1 # Differential effects of aerobic capacities on fight outcome in the

2 # Giant hissing cockroaches

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15 Running title: Contest behaviour in giant hissing cockroaches.

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19 **Lay Summary:** When animals fight over resources, why do some species invest

20 more in contest behaviours than others? Here we investigated an apparent disparity

21 in contest traits (weaponry, physiology and fighting tactics) in the Giant hissing

22 cockroaches. It appears that some species have evolved endurance-enhancing

23 adaptations to prime them for vigorous contests, leading to physically-fit 'fighters'.

Abstract

When animals engage in contests, 'fights' are usually non-injurious, involving either signals or trials of strength. Such fights end once an individual assesses that it is the weaker opponent, or it has reached the threshold level of costs it is prepared to pay. We investigated the agonistic behaviours performed and the relative costs paid by winners and losers during aggressive interactions in two species of Madagascan hissing cockroaches (the wide-horned cockroach, *Gromphadorhina oblongonota* and the flat-horned cockroach*, Aeluropoda insignis*). By combining contest observations with measures of energetic expenditure and using X-ray computed tomography to compare the internal structure of the respiratory systems of the competitors, we reveal the differential physiological investment associated with resource holding potential in these two species. Winners in *G. oblongonota* had larger respiratory volumes than predicted for their size compared to losers. *G. oblongonota* fought more aggressively and had larger respiratory systems overall than *A. insignis*, which engaged in less physical fighting. This indicates differential investment in fight tactics, and associated anatomy that may promote contest success. Invertebrate species at the upper limits of body size need to maintain efficient respiratory systems to support their size while also remaining subject to the influence of sexual selection.

Introduction

When animals engage in contests over resources, it is usually the larger contestant that wins. Thus there is a strong association between contestant size and their fighting ability or Resource-Holding Potential ('RHP'; Parker 1974). Furthermore, indices of RHP are strongly tied to body size (see Archer 1988), with many species exhibiting sexual size dimorphism indicating that one sex, usually the male, is more competitive than the other (reviewed in Andersson 1994). Larger size confers greater advantage during fights, including greater muscle power and thus greater strength (e.g. Mowles et al. 2011). Larger individuals can easily win against smaller ones, so that simple assessments of relative size can influence the likelihood of entering into a contest at all, with smaller individuals making immediate decisions to not engage in contests with larger rivals. For example, in female 'hermit' spiders, *Nephilengys cruentata*, intruders are more likely to leave a resident's web immediately if these have been constructed by individuals larger than themselves (Schuck-Paim 2000). This is likely due to assessment of the size of the web mesh. In fiddler crabs, *Austruca annulipes*, residents will form coalitions and engage with intruders based on size, which is likely assessed by the relative sizes of the sexually selected major claw (Detto et al. 2010). However, when competitor size is very similar, such disparities become harder to resolve and other factors are necessary to determine differences in RHP.

In addition to larger body sizes, males may possess weaponry for fighting over females, such as the enlarged claws of fiddler crabs (Jennions & Backwell 1996), antlers in deer (Clutton-Brock et al. 1979), and horns in other ungulates (Lundrigan

70   1996), in beetles (Ohde et al. 2018) and cockroaches (Clark & Moore 1994; Bell et

71   al. 2007), among other taxa (reviewed in Emlen 2008). Weaponry exists not purely to

72   injure opponents (although injuries do occur: e.g. Wilkinson & Shank 1976), but

73   rather to engage in a stereotyped 'trial of strength' or wrestling match where antlers

74   may be interlocked while the contestants push at one another, or where horns are

75   used by invertebrates in order to push or flip an opponent over (Goyens et al. 2015;

76   Durrant et al. 2016).

77

78   Trials of strength or endurance, while not necessarily resulting in death, are not

79   without costs, which are generally measured in time (extrinsic costs) and energy

80   (intrinsic costs) (Payne & Pagel 1996; 1997). The energetic costs of trials of strength

81   are incurred due to the performance of additional, often strenuous, dynamic

82   behaviours (e.g. pushing and wrestling). Thus, even non-injurious fights may be

83   metabolically demanding for contestants and their level of activity may be

84   constrained by their physical fitness. For example, the metabolic by-product lactic

85   acid has been shown to rapidly increase due to the vigorous contest behaviours

86   performed during hermit crab shell fights, limiting their performance (Briffa & Elwood

87   2005). Further, the aerobic capacity of hermit crabs limits their ability to persist in

88   contests and thus governs their chances of winning (Mowles et al. 2009). What

89   remains to be seen is which criteria are most important in determining contest

90   outcomes when animals are equally size matched. For example, size-matching may

91   result in the reduced ability for contestants to assess differences in RHP and thus

92   contests may be resolved by underlying physiological differences, with losers giving

93   up once they have reached a threshold of costs.

94

95   We investigated the relative importance of trials of strength and energetic capacity in

96   establishing dominance using two species of giant Madagascan hissing cockroach

97   (tribe: Gromphadorhini). These insects are useful for understanding sexual selection

98   as they are sexually dimorphic, with males being larger than females, and bear

99   sexually-selected, species-specific weaponry in the form of pronotal horns, with

100   which they engage in trials of strength over territories and access to females. Hissing

101   cockroaches produce a range of acoustic signals, by forcing air out of a specialised

102   4th pair of spiracles to generate a hissing sound (Nelson 1979; Nelson & Fraser

103   1980). Hissing is used in various contexts; it is produced in a 'soft' form during

104   courtship, and with greater amplitude during aggression and predator deterrence

105   (Nelson & Fraser, 1980). In aggressive interactions, males engage in 'butting'

106   contests where they attempt to butt and to flip their opponent over (Logue et al.

107   2011). Contest dynamics are known to vary within this tribe, with the large, wide-

108   horned hissing cockroach, *Gromphadorhina oblongonota*, engaging in vigorous

109   fighting and attempting to monopolise fertilisations via access to females, whereas

110   the smaller, flat-horned hissing cockroach, *Aeluropoda insignis*, is less aggressive,

111   but develops larger testes, suggesting that it monopolises fertilisations via post-

112   copulatory sperm competition (Durrant et al. 2016). However, all Gromphadorhini

113   species possess weaponry to some extent.

114

115   By staging encounters between size-matched dyads of male cockroaches, we

116   investigated the relative importance of known correlates of RHP. We measured

117   performance capacities (strength) and aerobic capacities (lactic acid generation and

118   respiratory system anatomy) to understand how underlying physical fitness may

119   determine the capacity to win contests in these two species. We predicted that in the

120 absence of size disparities, fights would likely escalate to trials of strength, and that

121 the winners of fights would have greater performance and aerobic capacities than

122 losers, indicating a higher physical fitness of the winner.

123

Methods

Study organisms

126 Cockroaches were sourced from breeding colonies at the University of Nottingham,

127 UK. Nymphs obtained from the colonies were sexed once they began to exhibit

128 sexually dimorphic subgenital plates, whereupon males were individually isolated in

129 tanks to mature in a temperature-controlled laboratory maintained at 28± 2 °C on a

130 12:12 hour reversed light/dark cycle. Each male was maintained in a plastic

131 container (185 mm L x 125 mm W x 75 mm D) containing beech chip substrate and

132 half a paper cup for shelter. Each male received two pieces of dry dog food once a

133 week and was provided with a piece of water-soaked cotton wool for moisture.

134 Isolation ensured that males matured without any prior fighting or mating experience.

135

Behavioural trials

137 Morphometric measurements were taken before any behavioural trials. Each male

138 was photographed alongside a scale bar to accurately measure body length using

139 ImageJ image analysis software (https://imagej.nih.gov/ij/). This allowed contest

140 dyads to be size-matched to within 0.3 % for *Gromphadorhina oblongonota* and

141 0.1% for *Aeluropoda insignis* (*G. oblongonota* = 46 males, resulting in 23 contests;

142 *A. insignis* = 38 males, resulting in 19 contests). Only animals without visible damage

143 were used in the behavioural trials. We marked each individual in a dyad with either

144 an arrow or a 'T' shape on its pronotal shield using white correctional fluid to identify

145 individuals during subsequent video analysis.

146

147 Contest trials were carried out between 09:00 – 15:00hrs under red lighting to

148 promote natural behaviours in these nocturnal animals (Koehler et al. 1987).

149 Interactions between size-matched dyads were staged in clear acrylic chambers (17

150 x 10 x 14.5cm high) with a white cardboard substrate to provide grip. Each

151 interaction was video recorded from above in high definition (HDRXR 160 EB video

152 camera, Sony Corporation, Japan).

153

154 Cockroaches were gently removed from their housing containers and simultaneously

155 placed into the contest arena such that their antennae touched on release. This

156 procedure promoted the initiation of contest behaviour over territory. Whilst many

157 contests featured vigorous fighting behaviour (butting and flipping the opponent onto

158 its back), many encounters involved 'low aggression' contest behaviours including

159 repeated antennations and approaches towards the opponent, which may retreat or

160 adopt a low posture (presumably to guard against being overturned). These low

161 aggression behaviours were often performed by both opponents to similar extents

162 during the encounter, necessitating a careful analysis of contests to determine which

163 individual displayed the more frequent dominant behaviours. Interactions were thus

164 recorded and terminated after 20 minutes had elapsed (*G. oblongonota* = 11

165 contests, *A. insignis* = 16), or once a clearly dominant individual emerged, with one

166 cockroach (the subordinate) attempting to escape the other, necessitating the

167 separation of the contestants for ethical reasons (*G. oblongonota* = 12 contests, *A.*

168   *insignis* = 3).

169

170   The recordings were reviewed at half-speed and scored using JWatcher version 1.0

171   event recording software (Blumstein & Daniel 2006) to record the occurrence of

172   individual behaviours. Actions reflecting aggressive/dominant or submissive

173   behaviours were scored for each animal. Variables scored were time approaching

174   rival (aggressive), time retreating from rival (submissive), time spent in low body

175   posture (submissive: protecting the legs beneath the body and avoiding being

176   flipped), antennations (aggressive: antennal whips directed at the opponent) and

177   butts (aggressive: hitting the opponent with the pronotal horns).

178

179   Principal components analysis was conducted in R version 3.4.1 to determine

180   whether the behaviours assumed to be dominant or subordinate formed a factor for

181   use in further analyses. As the amount of time approaching, retreating, or spent in

182   low posture will be dependent on interaction time, these were calculated as a

183   proportion of total interaction duration. Therefore, the factors in the models were:

184   proportion of time retreating, proportion of time approaching, proportion of time in low

185   posture, number of antennations performed and number of butts performed. For *G.*

186   *oblongonota*, PC1 had an Eigenvalue of 2.2 and explained 44% of the observed

187   variance (Table 1). The factor loadings identified a spectrum of submissive (low

188   values) to dominant (high values) behaviours. In *A. insignis*, PC1 and PC2 had

189   Eigenvalues of 1.3 and 1.2 respectively, explaining 26% and 25% of the variance in

190   the dataset. However, the component loadings of PC2 were the most similar to those

191   obtained for *G. oblongonota,* and biologically logical in terms of grouped behaviours.

192   Thus PC2 was retained, with low values indicating a subordinate individual, and high

193   values a dominant individual (Table 1).

194

195   Factor scores were extracted from the PCA to identify where each individual resided

196   on these behavioural axes and thus allowed the identification of dominant and

197   subordinate individuals where this had not been immediately apparent. Those with

198   the higher factor score in a dyad were identified as dominant and those with the

199   lower factor score were identified as subordinate.

200

201   **Physiological measures**

202   Immediately after each encounter, haemolymph samples ($\bar{x} \pm se = 0.1 \pm 0.006$ g)

203   were taken from each individual by piercing the arthrodial membrane at the base of

204   the second leg using a 0.6 x 30mm hypodermic needle (Becton Dickson, USA). We

205   alternated first drawing haemolymph from dominant or subordinate individuals (when

206   outcomes were obvious) to avoid order effects. Haemolymph was collected directly

207   into a pre-weighed 0.5 mL Eppendorf tube. Hissing cockroach haemolymph

208   coagulates rapidly and the wounds to the arthrodial membranes of each cockroach

209   sealed immediately following the procedure. All cockroaches were returned to their

210   individual housing containers after this procedure. They were given access to water

211   and food, with a rest period of at least seven days to recover prior to engaging in the

212   performance capacity trials.

213

214   The highly viscous nature of cockroach haemolymph necessitated a novel approach

215   to assaying haemolymph lactate. All haemolymph samples were frozen at -80 °C for

216   a minimum of 12 hrs before being freeze-dried for 12 hrs in a vacuum freeze dryer

217   (Birchover Instruments Ltd, UK). The resulting pellet was ground to a fine powder by

218   hand using a dissecting seeker inserted into the Eppendorf tube. Each sample was

219   then weighed to an accuracy of 0.0001 g and was analysed for *L*-lactate using the

220   perchloric acid extraction method following Neat et al. (1998). The powdered

221   haemolymph was suspended in 40-120 µl of chilled 0.3 M perchloric acid in a ratio of

222   1 mg powder to 3.3 µl acid. Suspensions were thoroughly mixed using a vortex mixer

223   and centrifuged for 10 minutes at 11,000 rpm. The resulting supernatant was

224   decanted into a clean 0.25 mL Eppendorf tube and stored at 4 °C. This process was

225   repeated, adding an identical volume of acid as before to the resulting pellet, which

226   was vortexed and centrifuged as before. The supernatant was decanted and added

227   to the previously-extracted volume for each sample. Samples were then neutralised

228   by adding 10-25 µl 2M potassium bicarbonate, vortexed and centrifuged as before.

229   This final supernatant was decanted and analysed for *L*-lactate following a

230   spectrophotometric method using a standard laboratory kit (Trinity Biotech PLC,

231   Bray, Co Wicklow, Ireland). *L*-lactate concentration was calculated as mg of lactate

232   per g dry haemolymph powder.

233

234   **Performance capacity trials**

235   Cockroach strength was measured using a tensile testing machine (5969, Instron,

236   USA) with a 50 N load cell. Each cockroach was tethered using a 60 cm length of

237   0.25 mm nylon fishing line attached to the dorsal surface of the second thoracic

238   segment using cyanoacrylate glue and a small piece of tape (see van Casteren &

239   Codd 2010). The tether was looped around a pulley and attached to the machine,

240   which slowly pulled the cockroach backwards at a rate of 9 mm per second. The

241 cockroach was placed on a textured rubber mat to provide grip and was provided

242 with half of a plastic cup for shelter, to promote forward motion towards the shelter

243 and away from the pulley. Strength trials were terminated once the animal was

244 drawn as far back as the pulley or let go of the mat. The maximum force (N) with

245 which the cockroach was able to resist the backwards pull from the machine was

246 recorded. The trial was repeated three times with approximately 24 hrs between

247 trials and the average tension of the three trials was recorded. The trial order of

248 animals was randomised for each repetition and cockroaches were kept in their own

249 enclosures with *ad libitum* access to food and water throughout the trial period. Due

250 to the amount of manipulation involved in the strength trials, it was necessary to

251 conduct these last so that the cockroaches would behave as naturally as possible

252 during their staged encounters.

253

254 **X-ray computed tomography**

255 To gain measurements of the respiratory systems of the individuals, they were first

256 killed by freezing at -80 °C. Cockroaches were scanned using X-ray computed

257 tomography (Phoenix v|tome|x m 240kV, Waygate Technologies, Wunstorf,

258 Germany) to produce internal and external morphometrics. Cockroaches were

259 wrapped in a thin sheet of X-ray translucent polystyrene foam and placed in a hollow

260 plastic tube of sufficient width to prevent specimen deformation. The tube was

261 mounted in the scanner. X-ray projection images were acquired in 250 ms intervals

262 (2638 in total over a 360° rotation) using an energy and current of 75 kV and 200 µA.

263 Scans were conducted in three sections, so as to image the entire body length at a

264 maximal spatial resolution of 30 microns per voxel. The three sections; head to

265   thorax, thorax to abdomen, and abdomen were automatically stitched together using

266   Phoenix Datos|x v2 software (Waygate Technologies, Wunstorf, Germany). Using

267   complete body scans, the hissing structure of the 4th spiracle and full tracheal

268   network of the respiratory system was identified and digitally segmented using

269   VGStudio MAX 2.2 (Volume Graphics GmbH, Germany). The tracheal network was

270   clearly identifiable from the surrounding tissues as the air delivery system is hollow

271   and unlike denser tissue. Measurements of total respiratory volume and total body

272   volume (mm^3) were recorded from the scan data.

273

Statistical methods

275   As the total respiratory volume of the cockroaches was found to be positively

276   correlated with the total body volume in both species (Spearman's rank correlations:

277   *G. oblongonota*: r_s = 0.935, *N* = 46, *P* < 0.0001; *A. insignis*: r_s = 0.857, *N* = 32, *P* <

278   0.0001), the residual respiratory volumes from these relationships were calculated in

279   order to investigate the extent to which the respiratory volumes of dominant and

280   subordinate individuals deviated from the allometric body volume-respiratory volume

281   relationship. Residuals were calculated from a simple regression for each species

282   with respiratory volume as the dependent variable and whole body volume as the

283   independent variable.

284

285   As the overall body volumes of the cockroaches were non-normally distributed, these

286   data were log-transformed, while the *L*-lactate data was multiplied by 10,000 then

287   transformed by calculating the square-root. Each *L*-lactate value was multiplied by

288   10,000 in order to ensure that the predictor variables in our models (*L*-lactate

289 concentration and residual respiratory volume) were on the same scale. To

290 investigate the effects of these respiratory variables on fight outcome, we used

291 binomial generalized mixed models with a logit link function and backwards stepwise

292 model reduction with ANOVA. 'Contest identity' was included as a random effect to

293 control for the non-independence of results from winners and losers interacting in the

294 same contest dyad while allowing us to retain the information for each individual.

295 Species were analysed separately. The factor in each model was contest outcome

296 (dominant or subordinate), and the variables were residual respiratory volume and

297 post-contest L-lactate concentration. Where transformation failed to normalise data,

298 non-parametric tests were used. All statistical analyses were conducted using R

299 version 3.4.1.

300

301 **Ethical note**

302 Engaging in contests is a noninjurious behaviour for hissing cockroaches. The

303 performance capacity trials were also noninjurious. In order to undergo X-ray

304 computed tomography, the subject must remain absolutely still and thus it was

305 necessary to kill the cockroaches prior to scanning. Cockroaches were humanely

306 killed by freezing at -80 °C. To minimize the number of individuals used we kept

307 sample sizes to the minimum suitable for the effective analysis of physiology,

308 performance capacities and of fight outcomes.

309

310

Results

Few individuals flipped their opponent during encounters, but *Gromphadorhina oblongonota* was more likely to do this than *Aeluropoda insignis* (*G. oblongonota* = 14, *A. insignis* = 1). Few interactions involved hissing, but *G. oblongonota* was less likely to perform this behaviour than *A. insignis* (*G. oblongonota* = 6, *A. insignis* = 12).

As the contestants were size-matched, there was no difference in body size between dominant and subordinate individuals in *Gromphadorhina oblongonota* (χ^2_{43} = -0.06, P = 0.952), or *Aeluropoda insignis* (χ^2_{29} = 0.583, P = 0.56). There was a positive correlation between body size and mean tension (N) of resistance produced by both *G. oblongonota* (Spearman's rank correlation, r_s = 0.508, N = 46, P < 0.001) and *A. insignis* (Pearson's product-moment correlation: r_{30} = 0.798, P < 0.001). As the strength of contestants was correlated with body size, and the contestants were size-matched, fight outcome was not related to individual strength in either *G. oblongonota* (Wilcoxon signed rank test: V_{21} = 145, P = 0.897) or *A. insignis* (Paired t-test: t_{17} = -1.077, P = 0.296).

Respiratory physiology and fight outcomes

When analysing *G. oblongonota* data, model reduction procedures indicated that *L*-lactate should be removed as a covariate (P = 0.085). However, interactions between this variable and respiratory volume may have relevant biological implications that are otherwise hard to detect. We therefore ran the model twice,

334   once with L-lactate retained, and once with it removed, and interpreted results from

335   both. In both cases, the main effect of respiratory volume remained.

336

337   From our CT-scans we were able to visualise the whole body, respiratory systems

338   and hiss-producing structures of both species (Figure 1). The specialised hissing

339   spiracle of the hissing cockroaches is identifiable as a scleratised void internal to the

340   4th spiracle, with a sphincter-like pinch-point connecting it to the rest of the

341   respiratory system (Figure 1). In *G. oblongonota*, there was a significant difference in

342   residual respiratory volumes between animals of differing dominance status.

343   Dominant individuals were found to have positive residual respiratory volumes,

344   whereas subordinate individuals were found to have negative residual respiratory

345   volumes (χ^2_{41} = -3.698, P < 0.001; Figure 2). In *A. insignis*, dominant and

346   subordinate individuals did not differ in residual respiratory capacity (χ^2_{27} = 1.203, P

347   = 0.229; Figure 2).

348

349   Post-contest levels of L-lactate between dominant and subordinate individuals did

350   not differ in either species (*G. oblongonota*: χ^2_{41} = -1.024, P =0.306; *A. insignis*: χ^2_{27}

351   = 0.760, P = 0.447). However, there was a significant interaction between residual

352   respiratory volume and L-lactate concentration on contest outcome in *G.*

353   *oblongonota* (χ^2_{41} = 2.907, P = 0.004), driven by different relationships between

354   residual respiratory volume and post-contest L-lactate concentration between

355   dominant and subordinate individuals. In dominant individuals, there is no significant

356   relationship between residual respiratory volume and post-contest L-lactate,

357   although the correlation coefficient is negative (Pearson's product-moment

358   correlation: r_{21} = -0.128, P = 0.56). In subordinate individuals, there is also no

359   significant relationship between residual respiratory volume and post-contest L-

360   lactate, although the correlation coefficient is positive (Pearson's product-moment

361   correlation: $r_{21} = 0.319$, $P = 0.138$). There was no significant interaction between

362   residual respiratory volume and L-lactate concentration on contest outcome in A.

363   *insignis* ($\chi^2_{27} = -1.384$, $P = 0.166$).

364

365   There was no difference in post-contest L-lactate concentrations between the

366   species (Welch's two sample t-test: $t_{73.3} = 1.346$, $P = 0.183$). *G. oblongonota* had

367   overall larger body sizes than *A. insignis* (Welch's two sample t-test: $t_{66.0} = -8.406$, P

368   < 0.0001) and larger raw respiratory volumes (Welch's two sample t-test: $t_{58.0} = -$

369   8.145, $P < 0.0001$). They also had larger relative respiratory volumes (respiratory

370   volume/body volume) than *A. insignis* (Kruskal-Wallis: $\chi^2_1 = 22.602$, $P < 0.001$;

371   Figure 3).

372

373   **Discussion**

374   In animal contests, it is usually the larger of the opponents that wins, and physical

375   fighting is usually avoided if clear size differences exist between opponents. We

376   have demonstrated that when opponents are size-matched, typical indices of RHP

377   (here size and strength) are no longer able to predict contest outcomes. In the

378   absence of such asymmetries, the underlying respiratory capacity of the competitors

379   differed between dominant and subordinate individuals, indicating its potential

380   importance in determining contest outcomes. We used two species of Madagascan

381   hissing cockroach to test the assumptions of differential investment in aggressive

382   behaviour. We have previously demonstrated that *Gromphadorhina oblongonota* is a

383   much more aggressive species than *Aeluropoda insignis*, with more elaborate

384   weaponry for use in aggressive encounters (Durrant et al. 2016). It appears that the

385   larger, more heavily weaponised male *G. oblongonota* competes for females via

386   competitive exclusion of rivals, whilst the smaller, less-aggressive male *A. insignis*

387   rely more on post-copulatory sperm competition to secure fertilisations,

388   demonstrated by their absolutely larger investment in testis size than their sister

389   genus *Gromphadorhina* (Durrant et al. 2016). What remained to be seen was how

390   aggressive encounters within each species were resolved, especially when

391   opponents were size-matched.

392

393   We found that the larger, more aggressive *G. oblongonota* had a greater relative

394   investment in its respiratory system than the less physically aggressive *A. insignis*.

395   Thus, the greater relative respiratory volumes of *G. oblongonota* may be required to

396   fuel its more vigorous, and potentially energetically demanding, contest behaviour.

397   Indeed, it was found that *G. oblongonota* individuals that had larger than predicted

398   respiratory capacities for their size were more likely to be dominant. Similar results

399   have been found in hermit crabs *Pagurus bernhardus*, where winners of contests

400   were found to have larger than expected concentrations of the respiratory pigment

401   haemocyanin than expected for their size (Mowles et al. 2009). In both of these

402   contest interactions, it appears that the contestants engage in energetically costly

403   behaviour that places demands on aerobic metabolism, and can force them into

404   anaerobic respiration as evidenced by the accumulation of lactic acid (Briffa &

405   Elwood 2005).

406

407   We have demonstrated that when size and strength differences are removed by

408   size-matching opponents, a potential effect of respiratory capacities on the outcome

409   of contests is revealed. Here, the ability to effectively deliver oxygen to the tissues

410   may enhance fighting ability by allowing longer lasting aerobic respiration.

411   Adaptations for prolonging aerobic respiration, such as larger tracheal systems,

412   probably evolved as a response to being a large invertebrate and to then maximise

413   oxygen exchange when burrowing through leaf litter. This is likely why we saw no

414   differences in *L*-lactate concentrations between competitors. Having the aerobic

415   capacity for their size and ecological niche could act as a substrate for sexual

416   selection and thus allow its further utility in male-male contests.

417

418   When investigating adaptations to contest behaviour, it is important to consider not

419   just the physical weaponry used by species, or the combative behaviours that they

420   may employ, but also the underlying physiology that may permit vigorous contest

421   behaviour to be performed. The relatively larger amount of body volume devoted to

422   the respiratory system in *G. oblogonota* than *A. insignis* suggests that *G. oblogonota*

423   has evolved not just larger weaponry for fighting, but also a system to more

424   effectively deliver oxygen to the tissues. Whilst this system may have evolved in

425   response to the problem of efficiently delivering oxygen to the tissues in an

426   increasingly larger-sized insect; such an adaptation may also support its ability to

427   engage in more vigorous contests. This could explain why dominant males in our

428   interactions had relatively larger respiratory systems than predicted for their size.

429

430   In the cockroach Order, there are many examples of sexually selected morphologies

431   and associated behaviours adapted to maximise fertilisation success. In the Cuban

432   burrowing cockroach, *Byrsotria fumigata*, the females are wingless and larger than

433   males, a typical feature of oviparous and ovoviviparous species, while the males are

much smaller and use their wings in a courtship display to attract females (Mowles and Jepson 2015). The wholly flightless tribe Gromphadorhini presents a very different strategy, with the males being larger than the females and furnished with varying degrees of species-specific weaponry with which to compete for access to females. Here, we have investigated two species at the extremes of this range, and while we urge caution in any two-species comparison (see Garland Jr & Adolph 1994), these extremes allow us to suggest how sexual selection may be driving different strategies within this tribe.

It appears that our two study species represent different approaches to gaining fertilisations and each possesses the necessary morphological and physiological adaptations to do so. *A. insignis* exhibits very little aggressive behaviour and typical correlates of RHP are not associated with dominance status as it likely obtains fertilisations via post-copulatory sperm competition (Durrant et al. 2016). Trials of strength are more common in the more aggressive *G. oblongonota*, which likely obtains fertilisations by fighting for access to and monopolisation of females (Durrant et al. 2016), necessitating more prominent weaponry and associated respiratory physiology. Thus, even within a family of animals known for their aggressive interactions (Clark & Moore 1994), different competitive strategies for obtaining fertilisations may be associated with differences in morphology, physiology and contest behaviours. Where male competitors engage in size-matched contests and thus size differences cannot be accurately used as indices of RHP, an examination of the underlying physiological and metabolic components of RHP is warranted to understand the evolution of such systems.

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552

553 **Table 1.**

554 Variable descriptions and their component loading of aggressive and submissive

555 behaviours in two species of giant hissing cockroach, as generated by principal

556 components analyses.

Variable description	Behaviour category	*G. oblongonota* Component loading	*A insignis* Component loading
Proportion of interaction spent in low posture	Submissive	-0.284	-0.377
Proportion of interaction spent retreating	Submissive	-0.236	-0.464
Proportion of interaction spent approaching	Aggressive	0.428	-0.115
Number of antennations performed	Aggressive	0.557	0.602
Number of butts performed	Aggressive	0.608	0.517

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Figure Legends:

Figure 1. The CT 3D rendering of **(a)** male *Gromphadorhina oblongonota* (left) and male *Aeluropoda insignis* (right) (entire body volumes), **(b)** male *G. oblongonota* full internal respiratory volume illustrating the location of the sclerotised 4th spiracles used in hiss production (orange), **(c)** the sclerotised 4th spiracle structure of a male *G. oblongonota*, and **(d)** the sclerotised 4th spiracle structure of a male *A. insignis*.
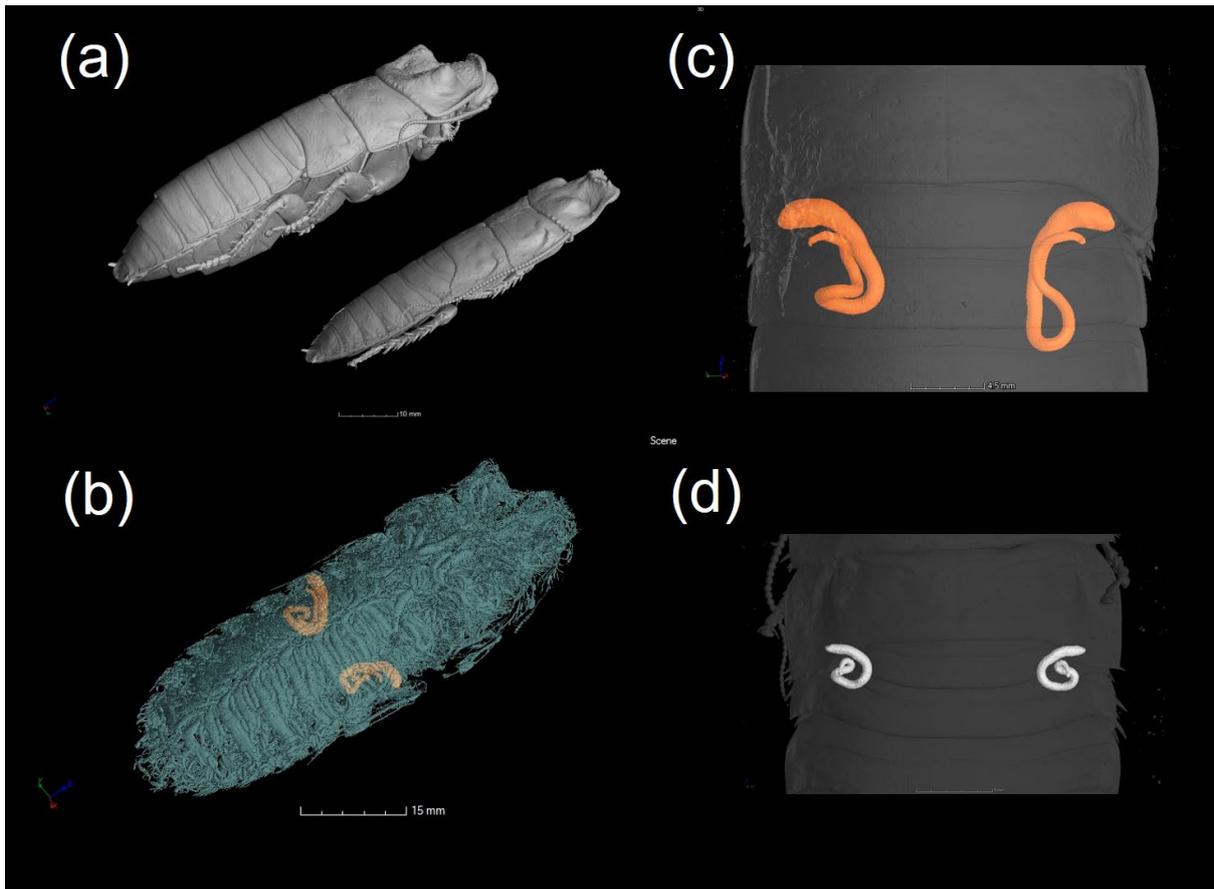
Figure 2. The residual respiratory volumes (mm^3) in males of two species of giant hissing cockroaches *Aeluropoda insignis* and *Gromphadorhina oblongonota* according to contest outcome.

Figure 3. Mean respiratory volume per body volume for males of two species of giant hissing cockroaches *Aeluropoda insignis* and *Gromphadorhina oblongonota*.
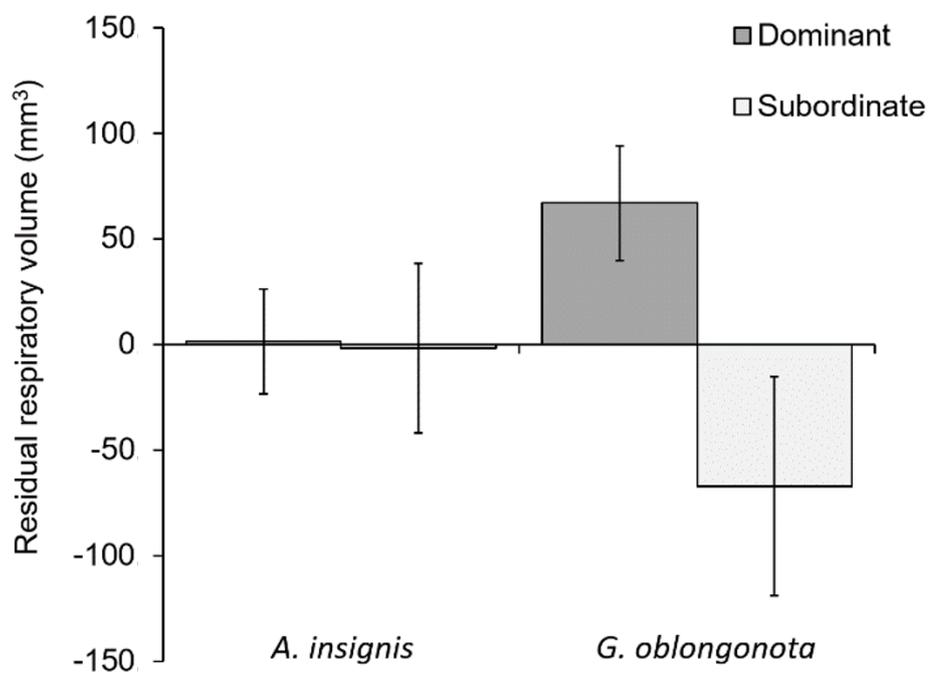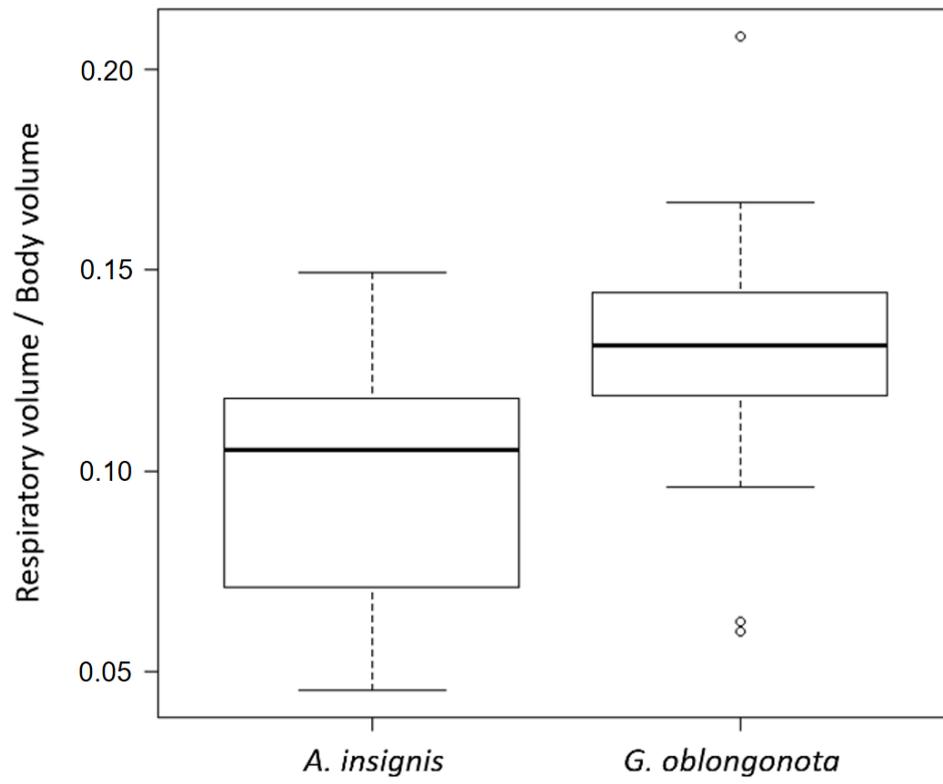
573 **Figure 1.**

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576   **Figure 2.**

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579 **Figure 3.**

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