genus
68 *Drosophila*. For example, experimental evolution studies have documented an evolutionary
69 response to manipulations of sexual selection in the CHC profiles of *D. serrata* (Chenoweth et
70 al., 2008), *D. pseudoobscura* (Hunt et al., 2012) and *D. simulans* (Sharma et al., 2012). These
71 studies provide important evidence for the influence of sexual selection on the evolution of
72 insect CHCs. Expanding beyond these study systems to other taxa will allow us to assess the
73 role of sexual selection in the evolution of insect CHCs more broadly.

74 The dung beetle, *Onthophagus taurus*, provides an excellent model to help address this
75 gap in our understanding. Sexual selection in this species is characterized by male-male
76 competition (Moczek & Emlen, 2000), and female choice based on a male's CHC profile and
77 his courtship behaviour (Kotiaho et al., 2001, Berson & Simmons, 2018b). Mating does not
78 appear to be harmful to females, rather females benefit from mating with multiple males
79 (Simmons & Garcia-Gonzalez, 2008, Simmons & Holley, 2011, McCullough et al., 2017).
80 Furthermore, both males and females assist in offspring provisioning in *O. taurus* (Hunt &
81 Simmons, 2002). Experimental evolution studies of this species that have manipulated sexual
82 selection through the enforcement of monogamy or maintenance of polygamy have found
83 divergence in sexual traits such as testes mass and sperm competitiveness (Simmons & Garcia-

84 Gonzalez, 2008), and both male and female genital morphology (Simmons & Garcia-Gonzalez,
85 2011). In the present study we asked whether this manipulation of the mating system resulted
86 in an evolutionary response in male *O. taurus* CHC profiles. We predicted that removing sexual
87 selection through enforced monogamy would result in an evolutionary response in the male
88 CHC profile given that females choose among males based on their CHC profiles (Berson &
89 Simmons, 2018b). Sexual selection on female CHCs is yet to be investigated in this species,
90 but is known from other taxa (Chenoweth & Blows, 2003, Thomas & Simmons, 2010).
91 Therefore we also looked for responses to the manipulation of sexual selection in the CHC
92 profile of females.

93

94

95 **Methods**

96 Full details of the initiation and maintenance of the experimental populations have been
97 described elsewhere (Simmons & Garcia-Gonzalez, 2008). Briefly, field caught beetles
98 underwent two generations of laboratory rearing before 60 males and 60 females were
99 randomly allocated to each of three replicate monogamous populations and three replicate
100 polygamous populations. All six populations were maintained in isolation from each other and
101 treated identically, except for a manipulation of the mating system. Monogamy was enforced
102 by randomly allocating one male to one female and allowing them to mate for one week. Within
103 each polygamous population, 10 males and 10 females were randomly assigned to one of six
104 30-L containers and left to interact freely for one week. Following these mating treatments, the
105 males were discarded and the females placed in individual chambers for one week and allowed
106 to produce brood masses (a single brood mass consists of an egg and dung resources for larval
107 development from hatching to adult emergence). For each population, the broods of 50 females
108 were combined from those females that produced ≥ 5 brood masses. Following the emergence

109 of offspring from their brood masses, beetles were kept in single-sex cultures for one week of
110 maturation feeding before the above procedures were repeated. Experimental evolution
111 proceeded for 21 generations (approximately 4 years of selection). A random sample of beetles
112 was frozen after maturation feeding each generation to track changes in traits of interest. The
113 effective population size of the monogamous populations was 100 (50 males and 50 females)
114 and for the polygamous populations was estimated to be ~ 106 (50 females ~ 56 males)
115 (Simmons & Garcia-Gonzalez, 2008). Maintaining similar effective population sizes ensured
116 that any observed evolutionary responses could be attributed to the different selection regimes,
117 rather than to differences in genetic drift or inbreeding. The intensity of sexual selection
118 operating in similar polygamous populations was estimated by Simmons et al. (2004), who
119 found that a male's mating success was positively related to his number of mates, with an
120 average (\pm SE) Bateman's gradient of 0.832 ± 0.019 across polygamous populations. Females
121 in the polygamous populations mated on average (\pm SE) with 4.5 ± 0.2 males (Simmons et al.,
122 2004, Simmons & Garcia-Gonzalez, 2008), compared with an estimate of 2.8 ± 0.17 males
123 from a field population (McCullough et al., 2017).

124 Five unmated males and five unmated females were randomly selected for CHC
125 analysis from single-sex cultures of each population after six, 12 and 21 generations of
126 experimental evolution, allowing us to track changes in male and female CHC profiles across
127 generations. CHCs were analysed by gas chromatography – mass spectrometry using a
128 Shimadzu QP2010 with protocols established for this species (Berson & Simmons, 2018b, see
129 their Figure 1 for an example of a typical chromatogram for *O. taurus*). Peaks were matched
130 to previously identified compounds (Berson & Simmons, 2018a). Peak areas were transformed
131 to logcontrasts (using *n*-tricosane as the divisor) (Blows, 1998), and a principal component
132 (PC) analysis performed on the correlation matrix to reduce the number of explanatory
133 variables. For data analysis, we retained only those PCs that had eigenvalues ≥ 1 and which

134 each individually explained $\geq 10\%$ of the variation. Although particular compounds may be
135 more important in sexual competition compared to others, individual CHCs are unlikely to
136 evolve independently. By using PCs we were able to test whether the aggregate CHC profile
137 responded to the manipulation of sexual selection. We conducted separate linear mixed effects
138 models for each retained PC as the response variable, with selection regime, generation and
139 sex as fixed effects, along with all possible interactions, and replicate population as a random
140 effect. We examined how the populations responded to selection over time by testing for a
141 significant selection regime x generation interaction. We also examined whether the CHC
142 profile of females evolved similarly (or otherwise) to males by testing for a significant
143 generation x sex interaction. Model validation was conducted using visual inspection of
144 diagnostic plots. Inspection revealed that models of the first PC (PC1) required a \log_{10} -
145 transformation of PC scores (we added 10 to the PC scores before \log_{10} -transformation to
146 account for negative values) and we report the results for PC1 using these \log_{10} -transformed
147 values. All analyses were performed in R version 3.3.3 (R Core Team, 2017), using the
148 *FactoMineR* package for PC analysis (Lê et al., 2008), *lme4* package for mixed model analyses
149 (Bates et al., 2015), the *car* package for testing the significance of model terms using chi-square
150 tests (Fox & Weisberg, 2011) and *ggplot2* for data visualization (Wickham, 2009).

151

152 **Results**

153 We analysed a total of 180 CHC profiles from the six experimental populations. The
154 PC analysis returned two PCs with eigenvalues ≥ 1 that each individually explained $\geq 10\%$ of
155 the total variation in CHC profiles (Table 1). We interpreted those eigenvector elements that
156 were $\geq 70\%$ of the highest element as contributing significantly to a PC (Mardia et al., 1979).
157 PC1 was weighted positively by all compounds except two, with the highest weightings tending
158 towards longer-chained CHCs. We therefore interpret this PC as representing greater

159 investment in a range of CHCs and particularly those with longer chain lengths. PC2
160 represented a contrast between several terminally branching shorter-chained compounds
161 (positive values) with two straight-chained alkanes (negative values).

162 All two-way interactions were significant for PC1 (Table 2). There was an overall
163 evolutionary response to the mating system manipulation (selection regime x generation
164 interaction), with the response of the sexes differing (sex x generation interaction). To see how
165 these significant interactions reflected the response of each sex to the different selection
166 regimes, we tested for an effect of generation on PC1 separately for each sex by selection
167 regime combination. There was a significant effect of generation on PC1 for monogamous
168 population males ($\chi^2_1 = 25.073$, $P < 0.001$) but not polygamous population males ($\chi^2_1 = 0.039$,
169 $P = 0.843$). In contrast, there was a significant effect of generation on PC1 for polygamous
170 population females ($\chi^2_1 = 20.308$, $P < 0.001$) but not monogamous population females ($\chi^2_1 =$
171 2.006 , $P = 0.157$). Plots of the population means at each generation separated by sex and
172 selection regime are shown in Figure 1. Thus, the removal of sexual selection through enforced
173 monogamy led to CHC profiles characterized by higher values of PC1 for males, but there was
174 no response to this manipulation in females. In contrast, the presence of sexual selection
175 (polygamous populations) led to CHC profiles characterized by lower values of PC1 for
176 females, but there was no response in males (Figure 1).

177 None of the interactions were significant for PC2, although there was a significant
178 effect of generation (Table 2). Plots of the population means for PC2 are shown in Figure 2.
179 There was a general tendency for PC2 scores to increase between generations six and 12, and
180 then to decrease at generation 21. The consistent pattern across the selection regimes and sexes
181 suggests that subtle differences in the environment across the generations may have had an
182 influence on PC2 scores, for example, differences in the quality of dung used to feed the

183 beetles. As our focus in this study was on the effect of selection, and there was no effect of the
184 selection regimes on PC2 scores, we restrict our Discussion to the results for PC1.

185

186 **Discussion**

187 By measuring the cuticular hydrocarbon (CHC) profiles of both sexes across multiple
188 generations of replicate populations evolving under either enforced monogamy or polygamy,
189 we have documented sex-dependent evolutionary responses to sexual selection in the major
190 axis of CHC variation (PC1) of the dung beetle, *O. taurus*. As would be expected from the
191 known action of sexual selection on male CHCs in this species (Berson & Simmons, 2018b),
192 an evolutionary response in the CHC profiles of males occurred when sexual selection was
193 relaxed (enforced monogamy) but not when it was allowed to continue (polygamy).
194 Unexpectedly, the CHC profiles of females did respond to the continued operation of sexual
195 selection. This suggests that despite polygamy representing the natural mating system of this
196 species, the specific polygamous experimental conditions employed in this study displaced the
197 female phenotype from that expressed in natural populations. The contrasting responses in
198 males and females suggests independence between the sexes in the evolution of their CHCs.
199 These findings provide important evidence in support of the role of sexual selection in the
200 evolution of insect CHC profiles.

201 The evolution of male PC1 scores provides evidence for the role of sexual selection in
202 the evolution of male *O. taurus* CHCs. As both inter and intra-sexual selection were either
203 present or absent in our experimental populations, it is not possible to determine the precise
204 mechanism that was responsible for the evolutionary divergence of male PC1 scores.
205 Nevertheless, the axis of CHC variation represented by PC1 in this study is broadly similar to
206 that represented by PC1 in a previous study that tested for sexual selection via female mate
207 choice on male *O. taurus* CHCs (Berson & Simmons, 2018b). This previous study did not find

208 evidence of female choice acting on PC1, indicating that female choice may not have played a
209 significant role in the evolution of male PC1 scores found here. Cuticular hydrocarbons are
210 associated with male dominance in some insects (Roux et al., 2002, Thomas & Simmons,
211 2011), and importantly, the form of sexual selection imposed on CHCs can differ for female
212 mate choice versus male contest competition (Lane et al., 2016). Mating success and fighting
213 success appear to be uncorrelated in male *O. taurus* (McCullough & Simmons, 2016), and
214 consequently any selection on CHCs imposed by male contest competition could differ to that
215 imposed by female mate choice. It is therefore possible that the removal of male contest
216 competition in the monogamous populations was responsible for the evolution of PC1 scores.
217 However, whether male contest competition favours lower PC1 scores for male *O. taurus*
218 remains to be confirmed.

219 The increase in male PC1 under enforced monogamy suggests that sexual selection in
220 this species may shift the male CHC profile away from its naturally selected optimum. In
221 addition to a sexual display function, CHCs provide a protective barrier against water loss
222 (Gibbs & Rajpurohit, 2010), with compounds of increased chain length associated with
223 decreased cuticular permeability (Gibbs & Pomonis, 1995). As the weightings on PC1 were
224 greater for longer-chained CHCs (Table 1), and enforced monogamy resulted in an increase in
225 male PC1 scores, the response of male CHCs may reflect evolution towards CHC profiles of
226 enhanced water-proofing properties following the relaxation of sexual selection. Nevertheless,
227 we suggest caution in this interpretation for two reasons. First, variation in PC1 will reflect
228 both variation in the compounds that weight significantly on PC1, as well as variation in the
229 compound used to standardize peaks across the CHC profile. Second, chain-length is just one
230 factor that can affect the water-proofing properties of insect CHCs, and the effect of CHC
231 composition on cuticular water-loss is not always clear (Gibbs & Rajpurohit, 2010). With these
232 caveats in mind, consistent with evidence for antagonistic natural and sexual selection on CHCs

233 in fruit flies (Hine et al., 2011, Sharma et al., 2012) and crickets (Berson et al., 2019), our
234 results suggest that natural and sexual selection may act antagonistically on *O. taurus* CHC
235 profiles.

236 We can offer at least two possible explanations for the evolutionary response seen in
237 female CHCs under polygamy. First, our polygamous treatment may have imposed similar
238 patterns of sexual selection to those acting on males in natural populations, but different
239 patterns on females. Despite the general focus on sexual selection acting on males, research
240 over recent decades has provided evidence for an association between female secondary sexual
241 traits and mating success (Amundsen, 2000, Bonduriansky, 2001, Clutton-Brock, 2007,
242 Clutton-Brock, 2009, Kraaijeveld et al., 2007, Edward & Chapman, 2011, Hare & Simmons,
243 2019), including female CHCs (Chenoweth & Blows, 2003, Thomas & Simmons, 2010).
244 Although the mating system of *O. taurus* has been characterized by sexual selection on males
245 (Moczek & Emlen, 2000, Kotiaho et al., 2001), females accrue benefits by mating with multiple
246 mates (Garcia-Gonzalez & Simmons, 2007, Simmons & Garcia-Gonzalez, 2008, McCullough
247 et al., 2017), particularly mates of high quality (Simmons & Holley, 2011). Recent behavioural
248 observations have also revealed aggressive interactions among females of this species (Beckers
249 et al., 2017). This raises the possibility that females might compete for access to high-quality
250 males, and that males may bias their mating activity towards females with particular CHC
251 profiles. It is possible that the particular polygamous experimental conditions employed in this
252 study increased competition among females for access to mates compared to that experienced
253 in the ancestral population, through limiting the number of available males, for example, and
254 shifted the female CHC profile in the polygamous populations towards that favoured by
255 elevated sexual selection. Alternatively, although all females were kept individually and given
256 equal resources when producing offspring, it is possible that the environmental conditions
257 during mating imposed natural selection on females. For example, in the polygamous, but not

258 the monogamous populations, females would have had to compete with other females and
259 males for access to food resources necessary for their future reproduction, and this competition
260 may have selected for changes in female CHC profiles. Regardless of the cause of the
261 evolutionary response in females, our results show that the observed evolution of female CHCs
262 was not simply a correlated response to the evolution of CHCs in males, and their independent
263 evolution may contribute to sexual dimorphism seen in insect CHC profiles (Thomas &
264 Simmons, 2008).

265 The CHC profiles of females in the polygamous populations evolved towards greater
266 negative PC1 scores, indicating that females with reduced expression of a range of longer-
267 chained CHC compounds had the greater reproductive success under the sexual conditions we
268 established in the polygamous populations. Male mate choice in insects is commonly biased
269 towards females of high fecundity (Bonduriansky, 2001) and evidence from the social insects
270 and one species of cricket suggests that female CHC profiles can act as honest signals of
271 fecundity (Holman et al., 2010, Van Oystaeyen et al., 2014, Berson & Simmons, 2019). The
272 production of CHCs appears to trade-off with egg production in several taxa (Schal et al., 1994,
273 Wicker & Jallon, 1995, Blows, 2002, Holman, 2012), and the reduced expression of a range of
274 CHC compounds could therefore signal increased investment in fecundity. If this were the case
275 for *O. taurus*, males may benefit by biasing their mating activity towards those females with
276 lower PC1 scores, leading to the observed evolutionary response of females in the polygamous
277 populations. Although consistent with our results, a fecundity-signalling role for CHCs in *O.*
278 *taurus* remains speculative, and further experiments are required to test this hypothesis.

279 In conclusion, our results provide an example of a manipulation of sexual selection
280 affecting the evolution of insect CHCs. Furthermore, our results suggest that sexual and natural
281 selection may act antagonistically on *O. taurus* CHC profiles, and that the CHCs of males and
282 females can evolve independently. Further work is required to fully understand how the

283 changes we observed in the CHC profile relate to variation in both male and female sexual
284 fitness, as well as resistance to desiccation in *O. taurus*.

285

286 **Data accessibility**

287 All data used to conduct the analyses will be uploaded to Dryad upon acceptance.

288

289 **Author's contributions**

290 LWS and JDB conceived the study. JDB collected the data, performed the analyses and wrote
291 the first draft of the manuscript. LWS and FGG performed the experimental evolution study.

292 All authors contributed to the final manuscript and agree to be held accountable for the content
293 therein.

294

295 **Acknowledgements**

296 We thank Maxine Lovegrove for assistance with maintaining the experimental evolution
297 populations and Robert Dugand for statistical advice. This work was supported through an
298 Australian Government Research Training Program Scholarship to JDB and funding from the
299 Australian Research Council to LWS. FGG was supported by grants (CGL2016-76173-P;
300 CGL2012-34685) from the Spanish Ministry of Economy (co-funded by the European
301 Regional Development Fund). The authors acknowledge the facilities, and the scientific and
302 technical assistance of the Metabolomics Australia Facility at the Centre for Microscopy,
303 Characterisation & Analysis, The University of Western Australia, a facility funded by the
304 University, State and Commonwealth Governments.

305 **References**

- 306 Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology & Evolution* **15**:
307 149-155.
- 308 Andersson, M. 1994. *Sexual selection*. Princeton University Press, Princeton, N.J.
- 309 Andersson, M. & Simmons, L. W. 2006. Sexual selection and mate choice. *Trends in*
310 *Ecology & Evolution* **21**: 296-302.
- 311 Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models
312 using lme4. *Journal of Statistical Software* **67**: 1-48.
- 313 Beckers, O. M., Kijimoto, T. & Moczek, A. P. 2017. doublesex alters aggressiveness as a
314 function of social context and sex in the polyphenic beetle *Onthophagus taurus*.
315 *Animal Behaviour* **132**: 261-269.
- 316 Berson, J. D. & Simmons, L. W. 2018a. A costly chemical trait: phenotypic condition
317 dependence of cuticular hydrocarbons in a dung beetle. *Journal of Evolutionary*
318 *Biology* **31**: 1772-1781.
- 319 Berson, J. D. & Simmons, L. W. 2018b. Sexual selection across sensory modalities: female
320 choice of male behavioral and gustatory displays. *Behavioral Ecology* **29**: 1096-1104.
- 321 Berson, J. D. & Simmons, L. W. 2019. Female cuticular hydrocarbons can signal indirect
322 fecundity benefits in an insect. *Evolution* **73**: 982-989.
- 323 Berson, J. D., Zuk, M. & Simmons, L. W. 2019. Natural and sexual selection on cuticular
324 hydrocarbons: a quantitative genetic analysis. *Proceedings of the Royal Society B:*
325 *Biological Sciences* **286**: 20190677.
- 326 Blomquist, G. J. & Bagnères, A.-G. (2010) Introduction: history and overview of insect
327 hydrocarbons. In: *Insect hydrocarbons: Biology, biochemistry, and chemical ecology*,
328 (Blomquist, G. J. & Bagnères, A.-G., eds.). pp. 3-18. Cambridge Univ Press,
329 Cambridge.

- 330 Blows, M. W. 1998. Evolution of a mate recognition system after hybridization between two
331 *Drosophila* species. *American Naturalist* **151**: 538-544.
- 332 Blows, M. W. 2002. Interaction between natural and sexual selection during the evolution of
333 mate recognition. *Proceedings of the Royal Society of London, Series B: Biological*
334 *Sciences* **269**: 1113-1118.
- 335 Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas
336 and evidence. *Biological Reviews* **76**: 305-339.
- 337 Chenoweth, S. F. & Blows, M. W. 2003. Signal trait sexual dimorphism and mutual sexual
338 selection in *Drosophila serrata*. *Evolution* **57**: 2326-2334.
- 339 Chenoweth, S. F., Rundle, H. D. & Blows, M. W. 2008. Genetic constraints and the evolution
340 of display trait sexual dimorphism by natural and sexual selection. *American*
341 *Naturalist* **171**: 22-34.
- 342 Clutton-Brock, T. 2007. Sexual selection in males and females. *Science* **318**: 1882-1885.
- 343 Clutton-Brock, T. 2009. Sexual selection in females. *Animal Behaviour* **77**: 3-11.
- 344 Cooper, I. A. 2010. Ecology of sexual dimorphism and clinal variation of coloration in a
345 damselfly. *American Naturalist* **176**: 566-572.
- 346 Darwin, C. 1871. *The descent of man, and selection in relation to sex*. J. Murray, London.
- 347 Edward, D. A. & Chapman, T. 2011. The evolution and significance of male mate choice.
348 *Trends in Ecology & Evolution* **26**: 647-654.
- 349 Fairbairn, D. J., Blanckenhorn, W. U. & Szekely, T. 2007. *Sex, Size & Gender Roles:*
350 *Evolutionary Studies of Sexual Size Dimorphism*. Oxford University Press, New York.
- 351 Fox, J. & Weisberg, S. 2011. *An R Companion to Applied Regression*, Second ed. Sage,
352 Thousand Oaks CA.
- 353 Garcia-Gonzalez, F. & Simmons, L. W. 2007. Shorter sperm confer higher competitive
354 fertilization success. *Evolution* **61**: 816-824.

355 Gibbs, A. & Pomonis, J. G. 1995. Physical properties of insect cuticular hydrocarbons: The
356 effects of chain length, methyl-branching and unsaturation. *Comparative*
357 *Biochemistry and Physiology B-Biochemistry & Molecular Biology* **112**: 243-249.

358 Gibbs, A. G. & Rajpurohit, S. (2010) Cuticular lipids and water balance. In: *Insect*
359 *Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*, (Blomquist, G. J. &
360 Bagnères, A.-G., eds.). pp. 100-120. Cambridge Univ Press, Cambridge.

361 Hare, R. M. & Simmons, L. W. 2019. Sexual selection and its evolutionary consequences in
362 female animals. *Biological Reviews of the Cambridge Philosophical Society* **94**: 929-
363 956.

364 Hedrick, A. V. & Temeles, E. J. 1989. The evolution of sexual dimorphism in animals:
365 Hypotheses and tests. *Trends in Ecology & Evolution* **4**: 136-138.

366 Hine, E., McGuigan, K. & Blows, M. W. 2011. Natural selection stops the evolution of male
367 attractiveness. *Proceedings of the National Academy of Sciences, USA* **108**: 3659-
368 3664.

369 Holman, L. 2012. Costs and constraints conspire to produce honest signaling: Insights from an
370 ant queen pheromone. *Evolution* **66**: 2094-2105.

371 Holman, L., Jorgensen, C. G., Nielsen, J. & d'Ettorre, P. 2010. Identification of an ant queen
372 pheromone regulating worker sterility. *Proceedings of the Royal Society of London,*
373 *Series B: Biological Sciences* **277**: 3793-3800.

374 Hunt, J. & Simmons, L. W. 2002. Behavioural dynamics of biparental care in the dung beetle
375 *Onthophagus taurus*. *Animal Behaviour* **64**: 65-75.

376 Hunt, J., Snook, R. R., Mitchell, C., Crudgington, H. S. & Moore, A. J. 2012. Sexual
377 selection and experimental evolution of chemical signals in *Drosophila*
378 *pseudoobscura*. *Journal of Evolutionary Biology* **25**: 2232-2241.

379 Kawecki, T. J., Lenski, R. E., Ebert, D., Hollis, B., Olivieri, I. & Whitlock, M. C. 2012.
380 Experimental evolution. *Trends in Ecology & Evolution* **27**: 547-560.

381 Kotiaho, J. S., Simmons, L. W. & Tomkins, J. L. 2001. Towards a resolution of the lek
382 paradox. *Nature* **410**: 684-686.

383 Kraaijeveld, K., Kraaijeveld-Smit, F. J. L. & Komdeur, J. 2007. The evolution of mutual
384 ornamentation. *Animal Behaviour* **74**: 657-677.

385 Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters.
386 *Evolution* **34**: 292-305.

387 Lane, S. M., Dickinson, A. W., Tregenza, T. & House, C. M. 2016. Sexual Selection on male
388 cuticular hydrocarbons via male-male competition and female choice. *J Evol Biol* **29**:
389 1346-55.

390 Lê, S., Josse, J. & Husson, F. 2008. FactoMineR: An R package for multivariate analysis.
391 *Journal of Statistical Software* **25**: 1-18.

392 Mardia, K. V., Kent, J. T. & Bibby, J. M. 1979. *Multivariate analysis*. Academic Press,
393 London.

394 McCullough, E. L., Buzatto, B. A. & Simmons, L. W. 2017. Benefits of polyandry:
395 Molecular evidence from field-caught dung beetles. *Molecular Ecology* **26**: 3546-
396 3555.

397 McCullough, E. L. & Simmons, L. W. 2016. Selection on male physical performance during
398 male-male competition and female choice. *Behavioral Ecology* **27**: 1288-1295.

399 Moczek, A. P. & Emlen, D. J. 2000. Male horn dimorphism in the scarab beetle,
400 *Onthophagus taurus*: do alternative reproductive tactics favour alternative
401 phenotypes? *Animal Behaviour* **59**: 459-466.

402 Pascoal, S., Mendrok, M., Wilson, A. J., Hunt, J. & Bailey, N. W. 2017. Sexual selection and
403 population divergence II. Divergence in different sexual traits and signal modalities in
404 field crickets (*Teleogryllus oceanicus*). *Evolution* **71**: 1614-1626.

405 R Core Team (2017) R: A language and environment for statistical computing. pp. R
406 Foundation for Statistical Computing, Vienna, Austria.

407 Rodríguez, R. L., Boughman, J. W., Gray, D. A., Hebets, E. A., Höbel, G. & Symes, L. B.
408 2013. Diversification under sexual selection: the relative roles of mate preference
409 strength and the degree of divergence in mate preferences. *Ecology Letters* **16**: 964-
410 974.

411 Rosenthal, G. G. 2017. *Mate choice: the evolution of sexual decision making from microbes*
412 *to humans*. Princeton University Press, Princeton, USA.

413 Roux, E., Sreng, L., Provost, E., Roux, M. & Clement, J.-L. 2002. Cuticular hydrocarbon
414 profiles of dominant versus subordinate male *Nauphoeta cinerea* cockroaches.
415 *Journal of Chemical Ecology* **28**: 1221-1235.

416 Schal, C., Gu, X., Burns, E. L. & Blomquist, G. J. 1994. Patterns of biosynthesis and
417 accumulation of hydrocarbons and contact sex pheromone in the female German
418 cockroach, *Blattella germanica*. *Archives of Insect Biochemistry and Physiology* **25**:
419 375-91.

420 Sharma, M. D., Hunt, J. & Hosken, D. J. 2012. Antagonistic responses to natural and sexual
421 selection and the sex-specific evolution of cuticular hydrocarbons in *Drosophila*
422 *simulans*. *Evolution* **66**: 665-677.

423 Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: A review of the
424 evidence. *Quarterly Review of Biology* **64**: 419-461.

425 Simmons, L. W., Beveridge, M. & Krauss, S. 2004. Genetic analysis of parentage within
426 experimental populations of a male dimorphic beetle, *Onthophagus taurus*, using

427 amplified fragment length polymorphism. *Behavioral Ecology and Sociobiology* **57**:
428 164-173.

429 Simmons, L. W. & Garcia-Gonzalez, F. 2008. Evolutionary reduction in testes size and
430 competitive fertilization success in response to the experimental removal of sexual
431 selection in dung beetles. *Evolution* **62**: 2580-2591.

432 Simmons, L. W. & Garcia-Gonzalez, F. 2011. Experimental coevolution of male and female
433 genital morphology. *Nature Communications* **2**: 6.

434 Simmons, L. W. & Holley, R. 2011. Offspring viability benefits but no apparent costs of
435 mating with high quality males. *Biology Letters* **7**: 419-421.

436 Slatkin, M. 1984. Ecological causes of sexual dimorphism. *Evolution* **38**: 622-630.

437 Steiger, S. & Stökl, J. 2014. The role of sexual selection in the evolution of chemical signals
438 in insects. *Insects* **5**: 423-438.

439 Svensson, E. I. & Gosden, T. P. 2007. Contemporary evolution of secondary sexual traits in
440 the wild. *Functional Ecology* **21**: 422-433.

441 Thomas, M. L. & Simmons, L. W. 2008. Sexual dimorphism in cuticular hydrocarbons of the
442 Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae). *Journal of*
443 *Insect Physiology* **54**: 1081-1089.

444 Thomas, M. L. & Simmons, L. W. 2009. Sexual selection on cuticular hydrocarbons in the
445 Australian field cricket, *Teleogryllus oceanicus*. *Bmc Evolutionary Biology* **9**: 12.

446 Thomas, M. L. & Simmons, L. W. 2010. Cuticular hydrocarbons influence female
447 attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus*. *Journal*
448 *of Evolutionary Biology* **23**: 707-714.

449 Thomas, M. L. & Simmons, L. W. 2011. Short-term phenotypic plasticity in long-chain
450 cuticular hydrocarbons. *Proceedings of the Royal Society B* **278**: 3123-3128.

- 451 Van Oystaeyen, A., Oliveira, R. C., Holman, L., van Zweden, J. S., Romero, C., Oi, C. A.,
452 d'Ettorre, P., Khalesi, M., Billen, J., Wackers, F., Millar, J. G. & Wenseleers, T. 2014.
453 Conserved class of queen pheromones stops social insect workers from reproducing.
454 *Science* **343**: 287-290.
- 455 Wicker, C. & Jallon, J. M. 1995. Influence of ovary and ecdysteroids on pheromone
456 biosynthesis in *Drosophila melanogaster* (Diptera: Drosophilidae). *European Journal*
457 *of Entomology* **92**: 197-202.
- 458 Wickham, H. 2009. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New
459 York.
- 460 Zuk, M. & Simmons, L. W. 2018. *Sexual Selection: A Very Short Introduction*. Oxford
461 University Press, Oxford, UK.
- 462

463 **Tables**

464

465 **Table 1.** Best matches for *Onthophagus taurus* cuticular hydrocarbons, their mean relative
 466 amounts, and the results of the principal component (PC) analysis. Eigenvectors are given
 467 under the headings PC1 and PC2, those in bold have values that are equal to or greater than
 468 70% of the highest eigenvector element (Mardia et al., 1979).

469

				PC1	PC2	
				Eigenvalue	13.711	5.294
				% variance	37.058	14.309
Peak	Compound	Mean	SE			
1	<i>n</i> -docosane	0.36	0.01	0.138	0.101	
2	<i>n</i> -tricosane	3.55	0.09			
3	11-methyltricosane	1.06	0.08	0.067	0.046	
4	2-methyltricosane	0.42	0.03	-0.049	0.343	
5	3-methyltricosane	0.44	0.02	0.013	0.335	
6	<i>n</i> -tetracosane	1.05	0.03	0.115	0.029	
7	2-methyltetracosane	0.31	0.02	-0.031	0.335	
8	<i>n</i> -pentacosane	11.32	0.25	0.132	-0.208	
9	11-methylpentacosane	2.57	0.10	0.114	0.236	
10	7-methylpentacosane	0.67	0.03	0.098	0.129	
11	2-methylpentacosane	1.08	0.04	0.102	0.247	
12	3-methylpentacosane	1.50	0.05	0.101	0.353	
13	<i>n</i> -hexacosane	1.17	0.03	0.172	-0.267	

14	3,7-dimethylpentacosane	0.58	0.03	0.160	0.071
15	3,7,11-trimethylpentacosane	0.22	0.01	0.177	0.062
16	2-methylhexacosane	0.47	0.02	0.127	0.002
17	<i>n</i> -heptacosane	10.74	0.43	0.118	-0.317
18	13-methylheptacosane	3.92	0.13	0.180	0.057
19	2-methylheptacosane	1.81	0.06	0.186	0.022
20	3-methylheptacosane	2.36	0.06	0.202	-0.043
21	<i>n</i> -octacosane	0.96	0.02	0.185	-0.232
22	14-methyloctacosane	0.52	0.02	0.190	0.047
23	<i>n</i> -nonocosane	3.61	0.14	0.122	-0.170
24	15-methylnonacosane	7.94	0.17	0.220	-0.025
25	11,15-dimethylnonacosane	0.53	0.02	0.211	-0.012
26	19,23-dimethylnonacosane	1.55	0.05	0.240	-0.037
27	5,15-dimethylnonacosane	2.62	0.11	0.180	-0.024
28	15-methyltriacontane	1.23	0.03	0.214	0.008
29	15-methylhentriacontane	16.82	0.34	0.201	-0.064
30	13,17-dimethylhentriacontane	0.64	0.02	0.222	0.005
31	9,21-dimethylhentriacontane	1.27	0.05	0.230	-0.005
32	7,17-dimethylhentriacontane	1.03	0.03	0.232	0.010
33	5,17-Dimethylhentriacontane	1.51	0.06	0.197	0.026
34	15-methyl-dotriacontane	0.82	0.03	0.180	0.028
35	15-methyltritriacontane	5.53	0.14	0.165	0.036
36	11,21-dimethyltritriacontane	4.02	0.18	0.197	0.108
37	15-methylpentatriacontane	0.86	0.04	0.054	0.167
38	11,21-dimethylpentatriacontane	2.94	0.16	0.154	0.150

471 **Table 2.** Results of the mixed effects model that included Selection regime (SR), Generation
 472 (G), Sex (S) and all possible interactions as fixed effects, and replicate population as a random
 473 effect.

474

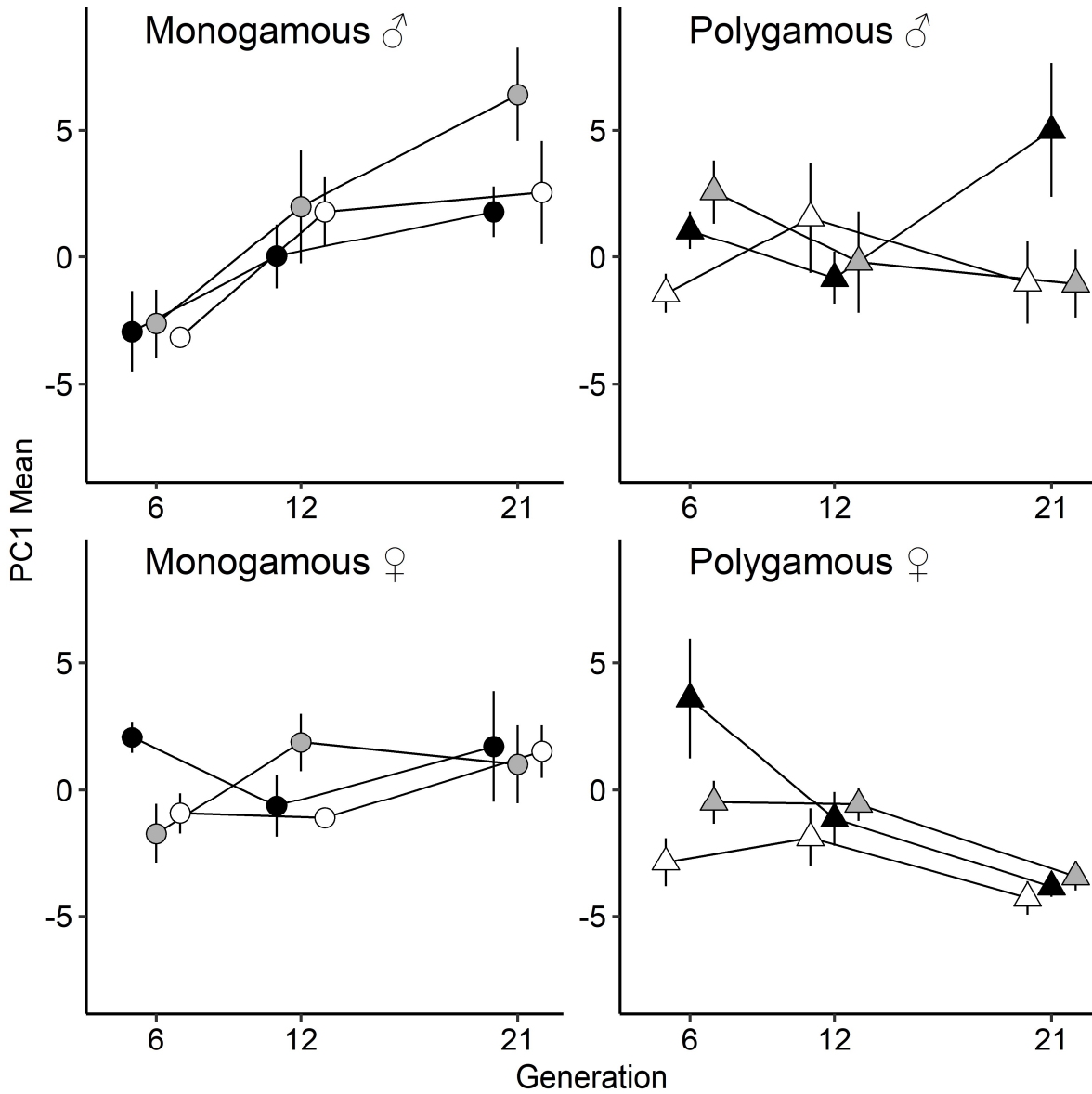
	PC1		PC2	
	χ^2_1	<i>P</i>	χ^2_1	<i>P</i>
Selection Regime (SR)	2.445	0.118	0.993	0.319
Generation (G)	1.813	0.178	16.550	<0.001
Sex (S)	5.006	0.025	3.189	0.074
SR x G	29.668	<0.001	0.097	0.756
SR x S	8.031	0.005	0.475	0.491
G x S	16.040	<0.001	3.775	0.052
SR x G x S	0.125	0.724	1.735	0.188

475

476 **Figures**

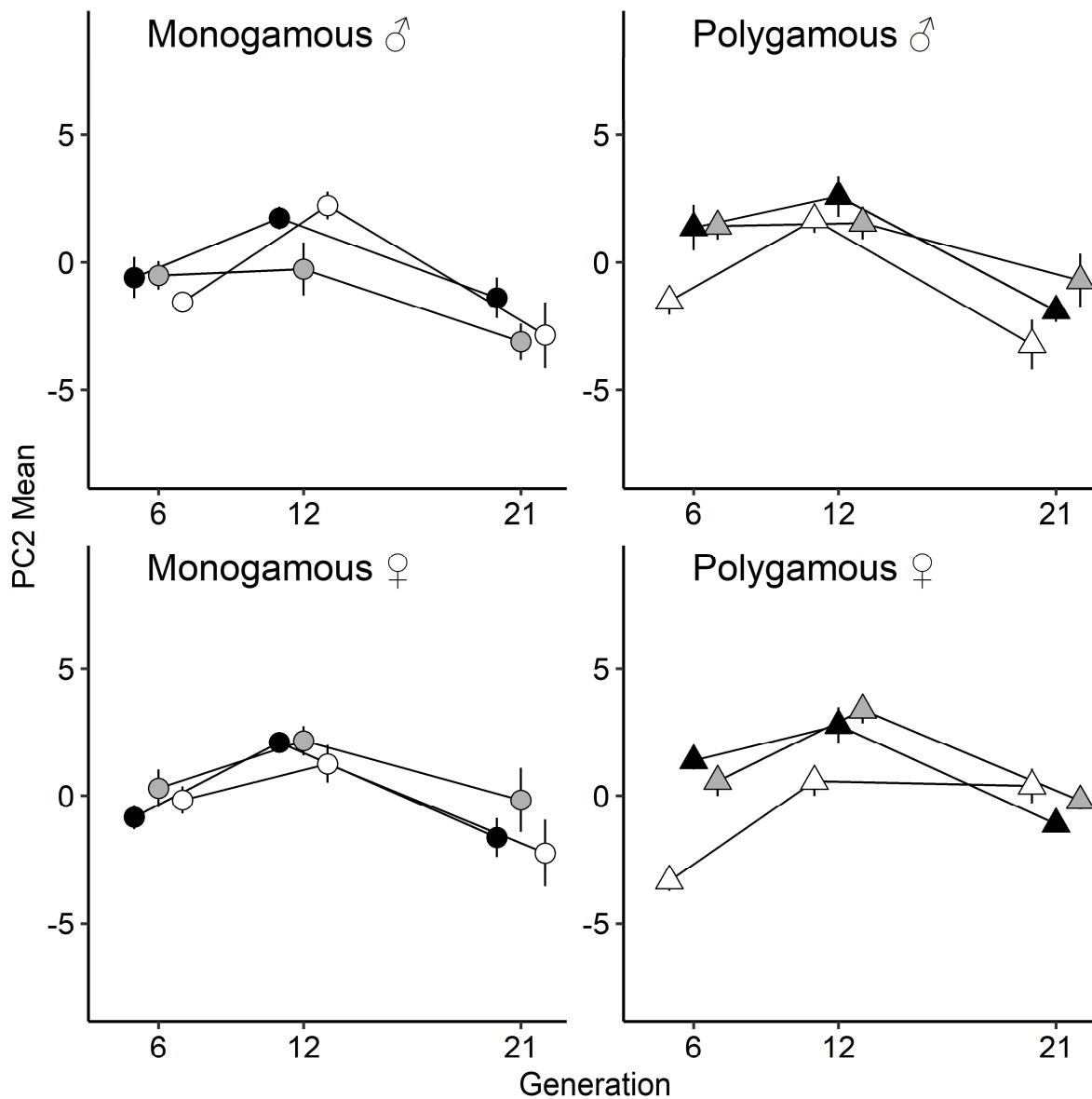
477

478 **Figure 1.** Plot of replicate population means at each generation for the first major axis of
479 cuticular hydrocarbon phenotypic variation (PC1) separated by sex and selection regime. Error
480 bars represent standard errors.



481

482 **Figure 2.** Plot of replicate population means at each generation for the second major axis of
483 cuticular hydrocarbon phenotypic variation (PC2) separated by sex and selection regime. Error
484 bars represent standard errors.



485