Differential effects of aerobic capacities on fight outcome in the Giant hissing cockroaches

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Running title: Contest behaviour in giant hissing cockroaches.

**Lay Summary:** When animals fight over resources, why do some species invest more in contest behaviours than others? Here we investigated an apparent disparity in contest traits (weaponry, physiology and fighting tactics) in the Giant hissing cockroaches. It appears that some species have evolved endurance-enhancing 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.

Key words: aggression, cockroach, *Gromphadorhina oblongonota*, repeated signal, resource holding potential, stamina, X-ray computed tomographyIntroduction

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 1996), in beetles (Ohde et al*.* 2018) and cockroaches (Clark & Moore 1994; Bell et al. 2007), among other taxa (reviewed in Emlen 2008). Weaponry exists not purely to injure opponents (although injuries do occur: e.g. Wilkinson & Shank 1976), but rather to engage in a stereotyped ‘trial of strength’ or wrestling match where antlers may be interlocked while the contestants push at one another, or where horns are used by invertebrates in order to push or flip an opponent over (Goyens et al. 2015; Durrant et al. 2016).

Trials of strength or endurance, while not necessarily resulting in death, are not without costs, which are generally measured in time (extrinsic costs) and energy (intrinsic costs) (Payne & Pagel 1996; 1997). The energetic costs of trials of strength are incurred due to the performance of additional, often strenuous, dynamic behaviours (e.g. pushing and wrestling). Thus, even non-injurious fights may be metabolically demanding for contestants and their level of activity may be constrained by their physical fitness. For example, the metabolic by-product lactic acid has been shown to rapidly increase due to the vigorous contest behaviours performed during hermit crab shell fights, limiting their performance (Briffa & Elwood 2005). Further, the aerobic capacity of hermit crabs limits their ability to persist in contests and thus governs their chances of winning (Mowles et al. 2009). What remains to be seen is which criteria are most important in determining contest outcomes when animals are equally size matched. For example, size-matching may result in the reduced ability for contestants to assess differences in RHP and thus contests may be resolved by underlying physiological differences, with losers giving up once they have reached a threshold of costs.

We investigated the relative importance of trials of strength and energetic capacity in establishing dominance using two species of giant Madagascan hissing cockroach (tribe: Gromphadorhini). These insects are useful for understanding sexual selection as they are sexually dimorphic, with males being larger than females, and bear sexually-selected, species-specific weaponry in the form of pronotal horns, with which they engage in trials of strength over territories and access to females. Hissing cockroaches produce a range of acoustic signals, by forcing air out of a specialised 4th pair of spiracles to generate a hissing sound (Nelson 1979; Nelson & Fraser 1980). Hissing is used in various contexts; it is produced in a ‘soft’ form during courtship, and with greater amplitude during aggression and predator deterrence (Nelson & Fraser, 1980). In aggressive interactions, males engage in ‘butting’ contests where they attempt to butt and to flip their opponent over (Logue et al. 2011). Contest dynamics are known to vary within this tribe, with the large, wide-horned hissing cockroach, *Gromphadorhina oblongonota*, engaging in vigorous fighting and attempting to monopolise fertilisations via access to females, whereas the smaller, flat-horned hissing cockroach, *Aeluropoda insignis*, is less aggressive, but develops larger testes, suggesting that it monopolises fertilisations via post-copulatory sperm competition (Durrant et al. 2016). However, all Gromphadorhini species possess weaponry to some extent.

By staging encounters between size-matched dyads of male cockroaches, we investigated the relative importance of known correlates of RHP. We measured performance capacities (strength) and aerobic capacities (lactic acid generation and respiratory system anatomy) to understand how underlying physical fitness may determine the capacity to win contests in these two species. We predicted that in the absence of size disparities, fights would likely escalate to trials of strength, and that the winners of fights would have greater performance and aerobic capacities than losers, indicating a higher physical fitness of the winner.

# Methods

## Study organisms

Cockroaches were sourced from breeding colonies at the University of Nottingham, UK. Nymphs obtained from the colonies were sexed once they began to exhibit sexually dimorphic subgenital plates, whereupon males were individually isolated in tanks to mature in a temperature-controlled laboratory maintained at 28± 2 °C on a 12:12 hour reversed light/dark cycle. Each male was maintained in a plastic container (185 mm L x 125 mm W x 75 mm D) containing beech chip substrate and half a paper cup for shelter. Each male received two pieces of dry dog food once a week and was provided with a piece of water-soaked cotton wool for moisture. Isolation ensured that males matured without any prior fighting or mating experience.

## Behavioural trials

Morphometric measurements were taken before any behavioural trials. Each male was photographed alongside a scale bar to accurately measure body length using ImageJ image analysis software (<https://imagej.nih.gov/ij/>). This allowed contest dyads to be size-matched to within 0.3 % for *Gromphadorhina oblongonota* and 0.1% for *Aeluropoda insignis* (*G. oblongonota* = 46 males, resulting in 23 contests; *A. insignis* = 38 males, resulting in 19 contests). Only animals without visible damage were used in the behavioural trials. We marked each individual in a dyad with either an arrow or a ‘T’ shape on its pronotal shield using white correctional fluid to identify individuals during subsequent video analysis.

Contest trials were carried out between 09:00 – 15:00hrs under red lighting to promote natural behaviours in these nocturnal animals (Koehler et al. 1987). Interactions between size-matched dyads were staged in clear acrylic chambers (17 x 10 x 14.5cm high) with a white cardboard substrate to provide grip. Each interaction was video recorded from above in high definition (HDRXR 160 EB video camera, Sony Corporation, Japan).

Cockroaches were gently removed from their housing containers and simultaneously placed into the contest arena such that their antennae touched on release. This procedure promoted the initiation of contest behaviour over territory. Whilst many contests featured vigorous fighting behaviour (butting and flipping the opponent onto its back), many encounters involved ‘low aggression’ contest behaviours including repeated antennations and approaches towards the opponent, which may retreat or adopt a low posture (presumably to guard against being overturned). These low aggression behaviours were often performed by both opponents to similar extents during the encounter, necessitating a careful analysis of contests to determine which individual displayed the more frequent dominant behaviours. Interactions were thus recorded and terminated after 20 minutes had elapsed (*G. oblongonota* = 11 contests, *A. insignis* = 16), or once a clearly dominant individual emerged, with one cockroach (the subordinate) attempting to escape the other, necessitating the separation of the contestants for ethical reasons (*G. oblongonota* = 12 contests, *A. insignis* = 3).

The recordings were reviewed at half-speed and scored using JWatcher version 1.0 event recording software (Blumstein & Daniel 2006) to record the occurrence of individual behaviours. Actions reflecting aggressive/dominant or submissive behaviours were scored for each animal. Variables scored were time approaching rival (aggressive), time retreating from rival (submissive), time spent in low body posture (submissive: protecting the legs beneath the body and avoiding being flipped), antennations (aggressive: antennal whips directed at the opponent) and butts (aggressive: hitting the opponent with the pronotal horns).

Principal components analysis was conducted in R version 3.4.1 to determine whether the behaviours assumed to be dominant or subordinate formed a factor for use in further analyses. As the amount of time approaching, retreating, or spent in low posture will be dependent on interaction time, these were calculated as a proportion of total interaction duration. Therefore, the factors in the models were: proportion of time retreating, proportion of time approaching, proportion of time in low posture, number of antennations performed and number of butts performed. For *G. oblongonota*, PC1 had an Eigenvalue of 2.2 and explained 44% of the observed variance (Table 1). The factor loadings identified a spectrum of submissive (low values) to dominant (high values) behaviours. In *A. insignis*, PC1 and PC2 had Eigenvalues of 1.3 and 1.2 respectively, explaining 26% and 25% of the variance in the dataset. However, the component loadings of PC2 were the most similar to those obtained for *G. oblongonota,* and biologically logical in terms of grouped behaviours. Thus PC2 was retained, with low values indicating a subordinate individual, and high values a dominant individual (Table 1).

Factor scores were extracted from the PCA to identify where each individual resided on these behavioural axes and thus allowed the identification of dominant and subordinate individuals where this had not been immediately apparent. Those with the higher factor score in a dyad were identified as dominant and those with the lower factor score were identified as subordinate.

## Physiological measures

Immediately after each encounter, haemolymph samples (*x̄* ± se = 0.1 ± 0.006 g) were taken from each individual by piercing the arthrodial membrane at the base of the second leg using a 0.6 x 30mm hypodermic needle (Becton Dickson, USA). We alternated first drawing haemolymph from dominant or subordinate individuals (when outcomes were obvious) to avoid order effects. Haemolymph was collected directly into a pre-weighed 0.5 mL Eppendorf tube. Hissing cockroach haemolymph coagulates rapidly and the wounds to the arthrodial membranes of each cockroach sealed immediately following the procedure. All cockroaches were returned to their individual housing containers after this procedure. They were given access to water and food, with a rest period of at least seven days to recover prior to engaging in the performance capacity trials.

The highly viscous nature of cockroach haemolymph necessitated a novel approach to assaying haemolymph lactate. All haemolymph samples were frozen at -80 °C for a minimum of 12 hrs before being freeze-dried for 12 hrs in a vacuum freeze dryer (Birchover Instruments Ltd, UK). The resulting pellet was ground to a fine powder by hand using a dissecting seeker inserted into the Eppendorf tube. Each sample was then weighed to an accuracy of 0.0001 g and was analysed for *L*-lactate using the perchloric acid extraction method following Neat et al. (1998). The powdered haemolymph was suspended in 40-120 μl of chilled 0.3 M perchloric acid in a ratio of 1 mg powder to 3.3 μl acid. Suspensions were thoroughly mixed using a vortex mixer and centrifuged for 10 minutes at 11,000 rpm. The resulting supernatant was decanted into a clean 0.25 mL Eppendorf tube and stored at 4 °C. This process was repeated, adding an identical volume of acid as before to the resulting pellet, which was vortexed and centrifuged as before. The supernatant was decanted and added to the previously-extracted volume for each sample. Samples were then neutralised by adding 10-25 μl 2M potassium bicarbonate, vortexed and centrifuged as before. This final supernatant was decanted and analysed for *L*-lactate following a spectrophotometric method using a standard laboratory kit (Trinity Biotech PLC, Bray, Co Wicklow, Ireland). *L*-lactate concentration was calculated as mg of lactate per g dry haemolymph powder.

## Performance capacity trials

Cockroach strength was measured using a tensile testing machine (5969, Instron, USA) with a 50 N load cell. Each cockroach was tethered using a 60 cm length of 0.25 mm nylon fishing line attached to the dorsal surface of the second thoracic segment using cyanoacrylate glue and a small piece of tape (see van Casteren & Codd 2010). The tether was looped around a pulley and attached to the machine, which slowly pulled the cockroach backwards at a rate of 9 mm per second. The cockroach was placed on a textured rubber mat to provide grip and was provided with half of a plastic cup for shelter, to promote forward motion towards the shelter and away from the pulley. Strength trials were terminated once the animal was drawn as far back as the pulley or let go of the mat. The maximum force (N) with which the cockroach was able to resist the backwards pull from the machine was recorded. The trial was repeated three times with approximately 24 hrs between trials and the average tension of the three trials was recorded. The trial order of animals was randomised for each repetition and cockroaches were kept in their own enclosures with *ad libitum* access to food and water throughout the trial period. Due to the amount of manipulation involved in the strength trials, it was necessary to conduct these last so that the cockroaches would behave as naturally as possible during their staged encounters.

## X-ray computed tomography

To gain measurements of the respiratory systems of the individuals, they were first killed by freezing at -80 °C. Cockroaches were scanned using X-ray computed tomography (Phoenix v|tome|x m 240kV, Waygate Technologies, Wunstorf, Germany) to produce internal and external morphometrics. Cockroaches were wrapped in a thin sheet of X-ray translucent polystyrene foam and placed in a hollow plastic tube of sufficient width to prevent specimen deformation. The tube was mounted in the scanner. X-ray projection images were acquired in 250 ms intervals (2638 in total over a 360° rotation) using an energy and current of 75 kV and 200 µA. Scans were conducted in three sections, so as to image the entire body length at a maximal spatial resolution of 30 microns per voxel. The three sections; head to thorax, thorax to abdomen, and abdomen were automatically stitched together using Phoenix Datos|x v2 software (Waygate Technologies, Wunstorf, Germany). Using complete body scans, the hissing structure of the 4th spiracle and full tracheal network of the respiratory system was identified and digitally segmented using VGStudio MAX 2.2 (Volume Graphics GmbH, Germany). The tracheal network was clearly identifiable from the surrounding tissues as the air delivery system is hollow and unlike denser tissue. Measurements of total respiratory volume and total body volume (mm3) were recorded from the scan data.

## Statistical methods

As the total respiratory volume of the cockroaches was found to be positively correlated with the total body volume in both species (Spearman’s rank correlations: *G. oblongonota*: rs = 0.935, *N* = 46, *P* < 0.0001; *A. insignis*: rs = 0.857, *N* = 32, *P* < 0.0001), the residual respiratory volumes from these relationships were calculated in order to investigate the extent to which the respiratory volumes of dominant and subordinate individuals deviated from the allometric body volume-respiratory volume relationship. Residuals were calculated from a simple regression for each species with respiratory volume as the dependent variable and whole body volume as the independent variable.

As the overall body volumes of the cockroaches were non-normally distributed, these data were log-transformed, while the *L*-lactate data was multiplied by 10,000 then transformed by calculating the square-root. Each *L*-lactate value was multiplied by 10,000 in order to ensure that the predictor variables in our models (*L*-lactate concentration and residual respiratory volume) were on the same scale. To investigate the effects of these respiratory variables on fight outcome, we used binomial generalized mixed models with a logit link function and backwards stepwise model reduction with ANOVA. ‘Contest identity’ was included as a random effect to control for the non-independence of results from winners and losers interacting in the same contest dyad while allowing us to retain the information for each individual. Species were analysed separately. The factor in each model was contest outcome (dominant or subordinate), and the variables were residual respiratory volume and post-contest *L*-lactate concentration. Where transformation failed to normalise data, non-parametric tests were used. All statistical analyses were conducted using R version 3.4.1.

## Ethical note

Engaging in contests is a noninjurious behaviour for hissing cockroaches. The performance capacity trials were also noninjurious. In order to undergo X-ray computed tomography, the subject must remain absolutely still and thus it was necessary to kill the cockroaches prior to scanning. Cockroaches were humanely killed by freezing at -80 °C. To minimize the number of individuals used we kept sample sizes to the minimum suitable for the effective analysis of physiology, performance capacities and of fight outcomes.

# 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* (χ243 = -0.06, *P* = 0.952), or *Aeluropoda insignis* (χ229 = 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: r30 = 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: V21 = 145, *P* = 0.897) or *A. insignis* (Paired t-test: t17 = -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, once with *L*-lactate retained, and once with it removed, and interpreted results from both. In both cases, the main effect of respiratory volume remained.

From our CT-scans we were able to visualise the whole body, respiratory systems and hiss-producing structures of both species (Figure 1). The specialised hissing spiracle of the hissing cockroaches is identifiable as a scleratised void internal to the 4th spiracle, with a sphincter-like pinch-point connecting it to the rest of the respiratory system (Figure 1). In *G. oblongonota*, there was a significant difference in residual respiratory volumes between animals of differing dominance status. Dominant individuals were found to have positive residual respiratory volumes, whereas subordinate individuals were found to have negative residual respiratory volumes (χ 241 = -3.698, *P* < 0.001; Figure 2). In *A. insignis*, dominant and subordinate individuals did not differ in residual respiratory capacity (χ 227 = 1.203, *P* = 0.229; Figure 2).

Post-contest levels of *L*-lactate between dominant and subordinate individuals did not differ in either species (*G. oblongonota*: χ 241 = -1.024, *P* =0.306*; A. insignis*: χ 227 = 0.760, *P* = 0.447). However, there was a significant interaction between residual respiratory volume and *L*-lactate concentration on contest outcome in *G. oblongonota* (χ 241 = 2.907, *P* = 0.004), driven by different relationships between residual respiratory volume and post-contest *L*-lactate concentration between dominant and subordinate individuals. In dominant individuals, there is no significant relationship between residual respiratory volume and post-contest *L*-lactate, although the correlation coefficient is negative (Pearson’s product-moment correlation: r21 = -0.128, *P* = 0.56). In subordinate individuals, there is also no significant relationship between residual respiratory volume and post-contest *L*-lactate, although the correlation coefficient is positive (Pearson’s product-moment correlation: r21 = 0.319, *P* = 0.138). There was no significant interaction between residual respiratory volume and *L*-lactate concentration on contest outcome in *A. insignis* (χ227 = -1.384, *P* = 0.166).

There was no difference in post-contest *L*-lactate concentrations between the species (Welch’s two sample t-test: t73.3 = 1.346, *P* = 0.183). *G. oblongonota* had overall larger body sizes than *A. insignis* (Welch’s two sample t-test: t66.0 = -8.406, *P* < 0.0001) and larger raw respiratory volumes (Welch’s two sample t-test: t58.0 = -8.145, *P* < 0.0001). They also had larger relative respiratory volumes (respiratory volume/body volume) than *A. insignis* (Kruskal-Wallis: χ21= 22.602, *P* < 0.001; Figure 3).

# Discussion

In animal contests, it is usually the larger of the opponents that wins, and physical fighting is usually avoided if clear size differences exist between opponents. We have demonstrated that when opponents are size-matched, typical indices of RHP (here size and strength) are no longer able to predict contest outcomes. In the absence of such asymmetries, the underlying respiratory capacity of the competitors differed between dominant and subordinate individuals, indicating its potential importance in determining contest outcomes. We used two species of Madagascan hissing cockroach to test the assumptions of differential investment in aggressive behaviour. We have previously demonstrated that *Gromphadorhina oblongonota* is a much more aggressive species than *Aeluropoda insignis*, with more elaborate weaponry for use in aggressive encounters (Durrant et al. 2016). It appears that the larger, more heavily weaponised male *G. oblongonota* competes for females via competitive exclusion of rivals, whilst the smaller, less-aggressive male *A. insignis* rely more on post-copulatory sperm competition to secure fertilisations, demonstrated by their absolutely larger investment in testis size than their sister genus *Gromphadorhina* (Durrant et al. 2016). What remained to be seen was how aggressive encounters within each species were resolved, especially when opponents were size-matched.

We found that the larger, more aggressive *G. oblongonota* had a greater relative investment in its respiratory system than the less physically aggressive *A. insignis*. Thus, the greater relative respiratory volumes of *G. oblongonota* may be required to fuel its more vigorous, and potentially energetically demanding, contest behaviour. Indeed, it was found that *G. oblongonota* individuals that had larger than predicted respiratory capacities for their size were more likely to be dominant. Similar results have been found in hermit crabs *Pagurus bernhardus*, where winners of contests were found to have larger than expected concentrations of the respiratory pigment haemocyanin than expected for their size (Mowles et al. 2009). In both of these contest interactions, it appears that the contestants engage in energetically costly behaviour that places demands on aerobic metabolism, and can force them into anaerobic respiration as evidenced by the accumulation of lactic acid (Briffa & Elwood 2005).

We have demonstrated that when size and strength differences are removed by size-matching opponents, a potential effect of respiratory capacities on the outcome of contests is revealed. Here, the ability to effectively deliver oxygen to the tissues may enhance fighting ability by allowing longer lasting aerobic respiration. Adaptations for prolonging aerobic respiration, such as larger tracheal systems, probably evolved as a response to being a large invertebrate and to then maximise oxygen exchange when burrowing through leaf litter. This is likely why we saw no differences in *L*-lactate concentrations between competitors. Having the aerobic capacity for their size and ecological niche could act as a substrate for sexual selection and thus allow its further utility in male-male contests.

When investigating adaptations to contest behaviour, it is important to consider not just the physical weaponry used by species, or the combative behaviours that they may employ, but also the underlying physiology that may permit vigorous contest behaviour to be performed. The relatively larger amount of body volume devoted to the respiratory system in *G. oblogonota* than *A. insignis* suggests that *G. oblogonota* has evolved not just larger weaponry for fighting, but also a system to more effectively deliver oxygen to the tissues. Whilst this system may have evolved in response to the problem of efficiently delivering oxygen to the tissues in an increasingly larger-sized insect; such an adaptation may also support its ability to engage in more vigorous contests. This could explain why dominant males in our interactions had relatively larger respiratory systems than predicted for their size.

In the cockroach Order, there are many examples of sexually selected morphologies and associated behaviours adapted to maximise fertilisation success. In the Cuban burrowing cockroach, *Byrsotria fumigata*, the females are wingless and larger than 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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# Table 1.

Variable descriptions and their component loading of aggressive and submissive behaviours in two species of giant hissing cockroach, as generated by principal 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 |

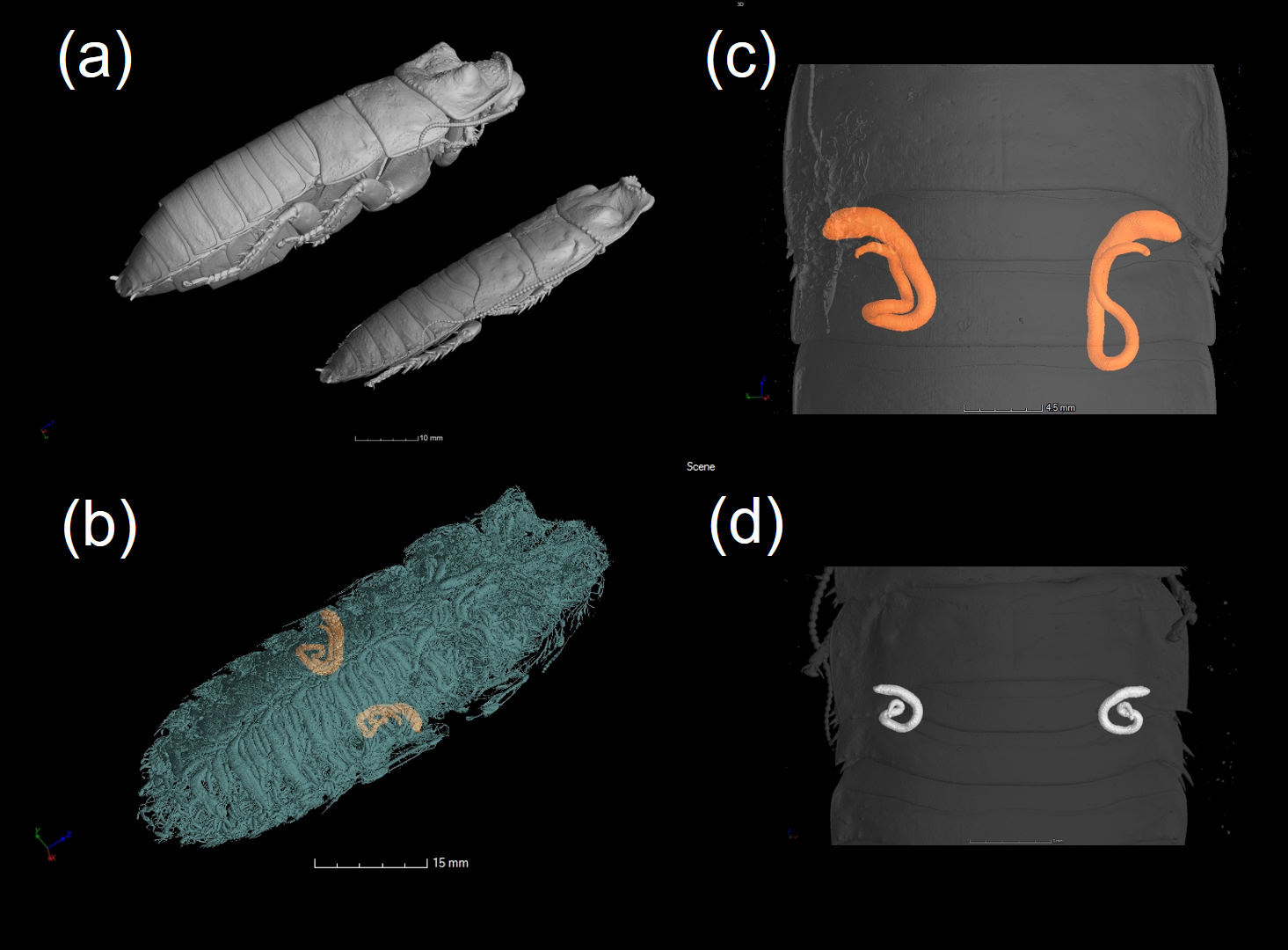
# Figure Legends:

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*.

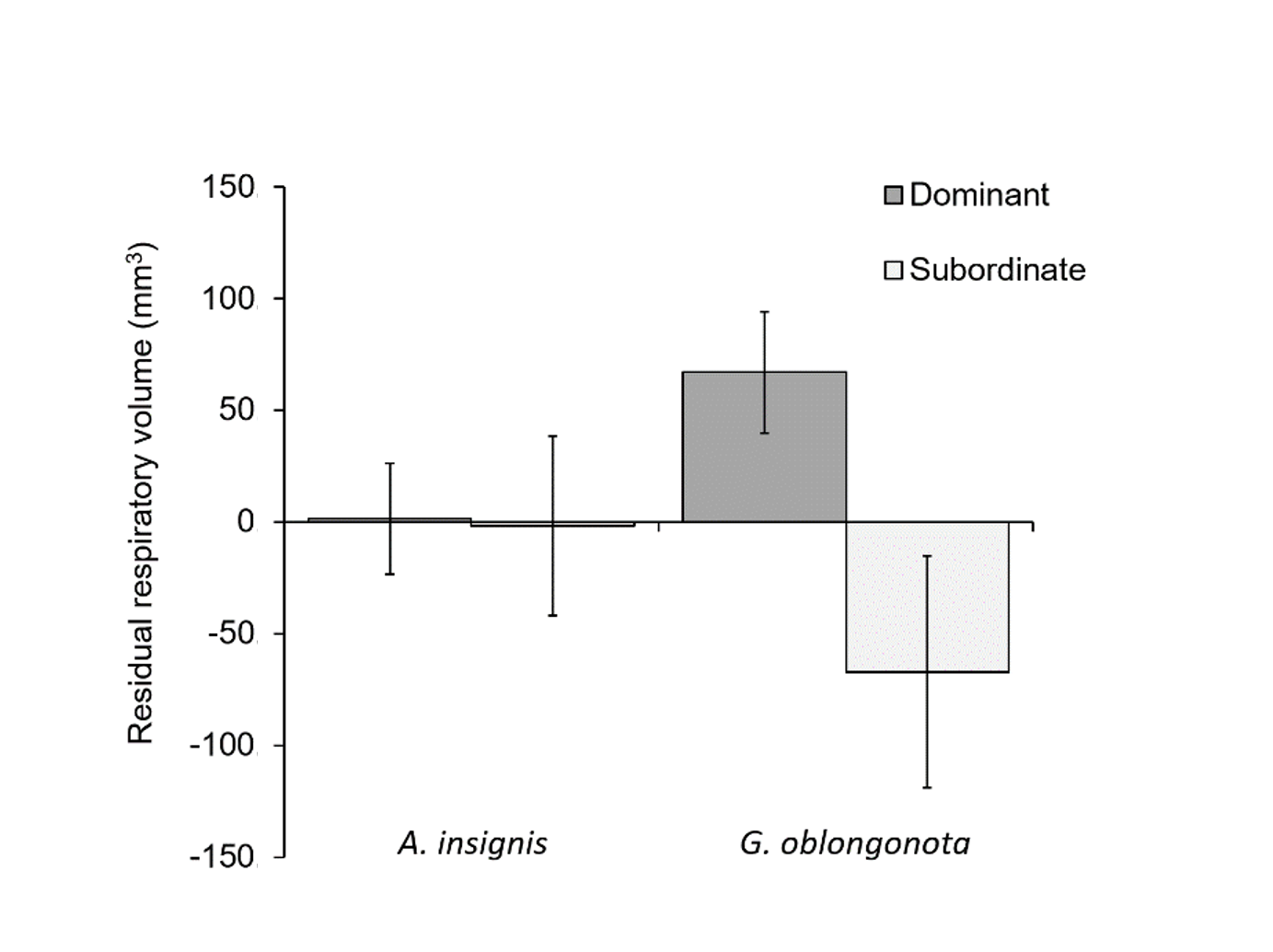
Figure 2. The residual respiratory volumes (mm3) in males of two species of giant hissing cockroaches *Aeluropoda insignis* and *Gromphadorhina oblongonota* according to contest outcome.

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

# Figure 1.



# Figure 2.



# Figure 3.

