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Comparative Analysis of Sexual Selective Traits in Giant Hissing Cockroaches (Tribe: Gromphadorhini)

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Abstract

Through male competition and female choice, sexual selection favours traits that are advantageous for mating success. Trait investment is costly; therefore, males invest a set amount of resources into pre-copulatory traits to aid in competition or post-copulatory traits to increase fertilisation success. The aim of this project was to investigate hissing cockroach sexual selection, looking at both female choice and post-copulatory sperm competition. Firstly, by manipulating horn length of models in two-way choice experiments to understand what role weaponry might play in competition and female choice, in both *Gromphadorhina oblongonota* and *Aeluropoda insignis* species. Secondly, by comparing post-copulatory traits and horn size across five hissing cockroach species to understand investment allocation and sperm competition pressures. Both *G. oblongonota* and *A. insignis* were able to distinguish between control and life-like models. However, when using life-like models with manipulated weaponry, females and males displayed no preference towards horn length. Horns are likely one part of a complex multi-modal signal in male-male interactions and females may not choose males based on this pre-copulatory trait. Comparing post-copulatory traits between the five species, *A. insignis*, *G. oblongonota* and *Elliptorhina javanica* differed significantly in horn length, relative testes mass, spermatophore weight, sperm number, acrosome and head length. However, these species are phylogenetically distant from one another, which could play a large role in driving their trait differences. Comparing sperm morphology and other post copulatory traits, similar patterns in trait investment were found and relationships often became non-existent when correcting for phylogenetic relatedness, suggesting these species may have similar levels of post-copulatory sperm competition. Due to sexual selection pressures being species specific, intraspecific patterns might be more revealing about response to local selection pressure rather than generic pressure amongst species as different species may respond to this shared pressure in different ways. These results imply a complex influence of phylogenetic dependence and sexual selection pressures on post copulatory traits, similar to other studies that correct for phylogenetic relatedness.

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Introduction

First described by Darwin (1859), sexual selection is a form of natural selection that can be driven by competition for mates (intrasexual selection) or mate choice (intersexual selection), favouring the evolution of traits advantageous for mating success (Kodric-Brown & Brown, 1987; Murphy, 1998). Mating success relies on single or multiple traits that provide an advantage to an individual, either assisting in attractiveness or winning competitions that lead to successful mating (Moore, 1990). The competition between males to mate with females causes a high reproductive variance among males, which leads to stronger sexual selection in males and sexually dimorphic traits (Bateman, 1948; Kodric-Brown & Brown, 1987).

Pre-copulatory sexual selection

Males can increase their chances of success by morphological, physiological and behavioural adaptations, and often develop strategies to outcompete rival males or make themselves seem more attractive to gain access to mates. The most common form of intrasexual selection is male-male competition. Male-male competition usually results in males acquiring traits that aid them whilst fighting a competitor, such as body size, horns and antlers (Berglund *et al.* 1996; Lailvaux *et al.* 2005; Kelly, 2006; Vanpé *et al.* 2008; Emlen, 2008; Miller, 2013). With the aid of these traits individuals can exclude their rivals and in doing so gain a greater chance of acquiring mates and fathering offspring, increasing their reproductive success.

Multiple mating strategies have evolved to gain access to females and increase an individual's chance of mating success (Gross, 1996; Wong & Candolin, 2005; McCulloch & Simmons, 2016). Monopolisation of an individual or groups is one way a male can increase his chances of reproductive success. An example of this is seen in harems, where a group of females are defended by a single male who monopolises the group and competes with other males for control (Modig 1996; Kelly, 2006). *Megalorchestia californica* amphipod males have enlarged gnathopods and red antennae to signal quality (Iyengar & Starks, 2008). Males use antennae and gnathopods in male-male competition, and to hold and guard female harems. Larger males with longer and brighter antenna outcompete rivals for bigger burrows and obtain larger clusters of females. In extreme cases, males can adapt to develop different mate strategies within the same species. This is often seen when monopolisation of one or multiple females by a dominant male reduces a lower quality male's chance of mating. Under these pressures, low quality males have evolved varied mating strategies which allow them to successfully copulate with females via alternate strategies. In *Onthophagus taurus* beetles, for example, horned male morphs physically compete with other males to control female access, while male morphs with no horns sneak copulations opportunistically and avoid competition (Moczek & Emlen, 2000).

Visual signals, such as ornaments and armaments, are traits that are selected for based on their attractiveness or effectiveness in beating males in competition. Ornaments are traits that are exaggerated in intricacy, size or colour and are used as attractive signals. Females have a selective preference for these

exaggerated traits. This causes the trait to be under sexual selection as females want to mate with more attractive males. For example, Brazilian whiptail lizards *Cnemidophorus ocellifer* females preferred males with the brightest UV reflectance compared with duller males, selecting for exaggerated reflectance and brightness (Lisboa *et al.* 2017). Armaments, on the other hand, are traits selected to better an opponent under male-male competition, aiding the organism in physical male-male combat. In *Sagra femorata* leaf beetles, for example, males have weaponised hind legs which they use in physical male-male competition to grasp, kick off and attack rival males that enter their territory (Katsuki *et al.* 2014). Males with larger hind legs win more fights than those with smaller hind legs. Therefore, the trait helps aid in fighting success to help guard their territory and mates. Armaments can also be used as a signal to show competitors their strength and fitness, allowing the competitors to assess their opponent's armaments and decide whether it is beneficial to engage in combat (Elias *et al.* 2008; Schnell *et al.* 2015). Females can generate selection on both armaments and ornaments. In the white-tailed deer *Odocoileus virginianus*, males produce antlers that they use in physical male-male competition; antler size is also used in female choice, as females choose males with larger antlers over males with smaller antlers (Morina *et al.* 2018).

Body size can also be a visual signal to both male competitors and choosy females. In most cases, organisms with greater body size have a competitive and attractive advantage, so that body size acts as a signal for fitness and strength. Many sexually selected traits tend to be positively correlated with body size

(Tokarz, 1985; Bonduriansky, 2007; Huyghe *et al.* 2012; Voituron *et al.* 2012; McCullough *et al.* 2015). However, body size can have no correlation at all with sexually selected traits and larger body size is not always more attractive (Knapp & Warner, 1991; Zhu *et al.* 2016).

Auditory signals may also be expressed in order to attract mates or as part of male-male competition (Mountjoy & Lemon, 1996; Iglesias & Hasson, 2017).

These signals are usually produced through the respiratory system and as such are usually a proxy for body size (Pfefferle & Fischer, 2006; Hal *et al.* 2013;

Rodriguez *et al.* 2014; Reber *et al.* 2017). Male grey tree frogs *Hyla versicolor* with longer mating calls are larger and more attractive to females and produce healthier stronger offspring with higher chances of survival (Welch *et al.* 1998).

This call acts as a cue that females can use to assess and choose males. In insects, acoustic signals are different, in the fact sounds are typically made from vibrations or rubbing of certain body parts to create noise (stridulation). For example, male house crickets *Acheta domesticus*, chirp during male-male competition, to attract mates or during courtship, by rubbing their wings together. When attracting mates, chirp complexity provides a signal to the female of male body size, with larger males producing a more complex song (Gray, 1997). Females prefer males with a more complex chirp than males with simple chirps, using this signal to select better quality males.

Signals can be complex, consisting of multicomponent or multimodal signals (Guilford & Dawkins, 1995; Holldobler, 1995; Rowe, 1999). A multimodal signal is where two or more types of signals (chemical, visual, auditory, behaviour or

tactile) are used together as a form of communication and can be categorised as multiple message signals, where each signal carries different information, or back-up signals, where multiple signals carry a single message (Johnstone, 1997; Candolin, 2003; Ronald *et al.* 2017). In courtship, male bush spiders *Schizocosa ocreata* use two modes of signals, visually raising and waving their front legs, and vibration signals. Females then assess males based on both these signals in unison whilst choosing a mate (Kozak & Uetz, 2016). Multicomponent signals are single sensory signals that convey two or more messages (Candolin, 2003). Greenfinches *Carduelis chloris* have visual cues on their tails and wings; wing colour signals the individual's age and tail colour indicates male parasite resistance (Lindstrom & Lundstrom, 2000). Both these cues are through one mode of signal (visual) and the female can use these visual cues to identify two separate pieces of information to assess overall male quality.

Signals (chemical, visual, auditory, motion or tactile) are often used to create maximum impact both in courtship displays and male-male competition. The benefits of using multimodal signals allow an individual to strengthen their display for attractiveness or in competition, allowing others to assess multiple qualities of the individual before making an informed decision. Rituals or mating displays often use a combination of signals, particularly acoustic and visual signals, together to create a greater display of attractiveness (Frith & Frith, 1987). For example, male túngara frogs *Engystomops pustulosus* produce acoustic whines as well as visual signals of inflating and deflating their vocal sac in mate attraction (Stange *et al.* 2016). Females use the visual and audio signal

separately when judging male attractiveness. Females prefer those with both complex song and visual cues, which enhances the male attractiveness to the females.

In male-male competition, assessment strategies can play an important part in contests. They can allow an individual to decide whether they are physically matched, assess their opponents in a mutual assessment, or the individual's own abilities in a self-assessment (Kelly, 2006; Schnell *et al.* 2015; Wofford *et al.* 2015). This is beneficial as it can be costly to engage in such contests, particularly if the odds of the opponent winning are small. Having multiple signals can aid in decision making to better assess the chance of winning against an opponent and work out the most beneficial course of action i.e. retreating or engaging. Male jumping spiders *Phidippus clarus* display both visual size and vibratory signals to one another before physically interacting. Body weight signals strength and contest success, while vibrations predict stamina and interaction time (Elias *et al.* 2008). Before physical contact, males make a self-assessment, based on both signals from the opponent, of whether they are likely to win. The complexity of multimodal signals can be a challenge to untangle. Some signals may be under differing forms of selection or under more than one form of selection and disentangling these can be tricky (Wilkins *et al.* 2015).

Post-copulatory sexual selection: Sperm competition

Intrasexual selection also occurs post-copulation in the form of sperm competition and cryptic female choice. Sperm competition is defined as a post-copulatory form of sexual selection that occurs when a female mates with two or

more males, causing multiple male ejaculates to compete for fertilization of the same female's ova (Parker, 1970). The strength of sperm competition is heavily influenced by mating systems and extra pair paternity. The more males a female copulates with the greater the amount of competition those sperm face to successfully achieve fertilisation; thus, sperm competition is driven by polyandrous mating (Tourmente *et.al.* 2013).

Traits are selected to increase the male's likelihood of reproductive success post-copulation, where individuals compete for fertilisation. Testes size is a trait known to be associated with sperm production and is related to sperm competition intensity. In male yellow-pine chipmunks *Tamias amoenus*, reproductive success is correlated to testes size (Schulte-Hostedde & Millar, 2004). Males with larger testes father more offspring than males with smaller testes as larger testes have a greater volume of sperm per ejaculate, suggesting that testes size is positively related to sperm competition outcome. Another common trait under sperm competition pressure is sperm length (Byrne *et al.* 2003; Gomendio & Roldan, 2008; Mossman *et al.* 2009; Helfenstein *et al.* 2010). In primates, sperm length differs between species in different mating systems, with longer sperm in species with polygamous mating systems, where sperm competition is higher, and shorter sperm in species with monogamous mating systems where sperm competition is lower (Gomendio & Roldan, 1991). Longer sperm were also found to swim faster. Therefore, sperm has adapted to be longer and faster to out-compete rival sperm under high levels of sperm competition.

Sperm competition is widespread across the Animal Kingdom, with a huge diversity of adaptations. The diversification of adaptation can be influenced by pressures from cryptic female choice, sperm competition and sperm storage (Kleven *et al.* 2008; Higginson *et al.* 2012; Tourmente *et al.* 2013; Bennison *et al.* 2015). Sperm competition can be categorised into defensive and offensive competition. Defensive adaptations are those that prevent sperm competition from occurring, by preventing another male from mating with the female. This can be done by mate guarding, when the male actively guards the female either physically (Benvenuto *et al.* 2009) or without contact (Alcock, 1982), or by mating plugs, which block the females reproductive tract preventing other males from mating without having to be present (Mangels *et al.* 2016). These strategies prevent sperm competition for a time, which increases the chances for the male's sperm to successfully fertilise the ova. A female will try to reject these strategies, often by expelling mating plugs or opportunistically sneaking off to allow other males to copulate with her, or physically wrestling off the male (Pitnick *et al.* 2001; Arnquist & Rowe, 2002; Nessler *et al.* 2007; Sentenska *et al.* 2015) .

Offensive adaptations include penile morphology, sperm morphology, sperm quality and number. These adaptations can be used to lower the intensity of sperm competition or outcompete rival male sperm. Penile morphology comes in a diverse array of forms (e.g. scoops, needles, barbs) that aid in sperm delivery and removal of rival sperm, cause damage to the female's reproductive tract to prevent further copulations or in extreme cases are used to traumatically

inseminate the female through her abdomen directly (Waage, 1979; Stutt & Siva-Jothy, 2001; Brennan *et al.* 2007; Eady *et al.* 2007). These penile functions reduce sperm competition and increase the male's chance of fathering offspring. In other extreme sperm competition cases, some species have developed multiple sperm types known as sperm heteromorphism, where eusperm that function in fertilisation and parasperm that aid eusperm or disable or block rival sperm are produced (Snook & Karr, 1998; Winik *et al.* 2000; Hayakawa *et al.* 2002; Hayakawa, 2006; Holman & Snook, 2008). Sperm gigantism is also found in extreme sperm competition, where males produce very few very large sperm that fill the female reproductive tract or storage tubules, preventing rival sperm access, to help increase their chance of successful fertilization (Pitnick & Markow, 1994; Vielle *et al.* 2016).

Males can increase fertilisation success by increasing copulation duration or the number of repeated copulations with a female, increasing their chance of fertilization by increasing the number of sperm transferred to the female and displacing rival male ejaculates (Parker & Simmons, 2000). These strategies work under the raffle model, where a male's chance of fertilization success increases with the more sperm he contributes to the competing ejaculate pool (Parker, 1982). The more sperm a male provides the greater the likelihood of one of his sperm fertilising the ova. Male Atlantic salmon *Salmo salar*, invest heavily in sperm number and quality under increased sperm competition (Vladic & Jarvi, 2001). Males with high numbers of better-quality sperm, fertilise a greater number of eggs. Under sperm competition, sperm are more uniform in their

morphology with less abnormality or damage, so each sperm cell they produce has an equal chance of success, increasing the males chance of fertilisation (Immler *et al.* 2008; Aron *et al.* 2016; Godwin *et al.* 2017). This quality control of creating equal sperm is a response to sperm competition. The greater the amount of high-quality sperm, the greater the chance one of these sperm is successful in fertilizing the ova (Parker & Simmons, 2000). Under relaxed sperm competition, sperm vary greatly in morphology with increased numbers of abnormal sperm. This is because selection on the male's sperm traits is reduced, and fertilization of an ovum can easily be achieved. Males do not have to spend resources on creating high-quality sperm when it is not necessary, due to the process being costly (Williams *et al.* 2005; Birkhead & Immler, 2007).

Sperm cells usually consist of a head containing the acrosome and nucleus, a midpiece containing mitochondria, and a flagellum known as the tail. In insects, such as *Drosophila*, sperm do not have a midpiece; instead their mitochondria are found in the tail (Shahaney *et al.* 1973; Laueinyecz *et al.* 2019). Sperm are extremely diverse in their morphology and energetics and differ dramatically across taxa. Variation in sperm morphology can be found both within and between species. The diversification of sperm traits is a response to post-copulatory sexual selection pressures. Sperm competition is thought to strongly influence sperm trait diversity, shaping sperm to maximise their fertilisation success (Kleven *et al.* 2008; Higginson *et al.* 2012; Tourmente *et al.* 2013; Bennison *et al.* 2015). Sperm morphological traits are the most commonly observed adaptation in response to sperm competition. Sperm competition

influences sperm morphology, generally causing tail and total sperm length to increase, and head length to either increase or decrease with increased sperm competition (Gomendio & Roldan, 1991; Anderson & Dixon, 2002; Fitzpatrick *et al.* 2009; Gómez-Montoto *et al.* 2011). These adaptations relate to motility, enhancing speed and endurance to help sperm swim faster and last longer, to outcompete rival sperm to successfully fertilise the ova. The relationship between motility and morphology has been well documented in many taxa, including rodents (Gómez-Montoto *et al.* 2011), cichlid fish (Fitzpatrick *et al.* 2009) and passerine birds (Lüpold *et al.* 2009; Rowe *et al.* 2012), with many studies finding that longer sperm swim faster, and sperm tail and total length is positively correlated with swimming speed (Gomendio & Roldan, 1991; Helfenstain *et al.* 2010). These traits allow the sperm to reach storage tubules or the ova quicker than shorter sperm (Rowe *et al.* 2012; Bennison *et al.* 2016). The effect of midpiece size on competitive ability is more complicated and its association with other traits varies between taxa. In Iberian deer *Cervus elaphus hispanicus* sperm, shorter midpieces have greater speed, whereas in house mice *Mus domesticus* sperm, longer midpieces have increased speed. Yet, in passerines midpiece length and speed have no association (Malo *et al.* 2006; Immler & Birkhead, 2006; Firman & Simmons, 2010; Rowe *et al.* 2012). Midpiece size is thought to be under pressure for speed and longevity in sperm storage tubules. The trade-off between speed to reach the storage tubule or the ova and energy storage for the sperm to last within storage tubules before racing to the ova could explain the diversity of trends found across taxa.

Female Mate Choice

In mate choice, females evaluate single or multiple signals or traits when choosing a male to mate with (Murphy, 1998). Female choice can also occur post-copulation, which allows her to influence the choice of sperm for fertilization; this is known as cryptic female choice (Pizzari & Birkhead, 2000). By being choosy, females can increase their reproductive success. Carefully selecting mates rather than mating at random can have direct and indirect benefits. Direct benefits include access to resources such as food, shelter and territory, paternal care of offspring, protection from infanticide or male harassment or disease/parasite avoidance from choosing healthy mates (Trivers, 1972; Forsgren *et al.* 1996; Oneal *et al.* 2007). Female Katydid *Conocephalus nigropleurum*, when presented with a choice of large and small males, choose large males as they gain a greater direct benefit from them (Gwynne, 1982). Larger males produce larger protein-rich nuptial gifts that increase egg production through female consumption.

Benefits can be indirect through offspring, such as producing sons with the same attractive traits of the father, which increase the sons' mating success known as the 'sexy sons' hypothesis, or choosing a male of high quality to increase offspring quality, survival and fitness (Fisher, 1915; 1958; Wilkinson & Reillon, 1994). In field crickets *Gryllus interger*, females choose males with longer calls (Hedrick, 1988). Offspring inherit their father's attractive call length, increasing their reproductive success. This complies with the sexy sons hypothesis, where females choose males with an attractive trait so that their sons will inherit the

trait and have greater reproductive success (Weatherhead & Robertson, 1979; Pai & Yan, 2002; Prokop *et al.* 2012). In the three-spine stickleback *Gasterosteus aculeatus*, females prefer males with bright red markings compared to duller males (Barber *et al.* 2001). Offspring of males with bright red colouration grow slower but become more resistant to disease and parasites and have a high white blood cell content than offspring of dull males, showing a health link to the sexually selected trait. By being choosy of red colouration, a female gains indirect benefits, improving her offspring's survival and disease resistance. Sons also show this bright red colouration that the females can use as a signal of fitness.

The good genes hypothesis proposes that variation among males provides females with information about the genetic quality that those males possess. From this, the female can choose a mate with good genes that can then be inherited by the offspring to indirectly increase female fitness (Zahavi, 1975; Kodrick-Brown & Brown, 1984; Anderson, 1986). Traits that indicate male quality are known as honest signals. Honest signals are phenotypic traits that differ in the population, are costly to produce and maintain and are strongly condition dependent. Males of good quality are able to displaying these traits. However, the traits are too costly for males of poorer condition and lesser quality. This is known as the handicap principle (Zahavi, 1975). Female yellowthroat warblers *Setophaga dominica* choose males based on traits that signal male health such as black mask and yellow bib size, with most attractive males having greater disease resistance and survival (Whittingham *et al.* 2015). This is a good example of how female choice with indirect benefits can link with an honest trait that shows that

signal. In the blue grosbeak *Passerina caerulea*, males with deep blue plumage are larger, possess bigger territories with good prey abundance and provided greater feeding rates than males with duller plumage, suggesting that male plumage is an honest signal of quality (Keyser & Hill, 2000). The trait needs to be costly in order to be honest to show the true quality of the male. In the scarlet-tufted malachite sunbirds *Nectarinia famosa*, females prefer males with bright long tail feathers; these males successfully find mates and produce offspring earlier (Evans & Hatchwell, 1992). Having long tail feathers reduces flight time and ability to catch prey, therefore, entails a cost and handicap to the trait. However, handicaps are not necessarily honest. With honest signals there will always be cheating; individuals of lower quality may put more resources into their signalling to appear high quality, thus defeating the handicap principle of honest signalling (Backwell *et al.* 2000; Bee *et al.* 2000; Moore *et al.* 2009). The cost of cheating needs to be considered as part of an honest signal, not just the cost of producing the signal directly (Weaver *et al.* 2017).

Trade-offs in sexual selection

Trade-offs are common in sexual selection and often shape the strategies and traits males and females use. Investment in traits is costly, and therefore it benefits an individual to invest in a way that gives them a sexual or competitive advantage. Males usually resolve trade-offs by investing a set amount of energy and resources in pre-copulatory or post-copulatory competition, enhancing their ability to compete with rival males. It is assumed that males cannot pay costs associated with both high-quality pre-copulatory traits to aid in winning male-

male competitions and high-quality post-copulatory traits to increase fertilisation success in sperm competition. If a male invests heavily in a pre-copulatory trait, such as armament size, then he has fewer resources and energy to invest in post-copulatory traits such as sperm number. This concept is known as the sperm competition hypothesis (Parker, 1970; Parker *et al.* 1997,2013).

Whether males invest more heavily in pre or post-copulatory traits depends on the number of males with which an individual competes, the extent to which males can monopolise females, and the environment (Parker *et al.* 2013; Lupold *et al.* 2014; Katsuki & Lewis, 2015). Female *Salvator rufescens* lizards mate with multiple males and can store sperm for large periods of time, and males have large jaw bulges that females find attractive. Males who have larger bulges have lower quality sperm compared to males with smaller bulges (Blengini *et al.* 2016). Due to the large amount of sperm competition these males face, males invest in sperm quality over attractive bulge size to increase their chance of fertilization success. Male bean beetles *Callosobruchus chinensis* have a trade-off between hind wing size used to find and attract females and sperm number (Katsuki & Lewis, 2015). Investment in the two traits is negatively correlated. Therefore, males can only invest maximally in one of the traits. In larger populations, males have higher sperm competition intensity and invest in sperm number to increase their chances of paternity. However, when the population is smaller there are fewer males, and males invest more in hind wing size and travel distance to find and attract a mate than in sperm number. It is not always the case that pre-copulatory and post-copulatory traits demonstrate a trade-off

in investment; they can be positively correlated with one another, whereby individuals invest in both pre and post-copulatory traits to increase total fertilization success or, the pre-copulatory trait acts as a signal of post-copulatory trait quality or reproductive condition (Shutler, 2011; Naretto *et al.* 2014; Supriya *et al.* 2018; Tuni *et al.* 2018).

Trade-offs are also found between different post-copulatory traits, such as sperm size and sperm number. For example, male passerine birds invest more in sperm number than sperm size. The more sperm they have the greater the chance of fertilization success. This is a better choice of investment under the raffle model, where fertilization success increases with the more sperm a male contributes to the competing ejaculate pool (Parker, 1982). However, *Drosophila* males invest more in sperm size than sperm number (Immler *et al.* 2011). Larger sperm have a greater chance of blocking up the female's reproductive tract, preventing other sperm from entering or fertilising. *Nauphoeta cinerea* cockroaches face multiple trade-offs between ejaculate traits (Moore *et al.* 2004). Males have limited resource investment to either produce lots of sperm or produce high-quality sperm. These traits are also negatively correlated with spermatophore size suggesting that producing a large spermatophore is costly and limits investment in sperm number and quality. Spermatophore investment reduces the chance of females re-copulating; therefore, lowers sperm competition, where sperm quality and number are not as important. However, investing in either sperm number or sperm quality is a beneficial investment

when females mate with multiple males. Trait investment must increase the males chance of outcompeting rival male sperm for the choice to be beneficial.

Use of models in behaviour

Model stimuli are used to understand behaviour by looking at an organism's reaction. There are many types of model stimuli used in behavioural studies today from live animals, to handmade physical and computer-animated models (Tinbergen, 1951; Dziewaczynski & Leopard, 2010; Iglesias & Hasson, 2017; Ronald *et al.* 2018). Handmade model stimuli are diverse and can be used to examine the role of single and multimodal/multicomponent signals in competition and mate choice. Tinbergen (1951;1952) was one of the first to use models to understand animal behaviour, to investigate red colouration and male aggression in three-spine sticklebacks. Models ranging from realistic to abstract shapes, with or without red displays, were presented to males. Males in breeding condition attacked any shaped model with red colouration, demonstrating colour alone provokes an aggressive reaction in sticklebacks.

Handmade model stimuli, often known as dummies or decoys, are commonly used in behavioural research. Dummies are handcrafted from materials to represent an animal and are made from scratch, casting dead animals or taxidermized specimens (Dziewaczynski & Leopard, 2010; Locatello *et al.* 2012). This method allows researchers to look at specific colour-based questions such as mate choice, as the dummies can be painted with variations of colour or patterns based on the questions being asked. For example, blenny *Salaria pavoale* male dummies were used in a two-way choice test to look at female

mate preference of size, yellow spot coverage and yellow patch intensity (Locatello *et al.* 2015). This shows dummies can work well for the use of visual stimuli to look at sexually selected traits.

Tactile auditory and olfactory stimuli can also be used in conjunction with visual stimuli (Klein *et al.* 2012; Callander *et al.* 2013; Stewart *et al.* 2017). This approach makes the stimuli more realistic and relatable to live stimuli and allows a wide variety of different questions to be addressed. Acoustics are often used with models to look at both acoustic and visual signals. One study used visual stimuli with audio recording to look at different combination of song complexity and visual display complexity in cowbirds *Molothrus ater* (Ronald *et al.* 2018). This allowed the researchers to look at both visual and auditory stimuli together to better understand mate preference using multimodal signals.

Recently, studies employing models have exploited modern techniques such as robotics, 3D printing and laser sintering (Klein *et al.* 2012; Callander *et al.* 2013; Igic *et al.* 2015; Stewart *et al.* 2017). Models can be created from CT or 3D scans of live or dead animals, and then 3D objects can be constructed using a software or files from a database. Laser sintered 3D models can provide more precise replicas of natural occurring stimuli than traditional physical models (Igic *et al.* 2015; Stewart *et al.* 2017). These models can be manipulated in different ways (e.g. body size and the size of features can be altered, or features can be removed or added entirely) to address different questions (Bartolini *et al.* 2016). Studies looking at sexual selection have used 3D model stimuli in both male and female mate choice experiments. 3D printed female turtles of large and small

sizes were used to look at male mating preference in the wild, revealing that males prefer larger females (Bulté *et al.* 2018). This shows 3D models work in the wild as well as controlled environments. 3D models of male guppies were printed and painted with different colourations in order to look at female choice with both colour and pattern (Heinen-Kay *et al.* 2014). Measuring the amount of time a female spends when given choice between different males, allows an assessment of female preference while controlling for size of the male models to look at selected traits. 3D models can also be applied in a teaching environment. Male guppy models of differing size were created to test female preference, and students then manipulated other traits such as coloration to create different projects, building a large database of traits and mate choice (Stewart *et al.* 2017).

Colour models have been used to act as visual stimuli to answer a range of behaviour questions about sexually selected traits in many species from fish to reptiles (Dziewaczynski & Leopard, 2010; Heinen-Kay *et al.* 2014). However, insects are one group where models are not often used in behavioural research, particularly sexual selection (Zagatti & Castel, 1987; Ruther *et al.* 2002). The studies that use models tend to look at defence and predatory behaviour by providing model prey, such as plasticine caterpillars and model moths (Rebora *et al.* 2004; Cuthill *et al.* 2005; Fan *et al.* 2009). An example of this is model caterpillars coated in *Mamestra brassicae* moth larvae haemolymph, acting as visual and scented stimuli among dead and live individuals to look at predation

preference and recognition by the *Pterostichus melanarius* beetle (Ferrante *et al.* 2017).

Madagascan hissing cockroaches

Hissing cockroaches are a small nocturnal tribe (Gromphadorhini) indigenous to Madagascar and Europa Island, known for their acoustic signals, which are created by forcing air through modified abdominal spiracles, creating a hiss like sound (Schal *et al.* 1984; Clark & Moore, 1995a; Clark & Moore, 1995b; Sueur & Aubin, 2006). Unlike other insects that create sounds through vibration and movements of body parts, hissing cockroaches produce acoustic signals through their respiratory system much like vocalisations in birds, mammals and amphibians. Males, females and juveniles produce hiss-like acoustics in a defensive manner to warn off other cockroaches and predators (Frazer & Nelson, 1984). Males also produce hisses during antagonistic interactions with other males, and during courtship and mating with females (Clark & Moore, 1994; Sueur & Aubin, 2006; Logue *et al.* 2009; Clark, 2010; Hunsinger *et al.* 2017). Being nocturnal, hissing could be helpful by providing information to potential opponents or mates, as sight is limited during active hours.

The males and females are sexually dimorphic, with males producing large pronotal shields and horns that differ with species. The two hissing cockroaches that differ the most in both pronotal shield shape and horn length are *Aeluropoda insignis* and *Gromphadorhina oblongonota*. *A. insignis* have flat horns and an elaborate pronotal shield with nodules along its rim that extend at

each side of the head. *G. oblongonota* have large horns and their pronotal shield forms a hood over the head of the cockroach (Figure. 1).

Males are known to compete both acoustically and physically against other males. During male-male competition males give aggressive displays, including thrashing their abdomen, walking over other males, hissing and more physical interactions such as butting and flipping (Schal *et al.* 1984; Durrant *et al.* 2016; Hunsinger *et al.* 2017). During antagonistic interactions between male *Gromphadorhina portentosa*, the male that hisses more tends to win the interaction (Clark & Moore, 1995b). Antagonistic hisses are variable and are correlated to male body size, with larger males producing lower frequency and longer hisses that dominate smaller males. Hissing could allow the males to assess their opponents relevant to their own competitive ability. This would benefit both males by avoiding unnecessary physical harm (Clark and Moore, 1994).

When comparing two different species of hissing cockroaches that use different strategies for antagonistic interactions, larger-horned cockroaches *G. oblongonota* were more aggressive, physically butting and flipping their opponents, whereas the flat-horned cockroaches *A. insignis* were less aggressive and preferred to hiss (Durrant *et al.* 2016). These two species were also found to invest differently in the pre-copulatory trait horn length and post-copulatory trait testes mass, with *A. insignis* investing more in testes mass and *G. oblongonota* investing more in horn length. The use of different strategies between species

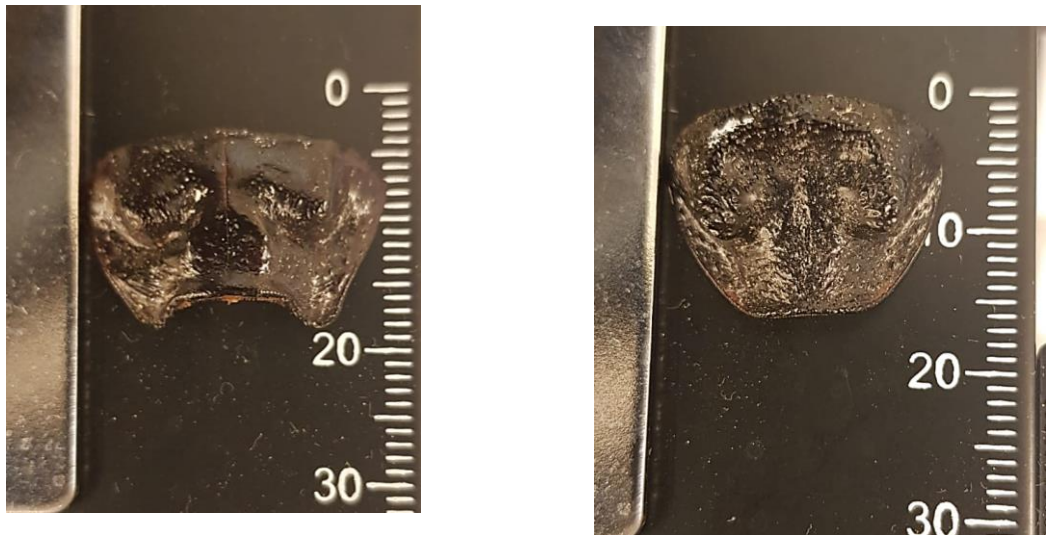


Figure. 1 Male flat horned hissing cockroach *Aeluropoda insignis* pronotal shield (Left) and long horned hissing coach male *Gromphadorhina oblongonota* pronotal shield (Right). Ruler scale in mm.

could explain the diversity in pronotal shield shape and horn size we see between hissing cockroaches.

It is not known, however, whether other postcopulatory traits such as sperm morphology and number differ across hissing cockroach species or relate to pre-copulatory traits. Comparing post-copulatory traits will provide a further understanding of what sexual selection pressures hissing cockroach species are under and the mating strategies they choose.

Males and females are known to engage in courtship before mating. This has been documented in *G. portentosa* (Schal *et al.* 1984; Frazer & Nelson 1984; Leibensperger *et al.* 1985). During courtship, antennation between males and females releases a pheromone emission that creates an initial attraction, then males adopt a specific posture and display to the female. When males are in the courtship posture, hissing often accompanies this (Schal *et al.* 1984; Frazer & Nelson, 1984; Leibensperger *et al.* 1985). Females discriminate between hissing and non-hissing males, as only males that hiss during courtship are successfully mated with (Frazer & Nelson, 1984; Clark & Moore, 1995a; Logue *et al.* 2009; Clark, 2010). It is still unknown if physical traits play a role in female choice. Traits such as body size, pronotal shield shape and horn length could provide signals females can use to assess males.

A better understanding of female responses to visual traits, as well as comparing post-copulatory traits and mating strategies between hissing cockroach species, could provide valuable information about the evolution of mating strategies in these species, where we know little about natural courtship. Hissing cockroaches

make an ideal study system with which to test hypotheses about sexual signals, as they are easily maintained and thrive in lab conditions. Detailed ethograms have been developed to characterize behavior in these species, and they are highly social and not easily stressed (Logue *et al.* 2009; Durrant *et al.* 2016).

Aims and Objectives

This study seeks to understand hissing cockroach sexual selection, looking at both female choice and post-copulatory sperm competition across hissing cockroach species. Firstly, female choice and male interactions were investigated in *G. oblongonota* and *A. insignis* to find out what role horn length plays in mate choice and competition. *G. oblongonota* and *A. insignis* are already known for differing in male-male competition strategies and there is a trade-off in horn length and testes size. Using CT scans from Durrant *et al.* (2016), 3D printed models of males with manipulated horn and pronotal shield sizes were created and presented to live individuals to understand the role these signals might play in male competition and female choice. Secondly, I compared post-copulatory traits among five species of hissing cockroaches, to understand investment allocation, mating strategies and sperm competition intensity. Mitochondrial DNA were sequenced for each of the species, in order to enable a phylogenetically correlated analysis of trait values.

I hypothesized that post-copulatory traits among hissing cockroach species, particularly that of *A. insignis* and *G. oblongonota*, vary in accordance with the predictions of sperm competition theory (Parker, 1970), as some of these species are known to use different pre-copulatory strategies. Thus, I predict that the

species will invest differently in their post-copulatory traits. I also predict that females will exhibit a preference for shield horn length and that female *G. oblongonota* will choose longer horns as they show more physical strength and are likely to win in male-male competition, whilst I predict that *A. insignis* females will choose males with exaggerated pronotal shields and longer horns over those with reduced traits.

Methodology

Lab maintenance

Four cockroach species (*Gromphadorhina oblongonota*, *Gromphadorhina portentosa*, *Elliptorhina javanica*, *Eublaberus distantii*) were sourced from an online supplier BUGZ UK (www.bugzuk.com) and the other two species (*Princisia vanwaerebeki*, *Aeluropoda insignis*) from independent breeder hobbyists (Figure.

2). Each species was maintained in lab populations at the University of Nottingham in a temperature-controlled room, kept at a constant 26°C with 60% ± 10% humidity on a reversed 12:12h day: night cycle to mimic a comfortable environment for growth and survival. Maturing nymphs were sexed and separated into individual clear plastic containers measuring 12 x 18 x 7cm.

Containers allowed for isolation to ensure both males and females grew up as virgins while still seeing and smelling other cockroaches without direct contact until controlled behavioural interactions. Adults for MtDNA extraction were also placed in separate containers until euthanised. Others were kept in colonies to encourage the breeding of lab populations. Containers were set up with Eco bark rainforest substrate (Lucky reptile, LivefoodsUK) with egg carton shelters.

Cockroaches were fed an *ad libitum* diet of dried complete dog food and orange or strawberry jelly pots (LivefoodsUK.com) which were replenished on a bi-weekly basis. Cockroaches used for behaviour experiments were kept on the same consistent orange jelly flavour whilst males used for mating were constantly fed on strawberry, so that any potential nutritional differences in flavour would not affect any results, most importantly that of sperm and

spermatophore traits. Water was sprayed on lids of the containers weekly for humidity and hydration.

Laser sintering

Life-like models were created from CT scans of male cockroaches from Durrant *et al.* (2016). One file each from *G. oblongonota* and *A. insignis* was chosen at random and edited using 3DS Max (Computer software, Autodesk, USA) to create a solid mesh and converted to stereolithography (.STL) files suitable for laser sintering. Control models were formed using AutoCAD (Computer software, Autodesk, USA) to create a rectangular cuboid similar in size to the life-like models. The dimensions of the control models were set at the mean width, length and height of the two species individuals which provided the CT scans. Files were sent to print in a Formiga-P100 laser sintering machine (EOS, Germany) using Nylon-12 powder chosen for its absorbency, lack of odour and take to colour dyes. After a 6-hour printing phase models are found within the powder block and cleaned using pressured air and water to remove any loose powder.

Behavioural response to 3D laser sintered models

Pilot trials were conducted to test cockroach response to 3D laser sintered models to see if cockroaches interact and perceive them as conspecifics, in order to use models in choice experiments. To do this 3D models, from CT scans of male *G. oblongonota* and *A. insignis*, were created as representatives of the species. Live males from both species were introduced to the models in various

trials in relation to colour, texture, control objects, background contrasts and cuticular hydrocarbon scent to see if males would display behaviors towards the models as they would a conspecific, and to find out what visual stimuli are required for a successful interaction. Male cockroaches were used in these trials, as the supply of females was limited. We used males under the assumption that male and female vision, perception and general behaviour does not differ, and hence that, if a male recognises a model cockroach as a conspecific then a female will also respond in a similar manner.

Arena set up

Trials took place under red light to allow for natural nocturnal behaviour to occur in a clear 17 x 14.5 x 10 cm Perspex arena placed inside a polystyrene box to diffuse noise, with an open-top for video recording. A white piece of paper with a black cross in the middle for camera focus drawn in pen was placed on the arena floor and changed every trial. The paper allows for easy removal of cockroach scent between each trial as well as a surface for the cockroach to walk on. A further piece of white paper was used upright in the middle of the arena to create a partition barrier between the live male and the models; this was removed before interactions were recorded (see Appendix Figure. A). A clamp and stand held a video camera (HDRXR 160 EB, Sony, Japan) over the arena to give an aerial view recording of the interactions and a directional microphone (MKH70, Sennheiser, Germany) was placed through a 3cm hole in the polystyrene box up to a mesh-covered hole in the arena and connected to a solid-state recorder (PDM660, Marantz, Japan) to record any hisses.

Colour trial protocol

In this first experiment, the aim was to establish whether cockroaches can detect and respond to objects which resemble conspecifics visually, and whether colour affects this response. *A. insignis* (n= 60) and *G. oblongonota* (n= 60) male cockroaches were exposed to life-like and control models in each of three treatments: plain white undyed, matt brown dyed, to represent real cockroach cuticle colour but not gloss, and shiny brown, dyed and sprayed to represent the real colour and shine of the cockroach cuticle (Figure. 3). To dye the models, RIT dark brown powdered clothing dye was mixed with water as per instructions and heated under a hot plate. Models were then submerged in the boiling dye and left 24 hours for the colour to take, then oven dried. Dye was chosen due to its easy take to the nylon-12 models, and because it has no odour that could affect cockroach behaviour. Shiny models were sprayed with Plasticote clear shine, chosen for its aquarium safe recipe so no toxins or odour would affect cockroach behaviour. Colour and level of gloss were matched to cockroaches by eye, under the assumption hissing cockroaches don't have strong visual senses due to their nocturnal behaviour (Mishra & Meyer-Rochow 2008).

For each of the three colour treatments, cockroaches were randomly subjected to either the control or the life-like model to observe interactions. These cockroaches were then subjected to the corresponding life-like or control model, with the same treatment, at a later date, in a paired design. The time between the first and second trial for each cockroach was the same for each individual. Half of the trial population was given control models first, then life-like models

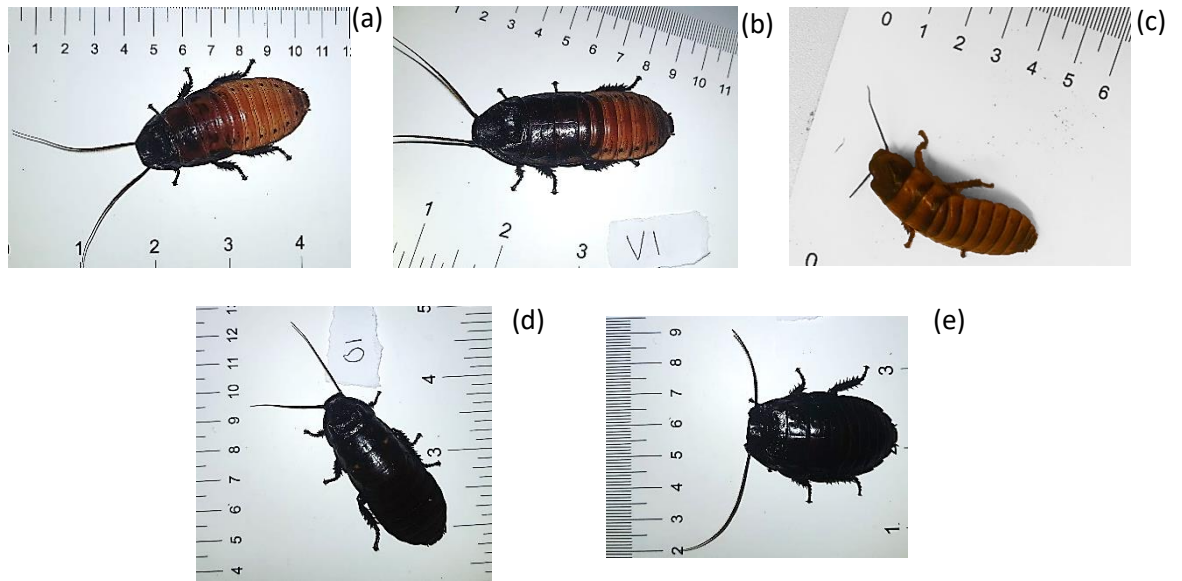


Figure. 2 (a) *Gromphadorhina portentosa*, (b) *Princisia vanwaerebeki*, (c) *Elliptorhina javanica*, (d) *Gromphadorhina oblongonota*, (e) *Aeluropoda insignis* males. Ruler scale in cm.

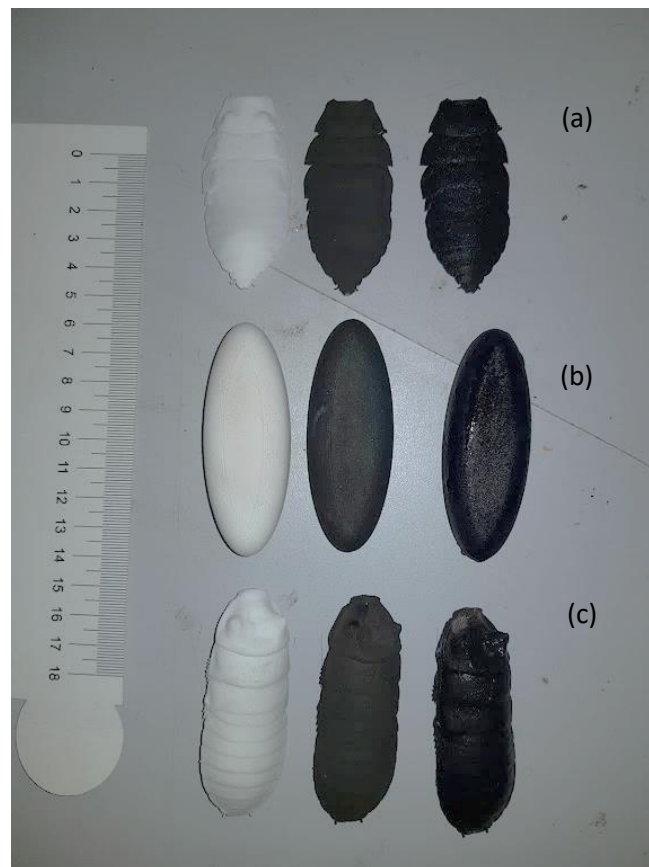


Figure. 3 *A. insignis* life-like (a), control (b) and *G. oblongonota* life-like (c) models remaining white (left), dyed matt brown (centre) and dyed brown and coated shiny (right) for visual experiments. Ruler scale in cm.

and the other half of the population were given the life-like models first. All trials were carried out between 9:00hrs and 17:00hrs.

Male cockroaches were removed from their housing with gloved hands and placed in one of the divided sections of the arena and left to acclimatise for 5 minutes with the model in the other divided section. The side of the arena in which the model and cockroach were placed was randomly switched. Video and audio were set to record just before the trial started and the barrier lifted. Trials were then recorded and terminated after 15 minutes. If cockroaches tried to exit the arena, they were tapped gently on their antennae or head to encourage them to return to the arena floor or they were picked up and placed back on their side of the arena. After each trial cockroaches were placed back in their housing, the paper was replaced, and models were cleaned by rigorous shaking in distilled water for 5 minutes to eliminate any cockroach odour. Video and audio were reviewed once trials were complete using a modified version of Durrant *et al's* (2016) male-male interaction ethogram in Jwatcher 1.0 (Table. 1).

Background contrast protocol

In this second experiment, the aim was to establish whether background contrast effects whether cockroaches can detect and respond to objects which resemble conspecifics visually.

To test whether contrast of models against the background mattered, matt brown life-like models, matt brown controls and white life-like models were

Table. 1 Ethogram of behaviours recorded during visual, background and scent experiments to look at cockroach interactions with models. Adapted from Durrant *et al.* (2016).

Observed Behaviour	JWatcher Category	Definition
Approach	State	A head-on walk towards the model.
Stationary	State	A cease in all movement.
Retreat	State	A swift movement away from the model after approach or interaction.
Low Posture	State	Flattening the body low to the ground and tucking antennae and legs under the body.
Antennation	State	Contacting the model with one or both antennae.
Contacting	State	Contacting the model with any body part other than the antennae.
Antennal thrashing	Event	A quick strike to the model using the antennae.
Hiss	Event	Hissing directed towards the model.
Butt	Event	A forceful motion, contacting the model with the head and pronotal shield.
Flip	Event	Forcibly turning the model around or over on to its side.
Abdominal thrashing	Event	Vigorous shaking of the abdomen towards or on to the model.
Walk over	Event	Walking over the model, approaching from the head across its body and back towards the head.
Movement	State	General movement in no relation to the model.

shown to *A. insignis* (n= 30) and *G. oblongonota* (n= 30) males on either a white or black paper background. Cockroaches were randomly subjected to white or black backgrounds with one of the models to observe interactions. These cockroaches were then subjected to the opposite background colour with the same model, at a later date, to get paired results to see whether interactions of the individual for each of the models differed on white and black backgrounds. The time between the first and second trial for each cockroach was the same for each individual. Half of the trial population was recorded on black backgrounds first, and the other half on white backgrounds first. All trials were carried out between 9:00hrs and 17:00hrs.

Protocol and data collection for male *A. insignis* and *G. oblongonota* individuals was identical to the first experiment.

Scent trials

In this experiment, the aim was to establish whether cockroaches can detect and respond more strongly to objects which resemble conspecifics when male or female scent is added to the models, and whether sex specific scent affects this response. Cuticular hydrocarbons were extracted from individuals via a non-lethal water-based method, following Roux *et al.* (2009). Scents were collected from five males per species through vigorous shaking of individuals in 500ml wide neck PP bottles (Aslon) filled with 100ml distilled water for 5 minutes. The supernatant (~5ml) was collected then rehydrated in 100ml distilled water for trials when needed. Male scent was mixed together for each species to create a generalized scent emulsion to minimise the effect of individual variation.

Another set of scents were extracted from five females using the same method. The emulsions were applied to the models using the same process of vigorously shaking the model in the emulsion for 5 minutes and leaving them to air dry to fix the scent to the models. The water based method was chosen due to being non-lethal and easy to carry out, as alternative solvent based methods require detailed steps with equipment and chemicals not accessible for this research (Roux *et al.* 2009; Turillazzi *et al.* 2010; Gerhardt *et al.* 2015; Inui *et al.* 2015).

Matt brown models were used for these trials after finding males of both species respond well to them. Individuals *A. insignis* (n= 30) and *G. oblongonota* (n= 30) males were then randomly given matt brown life-like models, painted with male scent, female scent or distilled water (control). All trials were carried out between 9:00hrs and 17:00hrs.

Protocol and data collection for male *A. insignis* and *G. oblongonota* individuals was identical to the first experiment.

Weaponry manipulation: male response and female choice

Two-way choice trials were conducted to test whether females have any preference towards males based on weaponry size, independently of any other cues (e.g. behavioural, olfactory or body size-related). To do this, models were manipulated to have either reduced, standard-sized or longer horns. Male response to weaponry size in conspecifics was also tested in the same way using two-way choice trials. Matt brown models were used as the experiments one and two above showed that cockroaches do interact with them as they would a

conspecific. Scent was not used as the scent extraction experiments did not increase behavioural response.

Both *G. oblongonota* and *A. insignis* cockroach horn sizes were manipulated using the 3DS Max Autodesk software, CT scans were uploaded into the program and adapted using a variety of different tools, first an edit poly modifier was applied to the surface of the cockroach generating over 600 points (vertices) on the cockroach that could be manipulated. The cockroaches were then scaled up to the largest male body length for each of the two species (Body length: *A. insignis* = 7cm, *G. oblongonota* = 8.5cm). The CT scans at this scale had horns which were close in length (within 3%) to the length predicted, based on body length, according to Durrant *et al.* (2016). Unmanipulated models printed at this scale were used in the standard-sized horn treatment. For horn manipulation, vertices at the tip of the horns were then selected with the soft selection tool applied with a falloff distance of 7mm, generating a mesh over the horns with the ability to effect all vertices in its selection zone so that when one vertex is moved they all move in relation to it, with vertices at the edge of the falloff zone moving less than those closer to the selected vertex stretching it from the base up (Figure. 4). The movement tool was selected and used to pull and stretch the horns to the maximum and minimum absolute horn lengths found in each species population (Durrant *et al.* 2016), while measuring the lengths with a guide block. In *A. insignis* due to naturally having very reduced horns and different shield shapes, the shield's outer edge with raised nodules was also stretched or reduced, to test if the shield size is a weapon or ornamental trait

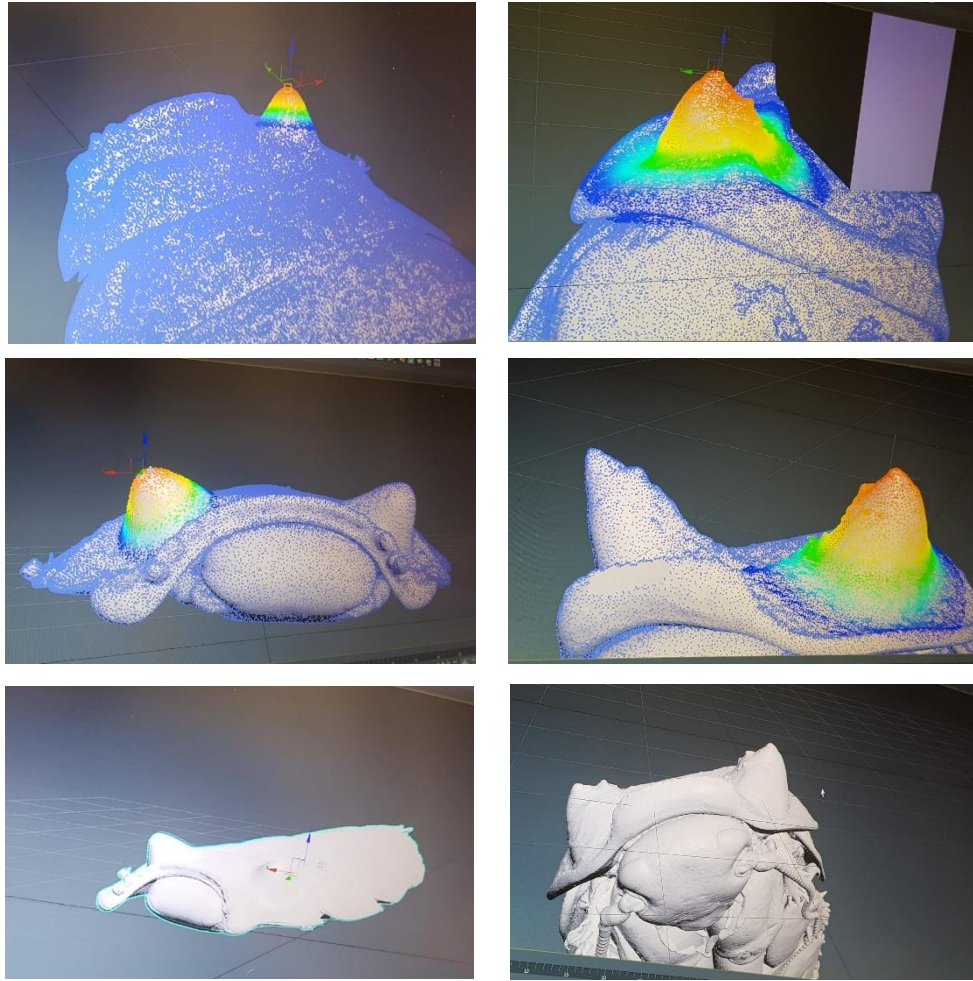


Figure. 4 Manipulation of horn size using 3DS MAX for *A. insignis* (left) and *G. oblongonota* (right). Mesh is selected around the horns to manipulate to the appropriate size using guide blocks set at chosen measurements (seen in top right image). Colour of the highlighted area indicates natural peaks and dips in the horns structure and keeps them in proportion whilst manipulation is carried out, so structure is not lost but is reduced or exaggerated as desired. Square grids are 2x2 cm.

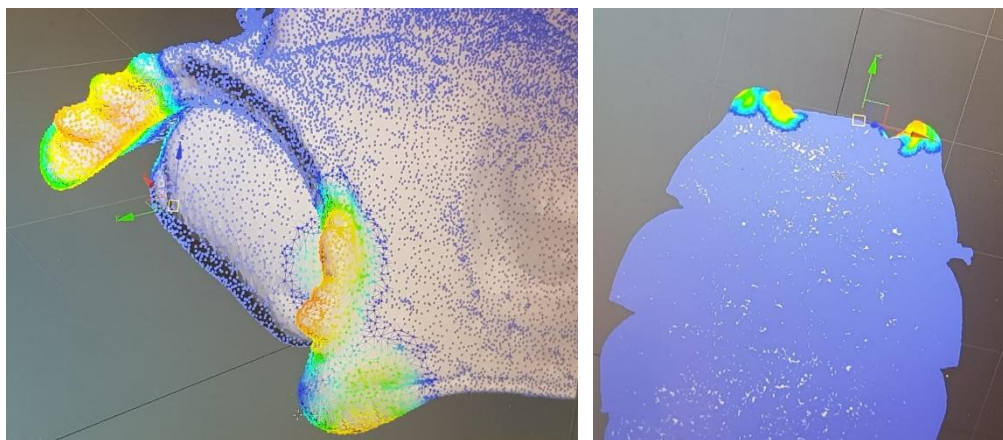


Figure. 5 Manipulation of shield ornaments in 3DS MAX for *A. insignis*. Square grids are 2x2 cm.

(Figure. 5). All other traits including body size of the models remained the same so horn size and *A. insignis* shield size were the only parts manipulated. These files were then exported as STL files for laser sintering and dyed in the same way as the pilot models above.

Trials took place under red light to allow for natural nocturnal behaviour to occur in a white Perspex Y-maze with 20 x 12cm sections partitioned with paper and an open top for video recording. White paper was placed on the floor of each section of the Y-maze and changed every trial. Vaseline was placed at the top of the Y-maze to discourage cockroaches from escaping. If cockroaches tried to exit the arena, they were tapped gently on their antennae or head to encourage them to return to the arena floor or they were picked up and placed back in the main section of the Y-maze. A video camera was held on a stand over the arena to give an aerial view for recording choice and any interactions.

Female choice trials

Female *A. insignis* (n= 50) and *G. oblongonota* (n= 50) cockroaches were randomly selected to receive a two-way choice trial involving either standard-sized horn models and long-horned models, or standard-sized horned models and short horned models. All trials were carried out between 9:00hrs and 17:00hrs.

Female cockroaches were picked up with gloved hands and placed in the main section of the Y maze to acclimatise for 5 minutes. In the two Y maze division sections, the standard-sized horned model and either long or short horned model were placed at either side section. The section in which each model was

placed was alternated to control for any bias of side preference. Videos were set to record just before the trial started and the barriers were removed simultaneously. Trials were terminated after 10 minutes to allow time for a response. The type of model approached first (when the female crosses over the border of the models section), the type of model interacted with first (antennation or contact) and any unexpected behaviour such as hissing, thrashing, attempting to shove or roll the model were noted in real-time. After each trial cockroaches were placed back in their housing, paper was replaced in each section and the models were cleaned by rigorous shaking in distilled water for 5 minutes to eliminate any cockroach odour and replaced. Video footage was then reviewed once trials were complete. Total interaction times for the two model choices were recorded using JWatcher 1.0 (Table. 2).

Male choice trials

Male *A. insignis* (n= 50) and *G. oblongonota* (n= 50) cockroaches were randomly subjected to either standard-sized horned models and long horned models or standard-sized horned models and short horned models in two-choice trials. All trials were carried out between 9:00hrs and 17:00hrs and completed in the same way as the female two-way choice trials above. As with the female choice experiment, the type of model approached first, the type of model interacted with first and any unexpected behaviour were noted in real-time. After each trial, the cockroach's body length was measured to find most males were of a similar length, few males were of short lengths and few males of long lengths. Males

Table. 2 Ethogram of recorded interactions during two-way choice trials.

Observed Behaviour	JWatcher Category	Definition
Movement	State	General movement in no relation to any of the models.
Stationary (centre)	State	A cease in all movement in the centre area.
Stationary (standard-size)	State	A cease in all movement in the standard-size horn model segment.
Stationary (manipulation)	State	A cease in all movement in the manipulated horned model segment.
Approach standard-size horn models	State	A head-on walk towards the standard-size horned model.
Approach manipulated horn models	State	A head-on walk towards the manipulated (small or large) horned model.
Standard-size horn models interaction.	State	All forms of interaction; contacting model with antenna or other body parts, butting or flipping the model with force using their head, hissing, thrashing abdomen at or on model, walking over model, lowering posture to the ground or deliberately retreating away from model after interacting or approaching.
Manipulated horn model interaction.	State	All forms of interaction; contacting model with antenna or other body parts, butting or flipping the model with force using their head, hissing, thrashing abdomen at or on model, walking over model, lowering posture to the ground or deliberately retreating away from model after interacting or approaching.

were categorized into small, medium or large to see if responses to the large models changes with size, as males tend to challenge those of a similar match (Schal *et al.* 1984; Clark & Moore, 1995a,1995b; Durrant *et al.* 2016; Hunsinger *et al.* 2017). Video footage was then reviewed once trials were complete. Total interaction times for each model presented were recorded using JWatcher 1.0.

Post-copulatory trait investment

Post-copulatory trait comparisons among five hissing cockroach species (*G. oblongonota*, *G. portentosa*, *E. javanica*, *A. insignis*, *P. vanwaerebeki*) were conducted to establish the level of sperm competition hissing cockroaches face, whether this differs between the species, what post-copulatory traits are influenced by sperm competition and whether there is a trade-off between post-copulatory traits and horn length. Understanding differences among individual post-copulatory traits can help depict the strategies these species choose by looking at trait investment. If species do not differ it is likely they are under similar strengths of sperm competition. MtDNA was taken from each of the species to create a phylogenetic tree so a phylogenetic-correlated analysis of trait values could be completed.

Hissing cockroach phylogenetic analysis

Two males and two females from each of the five species, along with a sixth species *E. distanti* as an outgroup for phylogenetic analysis, were humanely euthanized by freezing for 30 minutes at -18 °C prior to dissections. The back four legs were then removed from the body with scissors and the muscle tissue pried away and extracted with tweezers and put into an Eppendorf tube on ice.

Leg muscle was used to extract MtDNA following Bourguignon *et al.* (2018). Homogenization buffer along with four Precelly beads were then added to the samples and placed in Precelly's evolution tissue homogenization machine (P000062-PEVO0-A) to homogenise on a hard setting (8000rpm, 8 x 15sec cycle, 15sec pause). Salt extraction method for small-scale isolation of mitochondrial DNA was then carried out (see Ahmad *et al.* 2007). To amplify isolated MtDNA, the samples then went through PCR using 12S MtDNA primers (see Kambhampati & Smith, 1995). A sample from each of the PCR products was run on a gel to check for sufficient product before sending the sample off for sequencing at Macrogen Inc. (Amsterdam Netherlands). Sequences were then quality checked and clean sections were taken and blasted through the GenBank database (NIH genetic sequence database) to check sequences have a match to those published by Bourguignon *et al.* (2018). Unsuccessful or messy sequences were not included and samples from new individuals were taken and sequenced as replacements.

Neighbour adjoining trees, parsimony trees, distance-based and bootstrapped trees were then created to find the best fitting tree that had the outgroup in the correct place and results best match those of published trees (Kambhampati & Smith, 1995; Inward *et al.* 2007). To check species lines were not contaminated, all samples were used to make sure samples from each species clustered together. Once the quality of the samples was confirmed, the cleanest longest sequence from each of the species was used to represent the species to create a phylogenetic tree used for sperm trait analysis (Figure. 6).

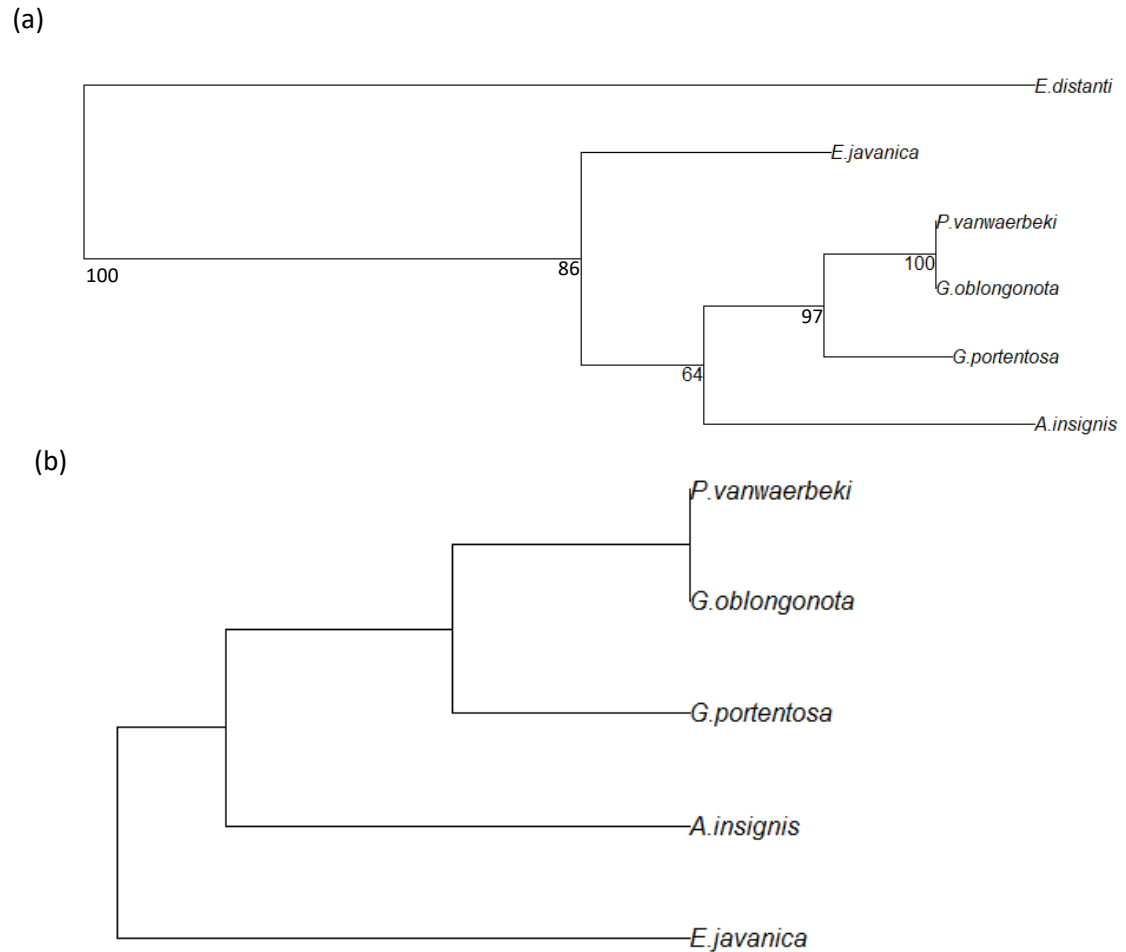


Figure. 6 (a) Distance-based tree with *Eublabeus distanti* as an outgroup to the five hissing cockroach species, numbers represent likelihood from 100 bootstrapped trees. (b) Reduced tree without outgroup *E. distanti*, used for phylogenetically independent analysis of post-copulatory traits among hissing cockroach species.

Sperm data collection

Mating protocol

Mating took place under red light to allow for natural nocturnal behaviour. Virgin males and females from isolated stock were placed into a Perspex arena set up identical to experiment one. This footage was then later used to calculate total copulation time for analysis. Mating interactions took place for roughly 15-30 minutes depending on whether the male and female both engaged with one another and successfully mated. Videos were terminated once mating was complete. Trials were stopped after 10-20 minutes if one or both individuals were not interacting with one another. Males and females were both replaced upon each unsuccessful or successful interaction, with the exception when stock became low. Where males were trying to mate with females unsuccessfully just the females were replaced in hope that the male would continue to court the new female to increase mating success. Mating's were carried out until 3-4 successful mating's were obtained per species (*G. oblongonota* n= 3, *G. portentosa* n= 4, *E. javanica* n= 3, *A. insignis* n= 4, *P. vanwaerebeki* n= 3).

Upon successfully mating, males and females were then humanely euthanized by freezing for 20 minutes. Males were weighed to the nearest 0.001g and their body length (from the top of their head to the end of the last body segment), shield width (bottom of the shield at the straightest point) and horn length were measured using dial callipers in millimetres. Males were then dissected to extract and weigh both left and right testes. Male ventral plates were cut off from the abdomen using scissors to open up the cockroach. Fatty tissue was pulled away

at each side of the last third of the abdomen, using a dissection pin, until testes at either side were visible. Testes could then be detached from the body by gently cutting away with the pin. Any remaining fat on the testes was removed before obtaining fresh weight of both right and left testes to the nearest 0.001g and stored in 10% formalin/PBS solution. Body weight and testes weight were then used to calculate mean relative testes mass (RTM) to account for body size effects on testes size.

Sperm extraction and preparation

Once the female was euthanized, the spermatophore placed by the male inside the female's primary genital pouch was quickly identified and removed. Females were placed on their dorsal side and the ventral plates were cut off and valves disconnected to expose the internal organs. The spermatophore could be detected easily at the end of the abdomen, half exposed and half in the genital pouch. Fatty tissue was gently moved away to expose the whole female reproductive structure. The genital pouch that contains the spermatophore was removed by cutting away from the rest of the body and picked up using tweezers. The spermatophore, partially exposed, was pulled out neatly using dissecting tweezers. In all but one of the hissing cockroach species (*A. insignis*) the spermatophore has an extra rubberised mass that fills the genital pouch; this part attaches itself to the female's pouch and is removed carefully with scissors. This mass of the spermatophore does not contain spermatozoa. Both parts of the spermatophore were photographed for measuring length and width to the nearest 0.001cm and weighed separately to the nearest 0.001g. The part that

contains the sperm was then placed in an Eppendorf tube with 1ml of PBS and broken apart with dissection pins to release the sperm.

From these sperm stocks, 300µl sub-sample was taken and fixed in 200µl formalin for concentration counts to work out sperm number. Another 300 µl was taken and mixed with 300 µl of Mitotracker Green dye to stain the mitochondria before being fixed in 10% formalin solution for morphology analysis. All samples were stored at room temperature in polystyrene boxes to prevent light exposure.

Sperm morphology

For all species, 5µl of sperm of each male was mixed with 2.5µl of Hoechst-33342 to stain the nuclear DNA (to highlight the head of the sperm) on a coverslip before being placed on a microscope slide and positioned on the microscope (ZEISS Exciter wide field). The stained sperm was viewed at 20 x 0.75 magnification and ten random healthy sperm, where morphology could be seen clearly for accurate measurement, were photographed using the attached HAL 100 camera, to provide a good estimate of within-male variation for trait lengths (Kleven *et al.* 2008). Photographs were taken under FITCI and DAPI fluorescent channels for each of the ten sperm per male to highlight the acrosome, head and tail of the sperm clearly (Figure. 7). ImageJ was then used to measure different sperm traits from the images to the nearest 0.001µm: (i) acrosome length, (ii) head length, (iii) tail length, (vi) total length. For each sperm trait, a mean from

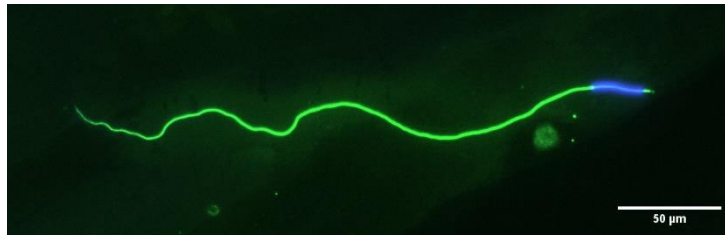


Figure. 7 Image of Hissing cockroach *G. portentosa* sperm under fluorescent light channels, head is stained blue, the tail and acrosome either side of the head is stained green making the sperm traits visible for measurement.

three measurements was taken per individual sperm. From this, a mean for each sperm trait per male was calculated to the nearest 0.001 μ m. Sperm measurements were highly repeatable for acrosome, head, tail and total length (ANOVA: $F= 2.22$, $df= 9$, $R^2= 0.99$, $p < 0.0001$; $F= 2.23$, $df= 9$, $R^2=0.992$, $p < 0.0001$; $F= 2.13$, $df= 9$, $R^2= 0.985$, $p < 0.001$; $F= 2.73$, $df= 9$, $R^2= 0.985$, $p < 0.001$).

Sperm count

Sperm count samples for each male were mixed to displace the sperm that sank to the bottom of the Eppendorf tube. For each sample, 8 μ l were pipetted into a Neubauer cell counting chamber under a microscope (Laboulx S) at x10 magnification under transmitted light. Sperm were counted by eye, using a clicker counter, in each section of the chambers until a full square had been counted. Counts were highly repeatable (ANOVA: $F= 2.87$, $df= 2$, $R^2= 0.999$, $p < 0.0001$). The sperm count and square sections were used to work out the concentration of sperm (concentration=number of sperm x 10000/number of squares).

This was repeated until there were three concentrations per male to calculate mean sperm concentration. From this concentration total sperm number produced by the male could be estimated.

Statistical analysis

To explore the effects of cockroach interaction for both *A. insignis* and *G. oblongonota* on model colour (matt brown, shiny brown, white) between control and life-like models for each treatment category, paired interaction and the

interaction between each of these factors, generalised linear mixed effects models (GLMM) were conducted with individual subjects used as the random effect. Total time spent interacting and interaction time for each behaviour category, aggressive (butt, flip, abdomen thrash, hiss, walk over, antennal thrash), submissive (low posture, retreat) and exploratory (antennation, approach, contact) were compared. Data were log-transformed (natural) to normalise the distribution.

To test whether background colour affects cockroach interactive response to models, GLMM analysis of the time spent interacting with models (life-like matt brown, life-like white, matt brown control), background (black, white), species, paired interactions and the interaction between each of these factors were conducted with individual subjects used as the random effect. Total time spent interacting and interacting time for each behaviour category aggressive, submissive and exploratory were compared. Data were log-transformed (natural) to normalise the distribution.

To understand if scent increases interactions with models for both species, GLMM analyses were used to compare the total time spent interacting and time spent interacting aggressively, exploratorily and submissively between male scented, female scented and distilled water scented models, species and the interacting between scented models and species with individual subjects used as the random effect. Data were log-transformed (natural) to normalise the distribution.

Two-way choice trials were conducted to understand if female and male *G. oblongonota* and *A. insignis* respond to horn size. First approach choice and first interaction choice were compared using Chi-square. The total time spent interacting with both models in each experiment were compared using intercept only models (Sefc *et al.* 2017; Muff *et al.* 2019). Due to *A. insignis* horns being reduced, pronotal shield size was also manipulated. For the intercept only models following Sefc *et al.* (2017), female interaction index (FII) from was calculated as $(\text{female interaction with standard-sized models} - \text{female interaction with manipulated models}) / (\text{female interaction with standard-sized models} + \text{female interaction with manipulated models})$ for each female to test whether the FII average in each experiment deviated significantly from zero and the male interaction index (MII) was calculated as $(\text{male interaction with standard-sized models} - \text{male interaction with manipulated models}) / (\text{male interaction with standard-sized models} + \text{male interaction with manipulated models})$ for each male to test whether the MII average in each experiment deviated significantly from zero. Deviations indicate bias towards either models, with positive values indicating standard-sized horns preference and negative values indication manipulated horn preference. As males varied dramatically in size, they were categorised in to large, medium and small groups to see if interactions differed between each size group. ANOVAS were conducted to see if body size of both *G. oblongonota* males and *A. insignis* males affected interaction choice. Both first choice and total interaction time are frequent methods used to analyse two-way choice (Sefc *et al.* 2017; Bulté, *et al.* 2018).

ANOVA was used to look at post-copulatory trait characteristics and horn length between hissing cockroach species, to find similarities and differences that could indicate different mating and trait investment strategies. Phylogenetic generalist least squares regressions (PGLSR) were conducted to explore relationships and patterns between post-copulatory traits of interest (relative testes mass, spermatophore weight, ampulla weight, sperm number), sperm characteristics (acrosome length, tail length, head length and total length), mating time, male weight and horn size between each species, whilst accounting for phylogenetic relationships. Male body size was used as a covariant. PGLS correlations were also used to look at the relationships between sperm characteristics across the five species of hissing cockroaches.

All data were analysed using R Studio version 1.2.1335 (Boston MA).

Phylogenetic trees were created using the packages *ape* (analyses of phylogenies and evolution 5.1) and *phangorn* (phylogenetic reconstruction and analysis 2.4.0). PGLSR was conducted using *ape* 5.1 (with nlme 3.1) and *geiger* (analysis of evolutionary diversification 2.0) packages.

Results

Behavioural response to 3D laser sintered models

Colour trials

Although individual males spent most of their total amount of time stationary (58%) or moving around the arena (29%) in the interaction, they displayed a wide range of behaviours directed at the models. The most common behaviours exhibited towards the models were apparently exploratory, such as using their antennae to touch the model (4%), making physical contact with their body (4%) or approaching the models (1%). Aggressive behaviours were less common, but included attempting to flip the model (0.05%), thrashing their antennae on the model (0.05%), hissing (0.02%), butting the model (0.1%), thrashing their abdomen (0.23%) and walking over the model as a sign of dominance (1.45%) (Schal *et al.* 1984; Durrant *et al.* 2016). Submissive behaviours were also less common but included lowering their posture to the ground and tucking in their antennae (2%) and retreating from the model upon approaching it (0.1%).

Male cockroaches spent a greater amount of time interacting with life-like models compared to control models, the total time spent interacting with the models during colour trials significantly differed depending on the shape of the models, independently of other factors (Table. 3, Figure. 8a). Total time spent interacting was not affected by model colour and species (Table. 3). However, the interaction between species and colour was significant, with *A. insignis* males spending

Table. 3 General linear mixed effects models (GLMM) of the time spent interacting against model colour (matt brown, shiny brown, white), model shape (life-like or control), species (*A. insignis*, *G. oblongonota*), paired interaction and the interaction between each of these factors. Significant effects are highlighted in bold. Data had been log-transformed (natural) to normalise the distribution.

Interaction	Term	Chi-squared	df	p
Total time spent interacting	Colour	2.982	2	0.225
	Shape	14.014	1	<0.001
	Species	0.36	1	0.548
	Colour x Species	6.231	2	0.042
	Colour x Shape	3.186	2	0.203
	Shape x Species	0.039	1	0.843
	Shape x Species x Colour	1.644	2	0.439
Exploratory interactions	Colour	3.004	2	0.222
	Shape	14.071	1	<0.001
	Species	1.678	1	0.195
	Colour x Species	6.852	2	0.032
	Colour x Shape	0.713	2	0.7
	Shape x Species	0.069	1	0.791
	Shape x Species x Colour	2.83	1	0.335
Aggressive interactions	Colour	3.421	2	0.18
	Shape	11.057	1	<0.001
	Species	0.062	1	0.803
	Colour x Species	2.568	2	0.276
	Colour x Shape	7.572	2	0.022
	Shape x Species	0.16	1	0.688
	Shape x Species x Colour	0.406	2	0.816
Submissive interactions	Colour	6.09	2	0.047
	Shape	12.07	1	<0.001
	Species	0.106	1	0.744
	Colour x Species	9.288	2	0.009
	Colour x Shape	3.86	2	0.145
	Shape x Species	0.978	1	0.322
	Shape x Species x Colour	7.029	2	0.029

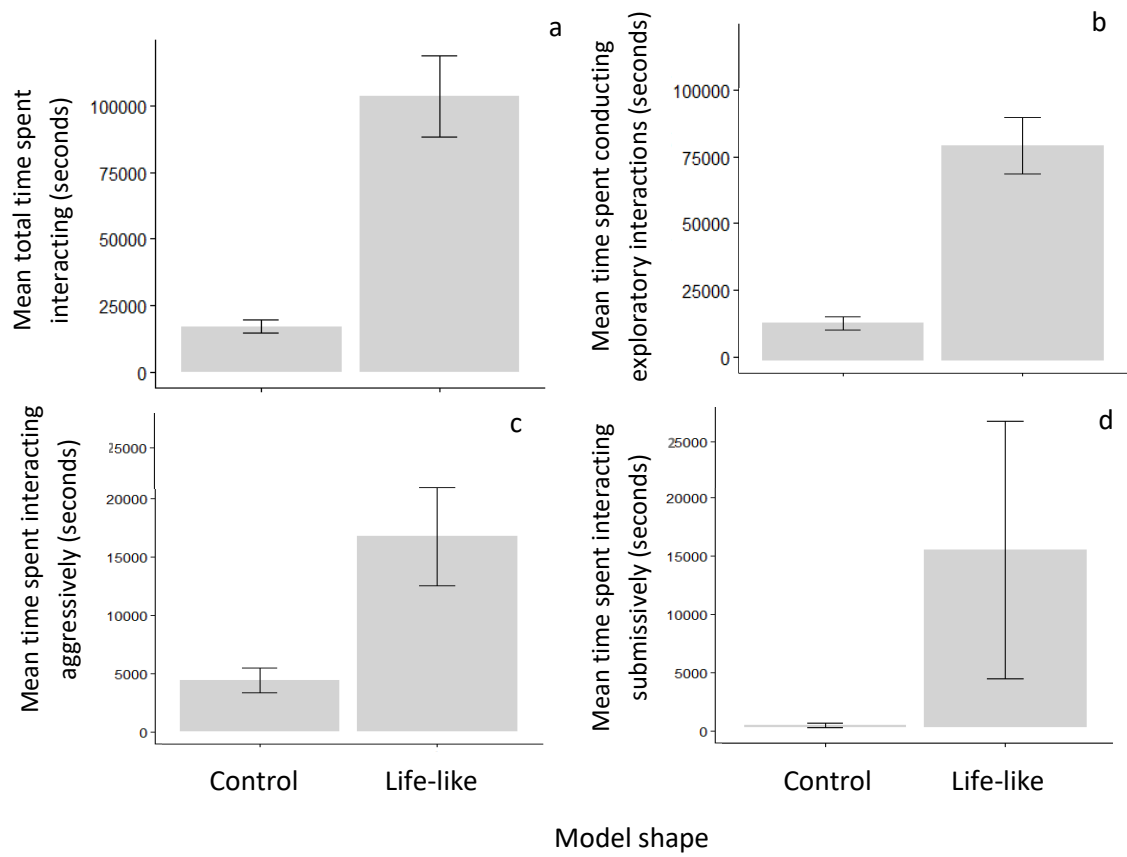


Figure. 8 (a) Mean total time male cockroaches of both species combined spent interacting, (b) time spent conducting exploratory interactions, (c) time spent interacting aggressively (d) and time spent interacting submissively between life-like and control models. Error bars represent $\pm 1SE$.

less time interacting with white models and more time with matt brown models, and *G. oblongonota* males spending less time with white models and similar time with matt and shiny brown models (Table. 3, Figure. 9a). There was no significant interaction between the effects of the colour and shape of the models on the total amount of time spent interacting, nor was there between shape and species, or colour, shape and species (Table. 3).

The time spent engaging in exploratory interactions significantly differed depending on the shape of the models, with male cockroaches spending a greater amount of time conducting exploratory interactions with life-like models compared to control models (Table. 3, Figure. 8b). Time spent conducting exploratory interactions was not affected by model colour or species independently (Table. 3). However, the interaction between species and colour was significant, with *A. insignis* males interacting less with white models and similarly with shiny and matt brown models, and *G. oblongonota* males spending differing amounts of time conducting exploratory interactions with models of each colour (Table. 3, Figure. 9b). There was no significant interaction between the effects of the colour and shape of the models on the amount of time spent conducting exploratory interactions, nor was there between shape and species, or colour, shape and species (Table. 3).

Overall, the time spent interacting aggressively with the models during colour trials was significantly affected by the shape of the models, independently of other factors, with male cockroaches spending a greater amount of time

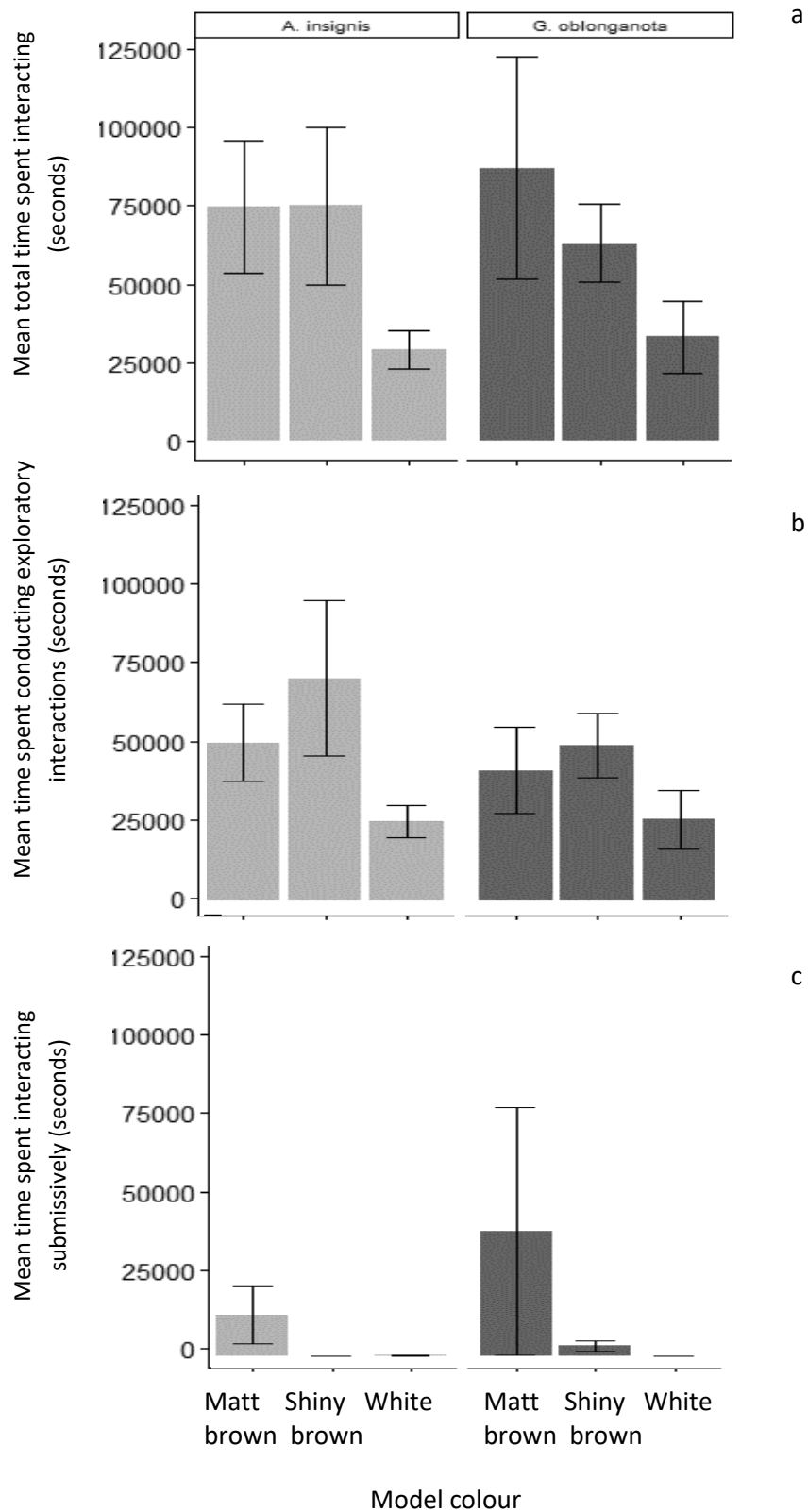


Figure. 9 (a) Mean total time male cockroaches spent interacting, (b) time spent conducting exploratory interactions, (c) and time spent interacting submissively (c) between matt brown, shiny brown and white coloured models for both *A. insignis* and *G. oblonganota* cockroach species. Error bars represent $\pm 1SE$.

interacting aggressively with life-like models compared to control models (Table. 3, Figure. 9c). Total time spent interacting aggressively was not affected by model colour or species independently (Table. 3). There was, however, a significant interaction between the effects of model colour and model shape on total time spent interacting aggressively, with little aggressive interaction between control models and cockroaches interacting more aggressively towards matt brown life-like models (Table. 3, Figure. 10). There was no significant interaction between the effects of the species and colour of the models on the total amount of time spent interacting aggressively, nor was there between shape and species, or colour, shape and species (Table. 3).

The time spent engaging in submissive interactions significantly differed depending on the shape of the models, with male cockroaches spending a greater amount of time interacting submissively with life-like models compared to control models (Table. 3, Figure. 8d). Time spent interacting submissively with the models was also significantly affected by the colour of the models independently, with cockroaches spending a greater amount of time interacting submissively with matt brown models compared to shiny brown and white models (Table. 3, Figure. 11). There was no effect on the time spent interacting submissively with cockroach species independently (Table. 3). There was no significant interaction between the effects of the colour and shape of the models on the total amount of time spent interacting submissively, nor was there between model colour and species (Table.3). There was a significant two-way interaction between the effects of model colour and cockroach species on the

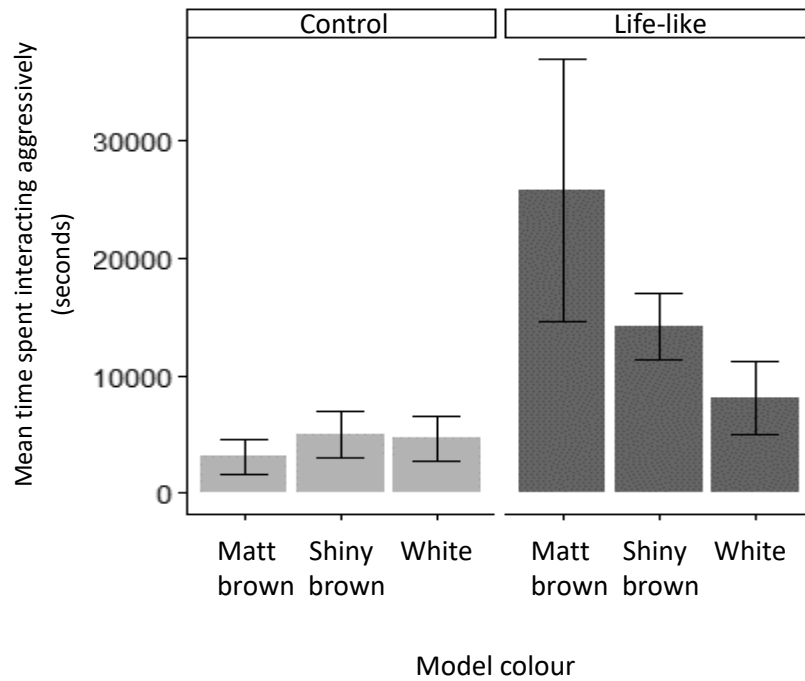


Figure. 10 Mean time male cockroaches of both species combined spent interacting aggressively between matt brown, shiny brown and white coloured control and life-like models. Error bars represent $\pm 1SE$.

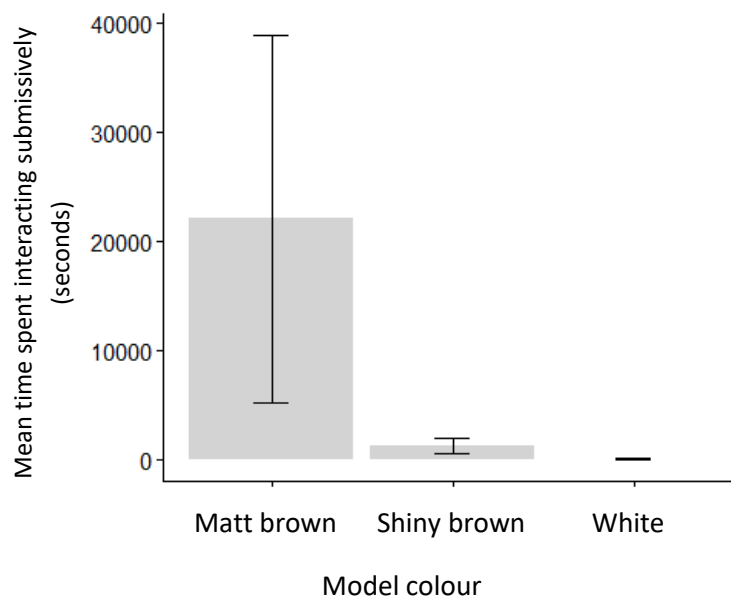


Figure. 11 Mean time male cockroaches of both species combined spent interacting submissively between matt brown, shiny brown and white coloured models. Error bars represent $\pm 1SE$.

time spent interacting submissively with the models, with *G. oblongonota* males spending a particularly long time interacting with the matt brown models (Table. 3, Figure. 9c). However, there was also a significant three-way interaction between species, model type and model colour: the increased time spent behaving submissively with the matt brown models by *G. oblongonota* males were more pronounced with the life-like models compared with the control models (Table. 3).

Background contrast trials

The results of the previous experiments showed that male cockroaches interacted more with life-like models over control models and generally matt brown models received more interactive responses and white models received less. To find out whether interactive responses were affected by the contrast of the models against the background colour, trials using a white life-like model, a matt brown life-like model and a matt brown control model were conducted on black and white backgrounds.

The total time spent interacting during background colour trials significantly differed between model types, with male cockroaches spending a greater amount of total time interacting with matt brown life-like models compared to white life-like models and matt brown control models (Table. 4, Figure. 12a). Total time spent interacting did not differ with background colour or species independently, and there were no significant interactions between any of the main effects of model type, species or background colour (Table. 4).

Table. 4 General linear mixed effects analysis (GLMM) of the time spent interacting with models (life-like matt brown, life-like white, matt brown control), background (black, white), species (*A. insignis*, *G. oblongonota*), paired interaction and the interaction between each of these factors. Significant effects are highlighted in bold. Data had been log-transformed (natural) to normalise the distribution.

Interaction	Term	Chi-squared	df	p
Total time spent interacting	Model	13.912	2	<0.001
	Background	0.054	1	0.814
	Species	3.817	1	0.051
	Model x Species	4.028	2	0.133
	Model x Background	4.556	2	0.102
	Background x Species	0.504	1	0.477
	Background x Species x Model	0.641	2	0.725
Exploratory interactions	Model	13.949	2	<0.001
	Background	0.253	1	0.614
	Species	4.225	1	0.039
	Model x Species	2.523	2	0.283
	Model x Background	3.378	2	0.184
	Background x Species	0.886	1	0.346
	Background x Species x Model	2.888	2	0.235
Aggressive interactions	Model	8.733	2	0.012
	Background	0.091	1	0.762
	Species	2.706	1	0.099
	Model x Species	9.208	2	0.01
	Model x Background	3.577	2	0.167
	Background x Species	0.723	1	0.395
	Background x Species x Model	7.29	2	0.694
Submissive interactions	Model	4.612	2	0.099
	Background	2.35	1	0.125
	Species	2.351	1	0.124
	Model x Species	4.997	2	0.082
	Model x Background	5.451	2	0.065
	Background x Species	2.923	1	0.087
	Background x Species x Model	6.312	2	0.042

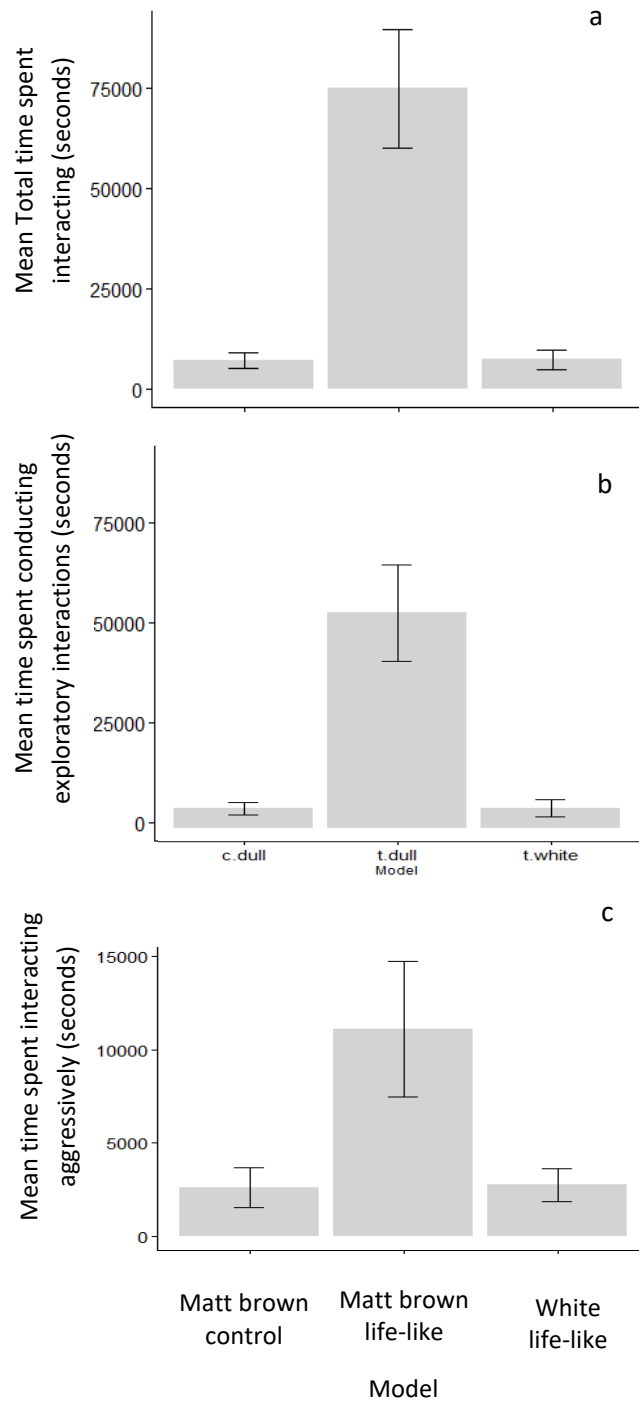


Figure. 12 (a) Mean total time male cockroaches of both species combined spent interacting, (b) time spent conducting exploratory interactions and (c) time spent interacting aggressively between matt brown life- like, matt brown control and white life like models. Error bars represent $\pm 1SE$.

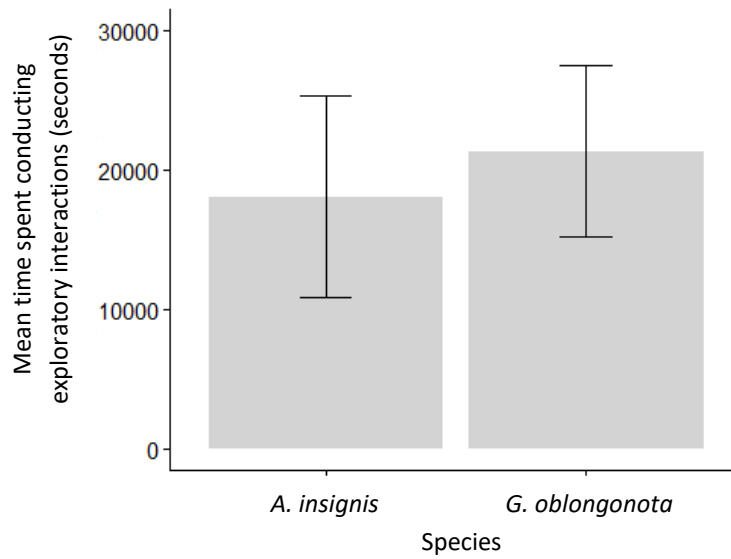


Figure. 13 Mean time spent conducting exploratory interactions between *A. insignis* and *G. oblongonota* males. Error bars represent $\pm 1SE$.

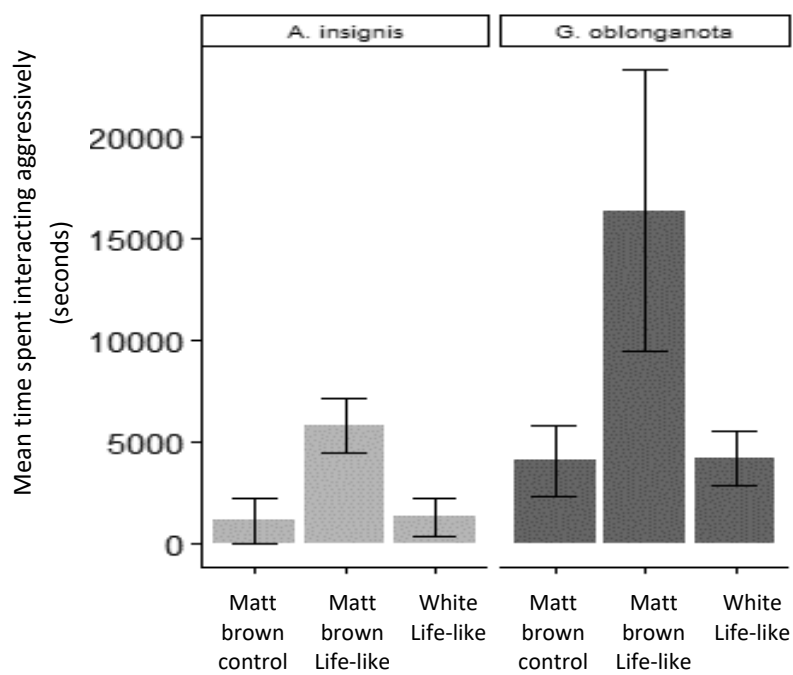


Figure. 14 Mean time *A. insignis* and *G. oblongonota* males spent interacting aggressively between matt brown control, matt brown life-like and white life-like models. Error bars represent $\pm 1SE$.

Time spent conducting exploratory interactions was significantly different between model types and species independently (Table. 4). Cockroaches spent a greater amount of time interacting normally with matt brown life-like models compared to white life-like models and matt brown control models (Figure. 12b). *G. oblongonota* males spent more time interacting than *A. insignis* males (Figure. 13). Time spent conducting exploratory interactions did not differ with background colour independently, and there were no significant interactions between any of the main effects of model type, species or background colour (Table. 4).

The time spent interacting aggressively during background trials significantly differed between model types, with cockroaches spending more time interacting aggressively towards matt brown life-like models compared to white life-like models and matt brown control models (Table. 4, Figure. 12c). Aggressive interaction time did not differ between species or background colour independently (Table. 4). There was no significant interaction between the effects of the models and background colour on the amount of time cockroaches spent interacting aggressively, nor was there between species and background colour, or background colour, model and species (Table. 4). However, there was a significant interaction between models and species on the time spent interacting aggressively, with *G. oblongonota* spending a greater amount of time interacting with the models and spending the most time interacting aggressively to the life-like matt brown models (Table. 4, Figure. 14).

There were no significant main effects of model type, species or background colour on the time spent interacting submissively, or any significant two-way interactions. There was, however, a significant three-way interaction, with *A. insignis* males spending more time interacting submissively with dull brown life like models, but more so when viewed against a black background.

Overall, both *A. insignis* and *G. oblongonota* interacted more with the dull brown life-like model compared to the control and white life-like model, regardless of background colour, suggesting background colour and model contrast does not affect interaction response to models.

Scent trials

To find out whether interactive responses increased when scents are added to the models, trials using matt brown life-like models with distilled water (control), female scent and male scent were conducted. The results of the previous experiments showed that dull brown life-like models received more interactive responses from cockroaches and background colour does not affect the level of interactive response, therefore this model was chosen for the scent experiment.

Overall, the total time spent interacting with the models during scent trials was not affected by model scent, nor species independently (Table. 5). However, the interaction between species and model scent was significant, with *A. insignis* interacting similarly with each scent, and *G. oblongonota* spending a greater amount of time interacting with female scented models (Table. 5, Figure. 15a).

The time spent engaging in exploratory interactions significantly differed depending on the scent of the models, with cockroaches spending a greater amount of time conducting exploratory interactions with female scented models compared to male scented and control models (Table. 5). Time spent conducting exploratory interactions was not affected by species independently (Table. 5). However, the interaction between species and model scent was significant, with *A. insignis* spending a similar amount of time conducting exploratory interactions across the scented models, and *G. oblongonota* spending a greater amount of time conducting exploratory interactions with female scented models (Table. 5, Figure. 15b).

The time spent interacting aggressively significantly differed between scented models, with cockroaches spending less time interacting aggressively towards female scented models compared to control and male scented models (Table. 5). Aggressive interaction time did not differ between species independently, nor was there a significant effect of model scent and species interaction on the time spent interacting aggressively (Table. 5). There were no significant main effects of model scent or species on the time spent interacting submissively, nor a significant two-way interaction between model scent and species (Table. 5).

The results showed both heightened exploratory and lower aggressive interactions, though time spent interacting with the models varied between *A. insignis* and *G. oblongonota*. However, the overall response was not changed by

Table 5. General linear mixed effects analysis (GLMM) of the time spent interacting with female scented, male scented and distilled water scented models, species (*A. insignis*, *G. oblongonota*), paired interaction and the interaction between species and scented models. Significant effects are highlighted in bold. Data had been log-transformed (natural) to normalise the distribution.

Interaction	Term	Chi-squared	df	p
Total time spent interacting	Scent	4.857	2	0.088
	Species	0.549	1	0.458
	Scent x Species	6.495	2	0.038
Exploratory interactions	Scent	10.455	2	0.005
	Species	2.625	1	0.105
	Scent x Species	12.203	2	0.002
Aggressive interactions	Scent	12.763	2	0.001
	Species	3.078	1	0.079
	Scent x Species	4.313	2	0.115
Submissive interactions	Scent	4.461	2	0.107
	Species	2.047	1	0.152
	Scent x Species	0.606	2	0.738

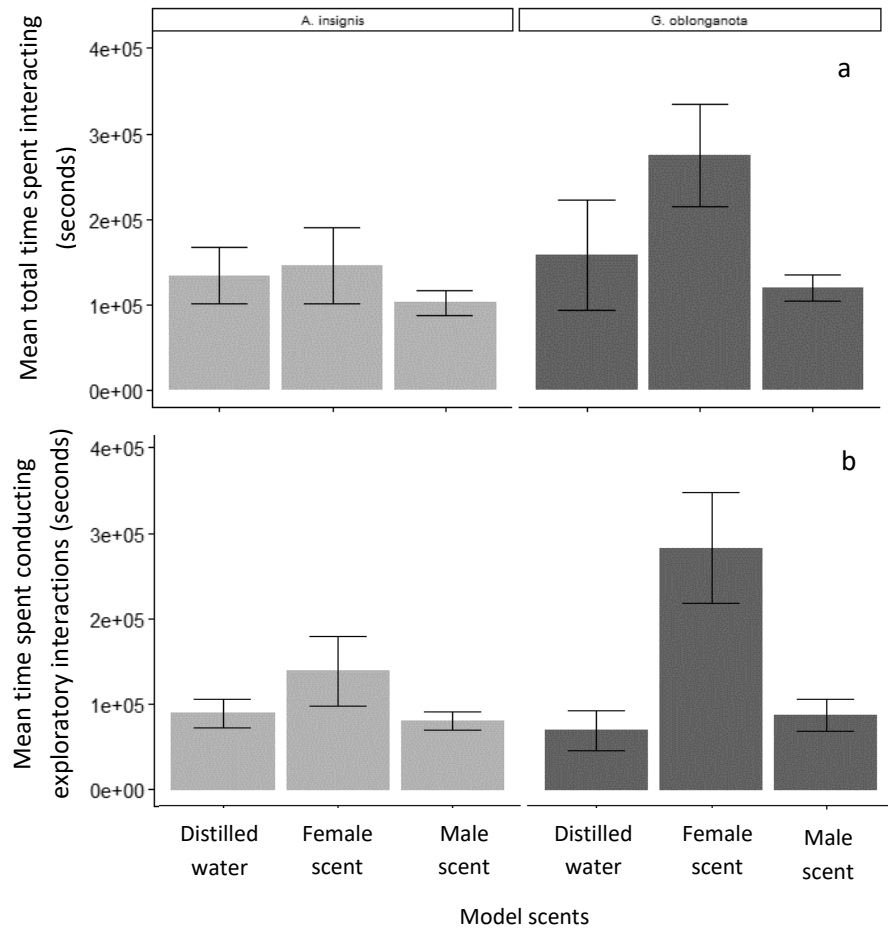


Figure. 15 (a) Mean total time spent interacting and (b) time conducting exploratory interactions between distilled water, female and male scented models for both *A. insignis* and *G. oblonganota* males. Error bars represent ± 1 SE.

the scents, suggesting this method does not increase male cockroach response to the models and will not be added to the models in the two-way choice experiments.

Weaponry manipulation: female choice

When *G. oblongonota* females were presented a two-way choice of a standard-size horned model and a short horned model they had no preference for models they approached (small $n=12$, standard-sized $n=11$) or interacted with (small $n=10$, standard-size $n=12$) ($X^2=0.043$, $df=1$, $p=0.834$; $X^2=0.181$, $df=1$, $p=0.663$).

Females interacted more with small horned models (Mean= 74680 seconds SEM= 23472.956) than the standard-size horned models (Mean= 69075 seconds SEM= 11488.448), but there was no preference of total interaction time for either model (intercept only model: estimate= 0.125, SE= 0.107, $t=1.174$, $p=0.252$) (Figure. 16a). When presented a two-way choice of a standard-size horned model and a long horned model, females had no preference for the type of model they approached (large $n=11$ standard-sized $n=11$) or interacted with (large $n=11$, standard-sized $n=10$) ($X^2=0$, $df=1$, $p=1$; $X^2=0.047$, $df=1$, $p=0.827$). Females tended to interact with large horned models (Mean= 53381 seconds SEM= 11937.864) more than standard-size horned models (Mean= 37472 seconds SEM= 9741.894), but there was no difference in total interaction time for either model (intercept only model: estimate= -0.093, SE= 0.108, $t=-0.863$, $p=0.397$) (Figure. 17a).

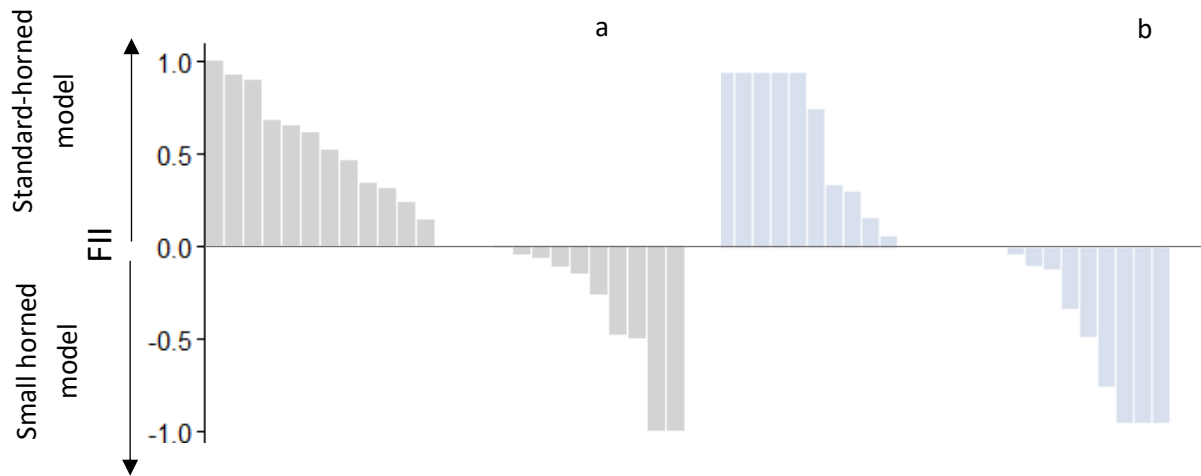


Figure. 16 Female interaction preference in two-way choice experiments for (a) *G. oblongonota* and (b) *A. insignis*. Values are plotted in rank order. Female interaction index (FII) values indicate the rate of interaction directed towards standard-horned models in relation to interaction directed to small horned models. Positive values= standard-horn preference. Negative values= small horn preference. Values are plotted in rank order. Each bar represents one two-way choice trial.

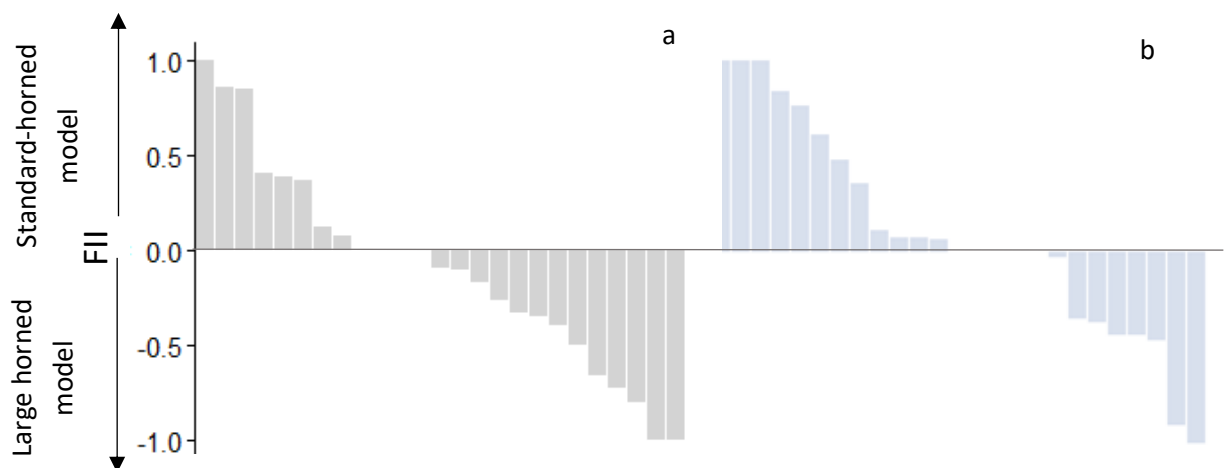


Figure. 17 Female interaction preference in two-way choice experiments for (a) *G. oblongonota* and (b) *A. insignis*. Female interaction index (FII) values indicate the rate of interaction directed towards standard-horned models in relation to interaction directed to large horned models. Positive values= standard-horn preference. Negative values= large horn preference. Values are plotted in rank order. Each bar represents one two-way choice trial.

When *A. insignis* was presented a two-way choice of a standard-size horned (and shield) and short horned models they had no preference for the type of model they approached (small $n=11$, standard-sized $n=8$) or interacted with (small $n=11$, standard-sized $n=8$); ($X^2=0.473$, $df=1$, $p=0.491$). Females tended to interact with standard-size horned models (Mean= 25191 seconds SEM= 13039.304) more than small horned models (Mean= 12169 seconds SEM= 3214.419), but there was no preference of total interaction time for either model (intercept only model: estimate= 0.017, SE= 0.127, $t=56$, $p=0.581$) (Figure. 16b). When presented a two-way choice of a standard-size horned model and a long horned model, females again had no preference for models they approached (large $n=13$ standard-sized $n=9$) or interacted with (large $n=11$, standard-sized $n=9$) ($X^2=0.727$, $df=1$, $p=0.393$; $X^2=0.2$, $df=1$, $p=0.654$). Females tended to interact with standard-size horned models (Mean= 23128 seconds SEM= 6598.247) more than long horned models (Mean= 19622 seconds SEM= 4363.442), but there was no preference of total interaction time for either model (intercept only model: estimate= 0.095, SE= 0.119, $t=0.855$, $p=0.401$) (Figure. 17b).

Overall females approached and interacted with both standard-sized and small-horned models and standard-sized and large horned models the same amount of times and did not show a preference to horn size.

Weaponry manipulation: male response

Body size did not affect total time *G. oblongonota* males spent interacting for both the long vs standard horn size (ANOVA: $F=6.278$, $df=2,2$ $p=0.137$) and the short vs standard horn size two-way choice experiments (ANOVA: $F=1.965$, $df=$

2,2, $p=0.337$). Body size also did not affect *A. insignis* males' interactions for both the long vs standard horn size (ANOVA: $F=2.333$, $df=2,2$ $p=0.299$) and short vs standard horn size two-way choice experiments (ANOVA: $F=12.741$, $df=2,2$, $p=0.072$).

Both *A. insignis* and *G. oblongonota* males were presented with either a standard-size horned model and a short-horned model, or a standard-size horned model and a long-horned model. When *G. oblongonota* was presented a two-way choice of a standard-size horned model and a short horned model they had no preference for the type of model that they approached (small $n=15$, standard-sized $n=7$) or interacted with (small $n=14$, standard-sized $n=8$) ($X^2=2.909$, $df=1$, $p=0.088$; $X^2=1.636$, $df=1$, $p=0.201$). Males interacted with both the small horned models (Mean= 61696 seconds SEM= 11884.535) and standard-size horned models (Mean= 52978 seconds SEM= 14284.934), but there was no preference of total interaction time for either model (intercept only model: estimate= -0.16, SE= 0.139, $t=-1.151$, $p=0.261$) (Figure. 18a). When presented a two-way choice of a standard-size horned model and a long horned model, males had no preference for the type of model that they approached (large $n=11$ standard-sized $n=8$) or interacted with (large $n=13$, standard-sized $n=7$) ($X^2=0.473$, $df=1$, $p=0.493$; $X^2=1.8$, $df=1$, $p=0.179$). Males again interacted with both large horned models (Mean= 37297 seconds SEM= 12755.71) and

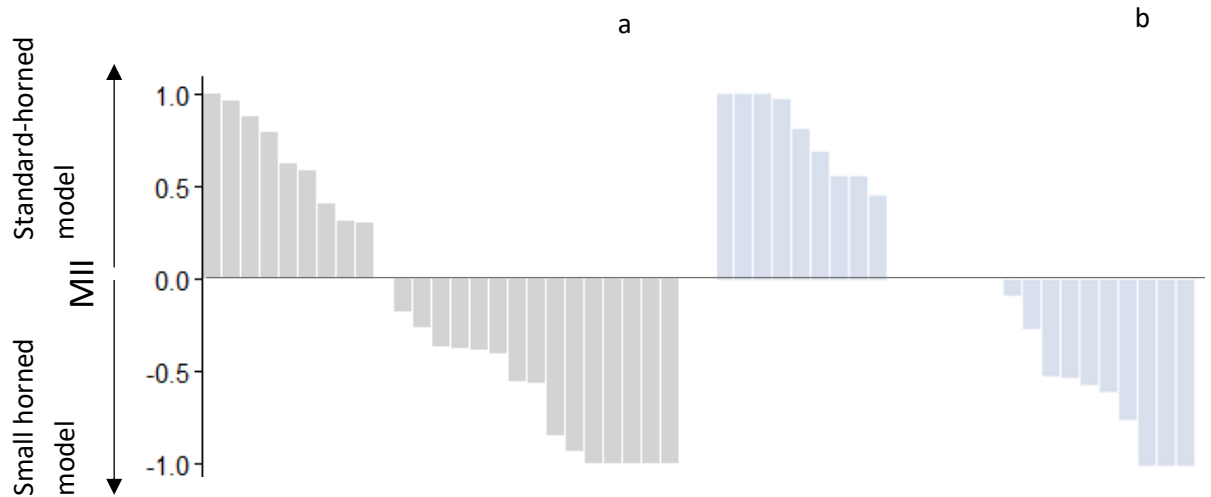


Figure. 18 Male interaction preference in two-way choice experiments for (a) *G. oblongonota* and (b) *A. insignis*. Male interaction index (MII) values indicate the rate of interaction directed towards standard-horned models in relation to interaction directed to small horned models. Positive values= standard-horn preference. Negative values= small horn preference. Values are plotted in rank order. Each bar represents one two-way choice trial.

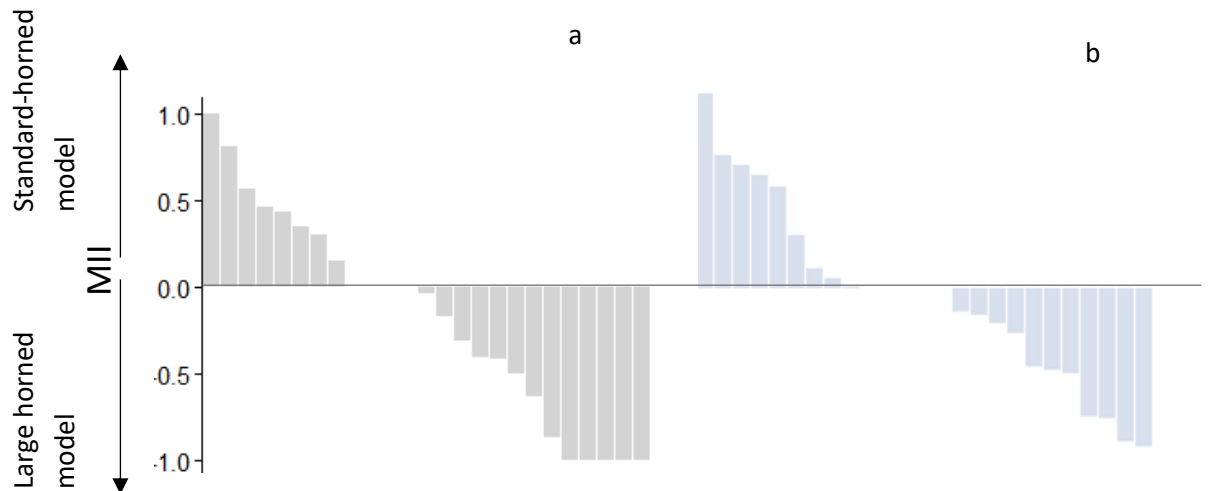


Figure. 19 Male interaction preference in two-way choice experiments for (a) *G. oblongonota* and (b) *A. insignis*. Male interaction index (MII) values indicate the rate of interaction directed towards standard-horned models in relation to interaction directed to large horned models. Positive values= standard-horn preference. Negative values= large horn preference. Values are plotted in rank order. Each bar represents one two-way choice trial.

standard-size horned models (Mean= 33487 seconds SEM= 8149.367), but had no preference in terms of total interaction time for either model (intercept only model: estimate = -0.17, SE= 0.128, $t = -1.406$, $p = 0.172$) (Figure. 19a). When *A. insignis* males were presented a two-way choice of a standard-size horned (and shield) models and a short-horned model they had no preference for models they approached (small $n = 11$, standard-sized $n = 6$) or interacted with (small $n = 9$, standard-sized $n = 10$) ($X^2 = 1.47$, $df = 1$, $p = 0.225$; $X^2 = 0.052$, $df = 1$, $p = 0.818$). Males interacted with both small horned (Mean= 14746 seconds SEM= 5207.47) and standard-size horned models (Mean= 16361 seconds SEM= 6524.769), with no preference of total interaction time for either model (intercept only model: estimate= 0.028, SE= 0.133, $t = 0.211$, $p = 0.834$) (Figure. 18b). When presented a two-way choice of a standard-size horned models and a long horned models, males had no preference for models they approached (large $n = 12$, standard-sized $n = 8$) or interacted with (large $n = 11$, standard-sized $n = 9$) ($X^2 = 0.8$, $df = 1$, $p = 0.371$; $X^2 = 0.2$, $df = 1$, $p = 0.654$). Males interacted with large horned models (Mean= 32155 seconds SEM= 5783.23) and standard-size horned models (Mean= 27116 seconds SEM= 12648.046), with no preference of total interaction time for either model (intercept only model: estimate= -0.007, SE= 0.094, $t = -0.394$, $p = 0.697$) (Figure. 19b).

Overall males approached and interacted with all models the same amount of times and did not show a preference to horn size when presented with a choice of models to interact with.

Post-copulatory trait investment

Hissing cockroaches significantly differed in horn length (ANOVA: $F = 4.226$, $df = 4, 12$, $p = 0.023$; Tukey multiple comparison test: $p < 0.05$) with *G. oblongonota* having significantly longer horns than *A. insignis*, which had the smallest horns (see Table. 6) Relative testes mass significantly differed amongst the hissing cockroach species (ANOVA: $F = 3.448$, $df = 4, 12$, $P = 0.042$; Tukey multiple comparison test: $p < 0.05$), with *E. javanica* having significantly larger relative testes mass and *G. oblongonota* having significantly smaller relative testes mass in relation to one another (see Table. 6). Copulation time between hissing cockroach species did not differ (ANOVA: $F = 2.968$, $df = 4, 12$, $p = 0.064$), suggesting it takes a similar amount of time to transfer a spermatophore over to a female regardless of the species. Total spermatophore weight significantly differed between the hissing cockroach species, with *A. insignis* having significantly lighter spermatophores and *G. oblongonota* and *G. portentosa* producing the heaviest spermatophores (ANOVA: $F = 5.703$, $df = 4, 12$, $p = 0.008$; Tukey multiple comparison test: $p < 0.05$, see Table. 6), but ampulla weight, where the sperm is stored, does not differ between the species (ANOVA: $F = 0.998$, $df = 4, 12$, $p = 0.445$). The number of sperm produced in a single mating differed significantly across hissing cockroach species, with *A. insignis* producing the least amount of sperm and *E. javanica* producing the greatest amount of sperm (ANOVA: $F = 4.507$, $df = 4, 12$, $p = 0.018$, see Table. 6). The coefficient of variance between male's sperm for each species were compared to see if variation within species differed significantly across species. All coefficient of variance for acrosome, head, tail and total length of sperm did not differ

Table 6. Post-copulatory trait characteristics and horn length means for five hissing cockroach species. Means followed by the same letter do not differ significantly (Tukey multiple comparison test, $p > 0.05$). RTM = Relative testes mass. SD= standard deviation. SE= standard error.

Trait	Species		Means	SD	SE
Horn length (mm)	<i>A. insignis</i>	B	2.024	1.109	0.277
	<i>G. oblongonota</i>	A	5.434	0.322	0.107
	<i>G. portentosa</i>	AB	3.635	1.32	0.412
	<i>P. vanwaerebeki</i>	AB	5.093	2.339	0.779
	<i>E. javanica</i>	AB	2.197	0.322	0.107
RTM (g)	<i>A. insignis</i>	AB	0.00731	0.001	0.0004
	<i>G. oblongonota</i>	B	0.00506	0.0007	0.0002
	<i>G. portentosa</i>	AB	0.00965	0.003	0.0005
	<i>P. vanwaerebeki</i>	AB	0.00737	0.001	0.0005
	<i>E. javanica</i>	A	0.0106	0.001	0.0003
Total spermatophore weight (g)	<i>A. insignis</i>	B	0.0135	0.005	0.001
	<i>G. oblongonota</i>	A	0.055	0.032	0.0109
	<i>G. portentosa</i>	A	0.0512	0.003	0.0009
	<i>P. vanwaerebeki</i>	AB	0.0383	0.011	0.003
	<i>E. javanica</i>	AB	0.0183	0.0008	0.002
Sperm number	<i>A. insignis</i>	B	2950833	277306.8	69326.71
	<i>G. oblongonota</i>	AB	3307778	470275.7	156758.58
	<i>G. portentosa</i>	AB	4054167	829468.3	201367.06
	<i>P. vanwaerebeki</i>	AB	3205556	645749.6	215249.88
	<i>E. javanica</i>	A	4951111	1041160.3	347053.44
Sperm acrosome length (μm)	<i>A. insignis</i>	A	2.193	0.968	0.242
	<i>G. oblongonota</i>	AB	2.936	1.12	0.373
	<i>G. portentosa</i>	AB	3.276	0.357	0.089
	<i>P. vanwaerebeki</i>	AB	3.2	0.109	0.036
	<i>E. javanica</i>	B	4.301	0.968	0.242
Sperm head length (μm)	<i>A. insignis</i>	B	20.938	2.196	0.549
	<i>G. oblongonota</i>	A	25.836	3.548	1.182
	<i>G. portentosa</i>	AB	22.217	0.872	0.218
	<i>P. vanwaerebeki</i>	B	20.18	1.455	0.485
	<i>E. javanica</i>	AB	21.678	2.196	0.549

between species (ANOVA: $F = 2.511$, $df = 4,12$, $p = 0.097$; $F = 1.409$, $df = 4,12$, $p = 0.285$; $F = 2.012$, $df = 4,12$, $p = 0.156$; $F = 0.887$, $df = 4,12$, $p = 0.501$). Therefore, species variation will not affect any between species analysis on sperm morphology characteristics. Sperm acrosome length did differ significantly between hissing cockroach species with *E. javanica* having a longer acrosome than *A. insignis* which had the shortest acrosome (ANOVA: $F = 3.739$, $df = 4,12$, $p = 0.033$, Tukey multiple comparison test: $p < 0.05$, see Table. 6). Sperm head length also differed across hissing cockroach species with *G. oblongonota* having the longer head in comparison to *A. insignis* and *P. vanwaerebeki* which had the shortest sperm head lengths (ANOVA: $F = 3.779$, $df = 4,12$, $p = 0.032$, Tukey multiple comparison test: $p < 0.05$, see Table. 6). Sperm total length and tail length did not differ between each of the hissing cockroach species (ANOVA: $F = 0.719$, $df = 4,12$, $p = 0.595$; $F = 0.647$, $df = 4$, $p = 0.639$).

There was no relationship between male weight, horn length or copulation time with any of the sperm characteristics (acrosome length, head length, tail length and total length) (see Table.7). Sperm number was not related to male body weight, horn length or copulation time (see Table. 7). There was also no relationship between total spermatophore weight or ampulla weight with male body size, male horn length or copulation time (see Table. 7). Male body size, horn length and copulation time did not relate to relative testes mass (see Table. 7). This suggests time spent copulating between species does not affect any post-copulatory characteristics and male horn length does not have any trade-offs with the post copulatory traits. This also shows that male weight did not affect

Table. 7 PGLS model estimates for relationships between sperm characteristics and male traits across five species of hissing cockroaches with body size as a covariate. SE= standard error. λ was set at best estimate.

Response variable	Coefficient	Estimate	SE	t- value	P- value
Acrosome length $\lambda = 1$	Male weight	-0.662	0.402	-1.646	0.347
	Horn length	0.85	0.764	1.113	0.465
	Copulation time	-0.042	0.192	-0.217	0.863
	Intercept	4.868	2.636	1.846	0.315
Head length $\lambda = 1$	Male weight	1.972	1.201	0.896	0.534
	Horn length	-1.766	4.182	-0.422	0.745
	Copulation time	0.623	1.055	0.59	0.66
	Intercept	7.531	14.42	0.522	0.693
Tail length $\lambda = 1$	Male weight	6.121	3.588	1.706	0.337
	Horn length	-13.948	6.817	-2.045	0.289
	Copulation time	2.449	1.720	1.424	0.389
	Intercept	254.065	23.507	10.807	0.058
Total sperm length $\lambda = 1$	Male weight	8.909	6.235	1.428	0.388
	Horn length	-16.823	11.848	-1.419	0.39
	Copulation time	3.218	2.989	1.076	0.476
	Intercept	2.63.201	40.852	6.442	0.098
Sperm number $\lambda = 1$	Male weight	-594975	598077	-0.994	0.501
	Horn length	724880	1136419	0.637	0.638
	Copulation time	-89210	206746	-0.311	0.808
	Intercept	6275990	3918358	1.601	0.355
Total spermatophore weight $\lambda = 1$	Male weight	0.008	0.005	1.499	0.374
	Horn length	0.005	0.02	0.265	0.763
	Copulation time	-0.002	0.006	-0.389	0.763
	Intercept	0.011	0.084	0.139	0.911
Ampulla weight $\lambda = 1$	Male weight	-0.003	0.001	-2.085	0.284
	Horn length	0.005	0.003	1.482	0.377
	Copulation time	-0.001	0.0008	-1.59	0.357
	Intercept	0.04	0.012	3.328	0.185
RTM $\lambda = 1$	Male weight	-0.002	0.0004	-5.689	0.11
	Horn length	0.002	0.0007	3.585	0.173
	Copulation time	-0.0007	0.0001	-3.941	0.158
	Intercept	0.024	0.002	9.106	0.069

any post-copulatory trait characteristic so any relationships that are found have no relation to the varied body sized of the five species.

When exploring the relationships between sperm morphology characteristics, PGLSR were conducted. Acrosome length showed no relationship with head length (PGLS: estimate= 1.961, SE= 1.402, t= 1.398, p= 0.395, λ = 0.999), tail length (PGLS: estimate= 1.762, SE= 1.256, t= 1.402, p= 0.39, λ = 0.999) or total length of sperm (PGLS: estimate= -1.679, SE= 1.186, t= -1.415, p= 0.391, λ = 0.999, see Table. 8). Therefore, acrosome length is independent of other sperm characteristics. Sperm head length was negatively related to tail length (PGLS: estimate= -0.895, SE= 0.053, t= -16.844, p= 0.037, λ = 0.999, see Table. 8). Sperm with longer tails have shorter heads when controlling for phylogenetic relatedness of species. Sperm head length and tail length were both positively related to total sperm length (PGLS: estimate= 0.852, SE= 0.031, t= -27.341, p= 0.023, λ = 0.999; estimate= 0.949, SE= 0.022, t= -42.723, p= 0.014, λ = 0.999), with longer sperm having longer heads and tails when controlling for phylogenetic relatedness of species (see Table. 8). All sperm morphology characteristics have a strong phylogenetic signal suggesting traits are dependent on phylogenetic relatedness λ = 0.999.

PGLSR were conducted to explore the relationships between sperm morphology characteristics and other post copulatory traits of interest (total spermatophore weight, ampulla weight, relative testes mass and sperm number) when controlling for phylogenetic relatedness of species. Acrosome length was not related to spermatophore total weight, ampulla weight or relative testes mass,

but was positively related to sperm number (see Table. 9, Figure. 20). Total spermatophore weight, ampulla weight, relative testes mass or sperm number were not related to sperm head length (see Table. 9). There was no relationship between tail length and total spermatophore weight, ampulla weight, relative testes mass or sperm number (see Table. 9). Total sperm length was also not related to total spermatophore length, ampulla length, relative testes mass or sperm number (see Table. 9). Overall no sperm characteristic length related to relative testes mass or total spermatophore weight.

Table. 8 PGLS correlations for relationships between sperm characteristics across five species of hissing cockroaches. Significant results highlighted in bold.

Variables	Acrosome length	Head length	Tail length	Total length
Acrosome length	-----			
Head length	-0.801	-----		
Tail length	-0.858	-0.941	-----	
Total length	0.837	0.894	0.949	-----

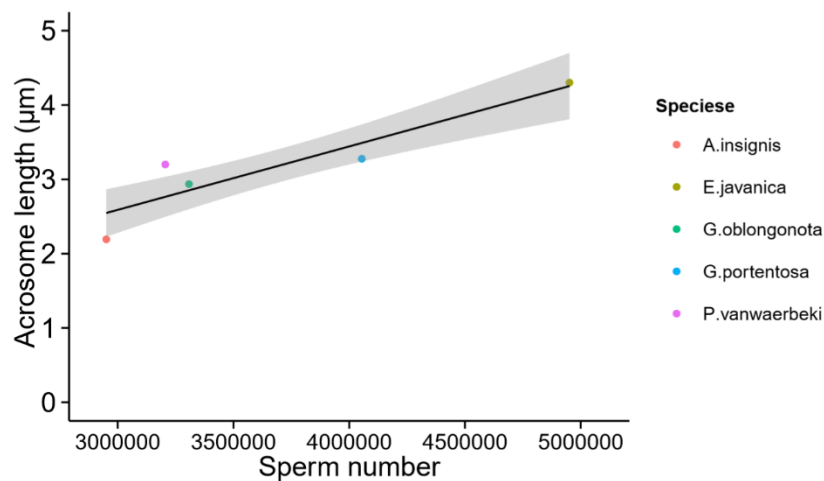


Figure. 20 PGLS Relationship between sperm acrosome length and sperm number of five hissing cockroach species. Line estimate using PGLS data parameters (line equations $y = -0.207 + 0.00008x$).

Table. 9 PGLS model estimates for relationships between sperm characteristics and post copulatory traits across five species of hissing cockroaches. RTM= relative testes mass. SE= standard error. Significant results highlighted in bold. λ was set at best estimate.

Response variable	Coefficient	Estimate	SE	t- value	P- value
Acrosome length	Total spermatophore weight	8.183	29.093	0.825	0.608
	Ampulla weight	-105.155	236.984	-0.443	0.734
	RTM	335.179	370.706	0.904	0.532
	Intercept	1.626	2.302	0.706	0.608
$\lambda = 1$	Sperm number	0.00008	0.000002	-4.438	0.021
	Intercept	-0.207	0.806	-0.257	0.813
Head length	Total spermatophore weight	61.804	11.845	5.217	0.12
	Ampulla weight	-795.538	96.483	-8.25	0.076
	RTM	355.434	150.926	2.355	0.255
	Intercept	28.478	0.937	30.385	0.021
$\lambda = 1$	Sperm number	-0.00001	0.000003	-0.648	0.777
	Intercept	25.465	10.403	2.447	0.091
Tail length	Total spermatophore weight	-291.892	143.617	-2.032	0.291
	Ampulla weight	-2120.132	1169.844	-2.325	0.258
	RTM	2845.347	1829.947	1.554	0.363
	Intercept	304.617	11.362	26.805	0.023
$\lambda = 1$	Sperm number	0.0001	0.0000005	0.0214	0.984
	Intercept	282.001	21.421	13.164	0.0009
Sperm total length	Total spermatophore weight	-226.585	168.215	-1.346	0.406
	Ampulla weight	-4188.072	702.898	-5.958	0.105
	RTM	3819.254	1691.518	2.257	0.265
	Intercept	341.868	12.495	27.36	0.023
$\lambda = 1$	Sperm number	0.0001	0.0000007	-0.157	0.885
	Intercept	313.902	27.965	11.224	0.001

Discussion

Hissing cockroaches were found to interact with 3D models as a conspecific.

Although, females and males did not have any preference of model interaction choice between manipulated weaponry in two-way choice experiments, suggesting horns as a signal alone are not related to male or female choice.

When comparing post-copulatory traits and horn length, *A. insignis*, *G.*

oblongonota and *E. javanica* had the greatest differences between species.

Comparing sperm morphology and other post copulatory traits between the five species of hissing cockroaches, sperm total length was positively correlated with head and tail length, although sperm head length was negatively correlated with tail length across species. There was also a positive relationship between sperm acrosome length and sperm number. Though, most traits were similar across species, suggesting these species may have similar levels of post-copulatory sperm competition. These results start to build a picture of mating strategies and trait investment across hissing cockroach species.

Model stimuli experiments

G. oblongonota and *A. insignis* were chosen for the model and two-way choice experiments due to their opposing strategies and visual characteristics, based on the work of Durrant *et al.* (2016). Throughout the colour experiments, both *A. insignis* and *G. oblongonota* interacted more with the 3D laser sintered life-like model compared to the control, suggesting that both species potentially recognise the model as a conspecific. *A. insignis* and *G. oblongonota* also spent a

greater amount of time interacting using exploratory behaviours such as antennation and contact, aggressively by walking over and butting, and submissively by retreating and adopting a low posture more frequently with the life-like model compared to the control. Matt brown or shiny brown models, both of which mimic cockroach cuticle colour, received greater amounts of interaction generally, with white models receiving the least amount of attention. This difference in response could be a visual problem with the perception of the cockroaches, as experiments were conducted on a white background, potentially making it harder for the cockroaches to identify the white model and control. Hissing cockroaches rely less on sight and more on chemical and mechanical senses to view their environment; therefore, the contrast between model colour and background may affect their visual perception (Dixon *et al.* 2015). However, having large compound eyes, hissing cockroaches do have some sense of sight and can use low level light to detect and see their environment (Mishra *et al.* 2008).

The importance of background contrast was tested by exploring interactions with white and matt brown models on black and white backgrounds. Background colour did not affect cockroach interactions with the models, and matt brown life-like models were interacted with more than white life-like models or control models. Therefore, it is unlikely that background colour affected cockroach interactions. When cockroaches moult to their next instar, the cockroach becomes white and vulnerable before the cuticle has hardened. In this state, other cockroaches tend to avoid these individuals (pers. obs.). Male *A. insignis*

and *G. oblongonota* may associate white life-like models with a recently moulted cockroach, which could explain these interactions. Submissive and aggressive interactions were rarely displayed throughout all trials. Visual cues alone may not threaten the male due to the model being stationary. Therefore, there is no need for males to act in a submissive or challenging manner as the model is not interacting with them. These results reveal that hissing cockroaches do recognise and interact with models as conspecifics. Generally, the matt brown life-like models, that mimic the hissing cockroach colour, provoked the greatest level of interaction. Models have been used on a range of taxa to answer a multitude of questions about specific traits to complex behaviours, including the response to visual cues in male aggression (Dziwaczynski & Leopard, 2010), as well as a range of other behaviours from mate choice to predatory behaviour (Rebora *et al.* 2004; Fan *et al.* 2009; Heinen-Kay *et al.* 2014; Locatello *et al.* 2015; Ferrante *et al.* 2017). Tinbergen (1951) used models ranging from life-like to circular shapes with and without red colouration to look at visual response to colour and shape in conspecific recognition and male aggression in the three-spine stickleback, *Gasterosteus aculeatus*. He found that colouration alone signalled male aggression as males acted aggressively towards any model shape with red colouration. There is less use of models on invertebrates and how they respond to models compared to other taxa, although model response by invertebrates is generally positive (Parish & Fowler, 1983; Frantsevich & Mokrushov, 1984; Gorb, 1998; Rutowski *et al.* 2001; Domingue *et al.* 2011). 3D printed models of emerald ash borers *Agilus planipennis*, attract female and more so males just as well as live beetles when displayed in traps compared to empty control traps, suggesting

models are perceived just as well as live individuals as conspecifics to both sexes of these species (Domingue *et al.* 2014). Models could provide a better alternative to dead or live insects as they have the potential to be perceived by individuals in the same way as conspecifics, creating an easy alternative to collecting live or dead specimens.

An issue that can arise with model stimuli, is that individuals may become habituated as the model cannot interact with the subject. If the subject spends a long time with the stimulus, it may start to behave differently to it or completely ignore it. Habituation can also change preference of traits, as the subject loses interest due to long exposure (Rowland, 2000). This can be seen in guppies *Poecilia reticulata* where females are exposed to males of the same colour pattern. Females lose interest in those males and prefer males with differing colour patterns. The first colour preference then increased again once the female is left without males of any colour (Daniel *et al.* 2019).

In the scent experiments, cockroaches spent less time interacting aggressively and a greater amount of time conducting exploratory interactions with female scented models. This is most likely due to the female scent, causing males to make contact and antennate with the female-scented model. As aggression is provoked by male-male competition, males may not feel challenged by female scent whereas visual cues of the model alone or with a male scent provokes an aggressive response. Sex specific scents are likely to create different signals to a male individual; therefore, males are likely to act differently with females. During courtship *G. portentosa* females produce sex pheromones that initially attract

the male (Fraser & Nelson, 1984). It is likely the extracted female scent has some attractive quality that could explain the increase in exploratory behaviour of the males with female scented models.

Scent adds another signal to the model to become a multi-modal signal of both visual and olfactory cues, which could create a stronger interactive response to the model, as cockroaches have two signals to interpret and act upon (Ward & Mehner, 2010; KonDo *et al.* 2012). Another explanation could be that scent is stronger than visual signals, therefore interactions are strongly influenced by scent over the visual model, hence why interaction towards the model changes despite its visual appearance as a male (Dixon *et al.* 2015). This change in response shows that adding scent provokes a greater level of response in *G. oblongonota* and could be applied to other questions related to scent-based cues. Scent play a varied role in cockroaches and other insects providing signals for a range of different messages from sex and age, group aggregation, male quality, dominance and kin recognition (Fraser & Nelson, 1984; Roux *et al.* 2002; Lihoreau & Rivault, 2008; Hamilton *et al.* 2019). Female *G. portentosa* have been found to discriminate between male and female scent as well as determine age and dominance rank of males through scent cues, preferring the scent of mature dominant males, using scent as a cue for mate choice (Leibensperger *et al.* 1985).

Although the results showed both heightened exploratory and lower aggressive interactions, time spent interacting with the models varied between *A. insignis* and *G. oblongonota*, with *G. oblongonota* displaying greater interaction times towards the male scented models. *A. insignis* spent a similar amount of time

interacting with male scented models, female scented models and the controls. Therefore, the response was not changed by scents, suggesting scent does not heighten response to models in this species. Alternatively, the water-based method used to extract the scent may have caused the lack of increased response from the hissing cockroaches. Roux *et al.* (2009) found that using water-based removal and application of hydrocarbons were quantitatively and qualitatively similar to solvent based removal and application. However, cuticular hydrocarbons act as water repellent compounds and due to their properties are not soluble in water, which could result in very little extraction success (Drijfhout *et al.* 2009). Studies generally use hexane-based methods of extracting cuticular hydrocarbons, which is more reliable, as well as using gas chromatography-mass spectrometry to analyse the level of cuticular hydrocarbons extracted (Turillazzi *et al.* 2010; Gerhardt *et al.* 2015; Inui *et al.* 2015). Using a solvent based method, i.e. hexane instead of water, is likely to have a greater chance of extracting cuticular hydrocarbons, additionally using gas chromatography-mass spectrometry to test the levels of cuticular hydrocarbons extracted would show if the method was successful.

Scent was not added to the models for the two-way choice experiments as the aim was to investigate visual signals alone.

Female trait preference

The overall results of the two-way choice tests suggest both *A. insignis* and *G. oblongonota* females do not have a preference regarding horn size. Females approached and interacted with both standard-sized and small-horned models

and standard-sized and large horned models the same amount of times. Total interaction times also did not differ between each two-way choice experiment. These results suggest females may not use horn length as a quality indicator trait. In nature, many armaments used for male-male competition are not a selective trait for the female (Gross, 1996; Wong & Candolin, 2005; Rios-Cardenas & Morris, 2011; McCulloch & Simmons, 2016). It's most likely that hissing cockroach horns are purely a weapon and are used to outcompete opponents in physical male-male competitions and are not assessed by the female when choosing a mate.

Horns are generally used as a weapon in hissing cockroach species, with males using their horns to butt and flip, dominating their opponents to gain access to females (Schal *et al.* 1984; Durrant *et al.* 2016; Hunsinger *et al.* 2017). Horn function can be easily observed in the long-horned cockroach *G. oblongonota*, but not so much in the flat horned cockroach *A. insignis* which differ in their investment in horn length. *G. oblongonota* invests more in horn length than testes mass and *A. insignis* invests more in testes mass than horn size. These two species also behave very differently when competing with other males. *G. oblongonota* regularly engage in physical male-male competition and *A. insignis* relying more on hissing than engaging in physical combat (Durrant *et al.* 2016). *A. insignis* have greatly reduced horns compared to other hissing cockroach species, whereby they appear as small flat mounds rather than horns. Interestingly this species also has a pronotal shield that is flatter, more ornate and protrudes out slightly at either side of the head. This ornate pronotal shield along with male-

male competitive strategy of auditory hissing rather than physical engagement makes it hard to assume its purpose is competition related, though the shield is known to be used to push opponents and objects (Durrant *et al.* 2016; Hunsiger *et al.* 2017).

The results do not suggest any female preference in horn size and shield size alone. However, male weaponry can be indicative of other traits such as body size that allow the female to assess the males current condition or potential genetic quality. Many sexually selected traits tend to be isometric with body size, so that larger individuals have proportionately larger ornaments or armaments (Tseng & Rowe, 1999; Bonduriansky, 2007). However, sexually selected traits can also be positively allometric, where larger individuals have larger than expected ornaments, or can be negatively allometric (Bonduriansky, 2007; Painting & Holwell, 2013; McCullough *et al.* 2015; Voje, 2016). These patterns tend to emerge when there is a trade-off between traits and traits are affected by genetic constraints, when traits are honest signals. In the heliconia bug *Leptoscelis tricolor*, males have a positive allometric relationship between body size and weaponised hind legs, where larger males produce heavier hind legs for their body size compared to smaller males (Somjee *et al.* 2018). Weaponised hind legs are costly to produce, but the cost is reduced in larger males allowing them to produce larger than expected legs.

In our experiments body size was not only controlled but only large male models were presented to females. If medium and small individuals with the same manipulated weaponry were also shown to females, we could assess whether

weaponry in relation to body size, body size alone or exaggerated weaponry is a selective trait. Acoustic hisses used in courtship may also be linked to horn and pronotal shield size and females may use this acoustic signal to interpret the male physical features or quality (Frazer & Nelson, 1984; Clark & Moore, 1995a; Logue *et al.* 2009). Concentrating more on pronotal morphological complexity and size, manipulating body size or adding mating hiss acoustics could help break down and understand what signals are related to female choice and understand the role of these traits, particularly pronotal shield morphological complexity.

Male response to manipulated weaponry

Both *A. insignis* and *G. oblongonota* males showed no difference in preference between models with manipulated and standard-sized horns, suggesting males may not assess other males based on horn length. It is likely horn length is only a small part of the signal males use to assess their opponent or themselves before deciding whether to challenge another male. Live males were categorized into small, medium, and large individuals, to see if their size affected their choice of model to interact with. There was no difference in either species for interaction times between small, medium or large males and the models. When a male interacts with a model, the model is stationary and does not respond as a live opponent might, which may have caused the male to stop interacting.

Movement of models using robotics has been found to evoke a greater response than static visual signals of stationary models, in species where males physically interact and conduct behavioural displays, such as frogs (Preininger *et al.* 2013; Stange *et al.* 2016) and lizards (Clark *et al.* 2019; Frohnwieser *et al.* 2019). When

Allobates femoralis frog models have added movement, male aggression and the probability of attack increase compared to static models, and any movement is enough to elicit an attack (Luna *et al.* 2010). Without movement the model may not trigger strong or prolonged aggressive behaviour.

Usually armaments are a signal to show competitors their strength and fitness without physical engagement or help the organism in combat. In male-male competition, assessment strategies can play an important part in contests. They can allow an individual to decide whether they are physically matched, assess their opponents in a mutual assessment or assess the individual's own abilities in a self-assessment (Kelly, 2006; Elias *et al.* 2008; Schnell *et al.* 2015; Wofford *et al.* 2015). This is beneficial as it can be costly to engage in contest behaviour, particularly if the odds are against them. Having multiple signals can aid in these decisions through increasing the response to one or both signals when displayed together (signal enhancement), or by providing the same information through multiple signals to backup that signal information (signal conformation), helping to better assess the odds of beating an opponent and to work out the most beneficial course of action (Johnstone, 1996; Cooper & Goller, 2004; Hebets & Papaj, 2005; Uetz *et al.* 2009).

It is likely that both *A. insignis* and *G. oblongonota* produce multimodal signals that males use to assess opponents, such as body size, hisses and visual displays e.g. abdomen thrashing (Schal *et al.* 1984; Durrant *et al.* 2016; Hunsinger *et al.* 2017). If medium and small models with the same manipulated weaponry were also presented to the males, we could assess whether weaponry in relation

to body size, body size alone or exaggerated weaponry are signals in male-male competition. Antagonistic hisses used during male-male competition may also be linked to weaponry size or body size, and males may use this acoustic signal to interpret the opponents physical traits or strength. In *G. portentosa* individuals hiss during male-male competition. Larger males produce lower frequency, longer hisses that cause smaller males to retreat (Clark & Moore, 1995b). Hiss duration and frequency can communicate male size that the males use to assess who is larger without physical contact. Only closely matched opponents engage in physical competition to determine who is dominant (Clark & Moore, 1994; 1995a; 1995b).

It is likely males could not assess and interact with the models in a similar way to live individuals due to missing multicomponent signals. Stomatopod *Neogonadactylus oerstedii* males have complex multimodal signals comprised of chemical, and visual cues that they display during contest, both these cues are used to assess their opponents fighting ability before deciding whether to engage in physical combat (Franklin *et al.* 2016). When either cue is taken away the decision response on whether to engage in offensive behaviours and speed of approach into competition changes. This is because males do not have all the information required to make an informed decision about its competitor and each signal separately only give part of the information required to make a beneficial decision.

Post-copulatory trait investment

When comparing post-copulatory traits and horn length, the results showed both differences and similarities between hissing cockroach species. Horn length differed between the species with *A. insignis* having the most reduced horns and *G. oblongonota* having the longest horns. *A. insignis* horn reduction may have a link to environmental factors such as habitat, as *A. insignis* look the most different from the other species, having a dorsoventrally flattened body shape and are broad laterally, making them lower to the ground. Not much is known about any hissing cockroach species' natural habitat or the densities they live in (Clark & Moore, 1995a; Clark & Moore, 1995b; Sueur & Aubin, 2006). However, if *A. insignis* live in enclosed and narrow habitats, large horns may prevent them from fitting in to these spaces (Goodman *et al.* 2008; Tomkins *et al.* 2011). *A. insignis* and *G. oblongonota* are quite distantly related with *A. insignis* the second most distant of the 5 species and *G. oblongonota* the most recent of the species along with *P. vanwaerebeki*. This phylogenetic gap may have some influence on the difference in horn length we see *G. oblongonota* invest heavily in horn length at a cost to testes mass, using their horns when engaging in male-male competitions (Durrant *et al.* 2016). *A. insignis* males engage less in physical competition and more in hissing to win male-male interactions and invest heavily in testes mass at a cost to horn size (Durrant *et al.* 2016). This suggests the difference in horn size is linked to the reproductive strategy of the species. In *Onthophagus* beetles, some males have horns on their heads, while others do not. Horned males often use their horns to physically attack others during male-male competition, while those without horns prefer to sneak copulations and

avoid competition. These different strategies relate to the horn phenotype of the beetles (Moczek & Emlen, 2000). This is most likely what is happening here with these two hissing cockroach species, where weaponry is only maintained at larger sizes, when it is used as a competitive strategy. Two species of closely related porcelain crabs *Petrolisthes spinifrons* and *Petrolisthes mitra* have different reproductive tactics like that of the hissing cockroaches, with *P. mitra* using male-male competition to gain access to females and *P. spinifrons* trying to copulate with females as the opportunity arises. Due to these differing strategies, *P. mitra*, which engages in male-male competition, have larger weapons, whereas *P. spinifrons* have reduced weapons (Baeza & Asorey, 2012).

Amongst the hissing cockroach species relative testes mass (RTM) was the most reduced in *G. oblongonota*, this is consistent with the findings of Durrant *et al.* (2016), where *G. oblongonota* invest heavily in horn size resulting in reduced testes mass due to the trade-off between the two traits. This trade off occurs due to a limited amount of investment in resources between pre-copulatory and post-copulatory traits and the level of pre-copulatory competition a male faces, when winning mostly relies on pre-copulatory trait investment (Parker *et al.* 2013).

Interestingly *E. javanica* had the greatest RTM producing similarly weighted testes to the other species, though their body size is 45% smaller than the next largest species, *A. insignis*. In the yellow dung fly, *Scathophaga stercoraria*, males faced with intense sperm competition developed heavier testes than those experimentally restricted to monogamy (low sperm competition) (Hosken &

Ward, 2001). *E. javanica* are likely under intense sperm competition to develop their larger testes relative to their body size, and sperm competition is likely to be more extreme in *E. javanica* than other hissing cockroach species, due to their significantly greater RTM. *E. javanica* also produced more sperm than other species and had the longest sperm acrosome length. This investment strategy works under the raffle model, where the more sperm a male provides the greater the likelihood of his sperm fertilising the ova (Parker, 1982). Sperm competition is likely to be intense in this species, with males investing heavily in testes mass and sperm number in order to increase chances of fathering offspring (Gomendio & Roldan, 1991; Schulte-Hostedde & Millar, 2004).

E. javanica horn length in relation to their body size is similar to that of the other hissing cockroach species, with the exception of *A. insignis*, which have reduced horns. This is interesting as it seems there may not be a strong trade-off between RTM and horn length for this species, unlike previous findings in *A. insignis* and *G. oblongonota* (Durrant *et al.* 2016). However, to see whether a trade-off exists in *E. javanica*, within species variation would need to be explored. *E. javanica* are not only the smallest of the species, they are also more colourful, producing yellow and brown banded stripes compared to the duller colouration of other hissing cockroaches and are most distantly related to the other four species. This gap could explain the size and coloration of the cockroach, and its relationship with the other cockroaches may affect its traits depending on if the traits have a strong genetic relation.

Acrosome length was found to be positively related to sperm number. This pattern was quite unexpected as the acrosome is not usually associated with any post-copulatory traits like sperm number or influenced by sperm competition and is usually not included or combined with head length in studies (Johnson & Briskie, 1999; Bennison *et al.* 2008; Hermosell *et al.* 2013). The acrosome function in fertilisation is to initiate a reaction in the zona pellucida membrane to break through the egg's protective barrier so that the sperm may enter. Across Muroid rodents, acrosome integrity calculated by the percentage of the acrosome that was intact pre-fertilisation, was positively correlated with sperm number, suggesting investment in sperm load positively relates to higher-quality sperm and the two trait investments are linked (Gómez-Montoto *et al.* 2011). High numbers of sperm in the oviduct could lead to polyspermy, where multiple sperm try to fertilise the egg at once. This can be a problem in some species, as the zygote contains more copies of chromosomes than required and is often deleterious causing death in the zygote. To prevent polyspermy, species either reverse their electrical charge across the egg's surface once a sperm has fused with the egg or create a chemical barrier around the egg or thicken the existing barrier to prevent sperm from penetrating (Jaffe *et al.* 1982; Primakoff & Myles, 2002; Halet & Wessel, 2004). Acrosome size could relate to acrosome reaction reducing the time it takes for a sperm to penetrate the egg (Eckelbarger, 1984; Familiari *et al.* 2007; Edward *et al.* 2015). Although it is not known if polyspermy is a problem in hissing cockroaches, it could explain why acrosome length is also longer when sperm number is greater.

A. insignis males produced lower quantities of sperm compared to the other species. This is unexpected as *A. insignis* invest heavily in RTM (Durrant *et al.* 2016). RTM usually positively correlates with sperm number (Gomez-Montoto *et al.* 2011). However, this is not the case in *A. insignis*. *A. insignis* sperm also had a shorter sperm acrosome length and sperm head length than other cockroach species. Shorter sperm heads have been associated with more intense sperm competition with shorter heads being linked to longevity of sperm and increased swimming velocity in house sparrow *Passer domesticus*. (Helfenstein *et al.* 2010) and the superb fairy-wren *Malurus cyaneus*. (Calhim *et al.* 2011). It may be that this is true of *A. insignis* sperm and they invest in creating faster and/or longer living sperm rather than in numbers or length, as investment in individual traits is costly. Understanding what benefits *A. insignis* get from investing in testes mass is not clear from this study but could be linked to velocity or longevity of sperm, which requires investigation. *A. insignis* is the second most distantly related of the hissing cockroaches after *E. javanica*, and its relation to the other cockroaches may bare some explanation to the different trait investments not just sexual selective pressures. However, its reduced horn and mating strategies and investment trade-off, suggest sexual selection does play a large role in its differing traits (Durrant *et al.* 2016), but phylogeny may play a greater role in post-copulatory trait investment as post-copulatory traits were found to be highly dependent on phylogenetic relatedness.

Interestingly sperm with longer tails have shorter heads across species when controlling for phylogeny. Generally, head length and tail length are positively

correlated, as longer heads help with sperm swimming velocity (Byrne *et al.* 2003; Gomendio & Roldan, 2008; Mossman *et al.* 2009; Johnson *et al.* 2012). However negative relationships between sperm head and tail length has also been found to be associated with sperm competition and swimming velocity. In Piterian boars *Sus scrofa domesticus*, males that are highly fertile have sperm with shorter head length and longer tail length than males with lower fertilising capacity (Kondracki *et al.* 2012). In house sparrows, *Passer domesticus*, pressure from strong sperm competition results in sperm with short heads and longer tails (Helfenstein *et al.* 2010). These sperm were found to have a greater swimming velocity due to smaller heads causing less drag resulting in faster sperm. This negative relationship is also associated with longevity as shorter sperm were found to live longer (Helfenstein *et al.* 2010). It is likely in these hissing cockroach species that longevity of sperm plays some role in shaping sperm traits as females can store sperm and use it for multiple clutches (pers. obs.). In the cockroach *Diploptera punctata*, females store sperm from a single male and do not mate again until the first batch of eggs are laid. After this, females are willing to mate with a new male. Using irradiated males Woodhead (1985) was able to demonstrate that females store the first male's sperm and use this sperm even after their second mating when producing their second batch of eggs, with one third of the eggs in the second batch being fathered by the first male. Non-virgin female *A. insignis* kept in isolation produced multiple (2-4) litters of offspring throughout the year, suggesting females store and use stored sperm to fertilise eggs over time (pers. obs.). Therefore, sperm with greater longevity would be

beneficial for males that could increase their paternity through utilisation of stored sperm.

Total spermatophore weight differed between the hissing cockroaches with *A. insignis* having the lightest spermatophores and *G. portentosa* and *G. oblongonota* producing the heaviest spermatophores. The weight of *A. insignis* spermatophore as a trait again differed significantly to the other four hissing cockroach species, due to *A. insignis* not producing the extra gelatinous mass around the ampulla. All other species produced a white gelatinous rubbery mass around the ampulla, function currently unknown, that filled the female reproductive pouch. This could be a part of the spermatophore itself to protect the ampulla, or may hold some nutritional benefit to the female or may be a way of preventing another male to mate with the female due to filling the whole pouch, acting as a sperm competition defensive strategy (Moore *et al.* 2004; Mangels *et al.* 2016). Interestingly, ampulla, where the sperm is held, weights did not differ between the five hissing cockroach species. Therefore, it is unlikely that total spermatophore weight has any relation to sperm number itself and suggest species produce a similar amount of sperm to the female and use similar strategies for sperm delivery.

The results also show many traits did not differ between species. Each species spent a similar length of time copulating, and courtship was similar in the way a male hissed and adopted a mating posture to attract a female (Schal *et al.* 1984; Frazer & Nelson, 1984; Leibensperger *et al.* 1985). This hiss became faster-paced and louder as the male tried to attach itself to the female. A few individuals of

each species did not hiss during courtship. Though this is not uncommon, females have been found to prefer males who were hissing over those that did not (Frazer & Nelson, 1984; Clark & Moore, 1995a; Logue *et al.* 2009). Two male *A. insignis* continued to hiss during copulation. This may be a strategy to give the female information about his quality and allow extended copulation times as she processes this information or may be a strategy to warn off rival males (Mountjoy & Lemon, 1996; Gray, 1997; Clark, 2010).

Sperm total length and tail length did not differ between species. This suggests males are likely to be under similar selective pressures. Sperm total length and tail length are commonly associated with pressure of sperm competition, where high sperm competition pressure causes sperm to be longer to swim faster (Gomendio & Roldan, 1991; Fitzpatrick *et al.* 2009; Lüpold *et al.* 2009; Holfenstain *et al.* 2010; Gómez-Montoto *et al.* 2011; Rowe *et al.* 2012). As cockroach species share similar sperm lengths, it's likely that all species are under high sperm competition as inter-sperm morphological variation is low. Male variance of sperm characteristics did not differ between species, which suggests males are facing strong selective pressures. If the individual species differed in sperm competition intensity then we would see a greater variance in those who are under relaxed sperm competition and a smaller variance in those species with greater sperm competition pressures (Parker & Simmons, 2000; Williams *et al.* 2005; Birkhead & Immler, 2006).

Many expected relationships did not appear between species. This could be because hissing cockroach species have similar post-copulatory selection

pressures causing species traits to be similar. It is likely any relationships between traits are within species. Across 83 mammalian species, there were positive associations between RTM and sperm length. However, when correcting for phylogenetic relatedness, there was no relationship between RTM and sperm lengths as sperm length was heavily dependent on phylogeny (Gage & Freckleton, 2002). Immler & Birkhead (2006) looked at the relationship between sperm traits and sperm competition intensity both within and between species of passerines. They found that when looking within individual species there were positive and negative associations between sperm morphology and RTM. However, when looking across species, these relationships did not occur.

In this study, when comparing horn size (a pre-copulatory trait) to RTM (a post-copulatory trait) across species, no patterns were found. However, a study looking at two species of hissing cockroaches found there was a negative relationship between horn length and RTM (Durrant *et al.* 2016). Therefore, this trade-off is likely found within species, but does not differ between species themselves when multiple species are examined. Often when looking across species, patterns within species are not reflected and can be masked (Gage & Freckleton, 2002; Immler & Birkhead, 2006). This could be because of phylogenetic constraints, limiting trait adaptations or local selection rather than generic pressure amongst species as different species may respond to this shared pressure in different ways (Gage & Freckleton, 2002; Badyaev *et al.* 2007; Hoffmann, 2012; Scordato *et al.* 2014; Servedio & Boughman, 2017).

Another explanation for lack of relationships could be that the traits of interest are influenced by another selection pressure such as cryptic female choice or sperm storage (Birkhead *et al.* 2006; Calhim *et al.* 2009). Sperm morphology is strongly influenced by time spent in female storage tubules rather than sperm competition in species of turtles (Zhang *et al.* 2016), Galliformes (Immler *et al.* 2007) and Passerines (Briskie & Montgomerie, 1992). Cockroaches may share similar effects from female storage tubules, as all species have sperm storage tubules present and personal observations have shown females from all species produced multiple batches of offspring (2-4) whilst isolated for over 10 months. Therefore, during this time sperm must face the pressure of surviving the storage tubules over this time period. Across 20 species of passerines researchers found no association between sperm length and sperm competition intensity, except a positive association between tail length and total length (Briskie & Montgomerie, 1992). Similarities were found in the hissing cockroaches, with no association between sperm length and RTM, positive relationships between sperm total length with head and tail length and negative relationship between tail and head length. Briskie & Montgomerie (1992) also found sperm length was positively associated with sperm length and storage tubule length, and the more storage tubules there were the shorter the sperm were across species. Like passerines it may be that some cockroach sperm traits are associated with manner of storage, swimming velocity or sperm longevity, which were not investigated in this study. It is often found when sperm traits are not influenced by sperm competition, they become influenced by cryptic female choice and sperm storage (Eberhard,

1996). It is possible that female storage and cryptic choice could influence post copulatory traits within and between hissing cockroach species.

Conclusion

This study sought to better understand sexual selection in hissing cockroaches, looking at both female choice and post-copulatory sperm competition across hissing cockroach species. As hissing cockroaches responded to models successfully, models produced using 3D laser sintering could potentially be applied to other insect species to look at single and multimodal/multicomponent traits, and address questions about competition and mate choice as well as other behaviours. Colour can be added to the models to look at colour preference and scent can be applied, providing possibilities to look at these stimuli in greater detail in relation to behavioural questions (Stewart *et al.* 2017). Although laser sintered models have rarely been used, they show potential and could provide a greater understanding of specific behaviours in male-male competition, mate choice and species recognition, by manipulating specific traits such as colouration, weaponry and body size to understand their importance. Horn length did not play a part in female choice or male interactions in *G. oblongonota* and *A. insignis* species. However, pre-copulatory trait manipulation of models may be a useful tool with potential to be applied to other insect species to understand the role they may play in mate competition and female choice whilst providing a standardized control of other traits. The role *A. insignis* shields play is still unclear, as neither males nor females interacted differently to the models with manipulated shields. Further work on *A. insignis* pronotal shields as well as

what cues could be used in female choice such as courtship hisses, is needed to understand mate choice in hissing cockroaches and why *A. insignis* pronotal shield shape differs to that of all other hissing cockroach species.

When comparing sperm morphology and other post copulatory traits between the five species of hissing cockroaches, similar patterns in trait investment were found suggesting these species may have similar levels of post-copulatory sperm competition. When controlling for phylogenetic association, relationships often became non-existent. This is often the case for studies that look across a group of species as it can be difficult to explain variation of post-copulatory traits, due to sexual selection pressures being species specific (Gage & Freckleton, 2002). Sexual selection, trait investment and strategies in hissing cockroaches may be better understood by comparing post-copulatory traits within species rather than between species. Ecological data for hissing cockroach species is missing and the density of hissing cockroach wild populations is unknown. This information is required to complete our understanding of what strategies are beneficial for hissing cockroach species.

This study has given an insight into post-copulatory trait investment to understand what mating strategies and sperm competition intensities these species face. It is clear that each species may have similar pressures amongst them. Although some trait investment patterns are shown and many post-copulatory traits differed between *A. insignis*, *G. oblongonota* and *E. javanica*. However, these species are phylogenetically distant from one another, which could play a large role in driving their trait differences, especially as results show

post-copulatory traits were highly phylogenetically influenced. Even so, this information provides an insight into hissing cockroach post-copulatory traits, which until now had not been uncovered. This adds to the growing body of literature (Gage & Freckleton, 2002; Immler & Birkhead, 2006; Kleven *et al.* 2008; Gómez-Montoto *et al.* 2011; Rowe *et al.* 2012; Higginson *et al.* 2012) that looks at sexual selection across species, whilst accounting for phylogenetic relatedness, to better understand if and what patterns form across a group of species, whether any general patterns emerge, and whether pressure and traits evolve among or between species.

References

Ahmad, S., Ghosh, A., Nair, D. and Seshadri, M. (2007). Simultaneous extraction of nuclear and mitochondrial DNA from human blood. *Genes and Genetic Systems*, 82(5): 429-432.

Alcock, J. (1982). Post-copulatory mate guarding by males of the damselfly *Hetaerina vulnerata selys* (Odonata: Calopterygidae). *Animal Behaviour*, 30: 99-107.

Andersson, M. (1986). Evolution of condition-dependent sex ornaments and mating preferences: Sexual selection based on viability differences. *Evolution*, 40(4): 804.

Anderson, M. and Dixson, A. (2002). Motility and the midpiece in primates. *Nature*, 416(6880): 496-496.

Arnquist, G. and Rowe, L. (2002). Correlated evolution of male and female morphologies in water striders. *Evolution*, 56: 936-947.

Aron, S., Lybaert, P., Baudoux, C., Vandervelden, M. and Fournier, D. (2016). Sperm production characteristics vary with level of sperm competition in *Cataglyphis* desert ants. *Functional Ecology*, 30(4): 614-624.

Backwell, P., Christy, J., Telford, S., Jennions, M. and Passmore, J. (2000). Dishonest signalling in a fiddler crab. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267(1444): 719-724.

Badyaev, A., Hill, G., Stoehr, A., Nolan, and McGraw, K. (2007). The evolution of sexual size dimorphism in the house finch. II. Population divergence in relation to local selection. *Evolution*, 54(6): 2134-2144.

Baeza, J. and Asorey, C. (2011). Testing the role of male-male competition in the evolution of sexual dimorphism: a comparison between two species of porcelain crabs. *Biological Journal of the Linnean Society*, 105(3): 548-558.

Barber, I., Arnott, S., Braithwaite, V., Andrew, J. and Huntingford, F. (2001). Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1462): 71-76.

Bartolini, T., Mwaffo, V., Showler, A., Macrì, S., Butail, S. and Porfiri, M. (2016). Zebrafish response to 3D printed shoals of conspecifics: the effect of body size. *Bioinspiration and Biomimetics*, 11(2): 026003.

Bateman, A. J. (1948). Intrasexual selection in *Drosophila*. *Heredity*, 2: 349–368.

Bee, M., Perrill, S. and Owen, P. (2000). Male green frogs lower the pitch of acoustic signals in defence of territories: a possible dishonest signal of size? *Behavioral Ecology*, 11(2): 169-177.

Bennison, C., Hemmings, N., Brookes, L., Slate, J. and Birkhead, T. (2016). Sperm morphology, adenosine triphosphate (ATP) concentration and swimming velocity: unexpected relationships in a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, 283(1837): 20161558.

Bennison, C., Hemmings, N., Slate, J. and Birkhead, T. (2015). Long sperm fertilize more eggs in a bird. *Proceedings of the Royal Society B: Biological Sciences*, 282(1799): e20141897.

Benvenuto, C., Knott, B. and Weeks, S. (2009). Mate-guarding behaviour in clam shrimp: a field approach. *Behavioral Ecology*, 20(5): 1125-1132.

Berglund, A., Bisazza, A. and Pilastro, A. (1996). Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society*, 58(4): 385-399.

- Birkhead, T. and Immler, S. (2007). Making sperm: Design, quality control and sperm competition. *Society of Reproduction and Fertility Supplement*, 65: 175–81.
- Birkhead, T., Immler, S., Pellatt, E. and Freckleton, R. (2006). Unusual sperm morphology in the Eurasian bullfinch (*Pyrrhula pyrrhula*). *The Auk*, 123(2): 383.
- Blengini, C., Naretto, S., Cardozo, G., Giojalas, L. and Chiaraviglio, M. (2016). Relationship between pre- and post-copulatory traits in *Salvator rufescens* (*Squamata: Teiidae*). *Biological Journal of the Linnean Society*, 119(4): 932-942.
- Bonduriansky, R. (2007). Sexual selection and allometry: A critical reappraisal of the evidence and ideas. *Evolution*, 61(4): 838-849.
- Bourguignon, T., Tang, Q., Ho, S., Juna, F., Wang, Z., Arab, D., Cameron, S., Walker, J., Rentz, D., Evans, T. and Lo, N. (2018). Transoceanic dispersal and plate tectonics shaped global cockroach distributions: evidence from mitochondrial phylogenomics. *Molecular Biology and Evolution*, 35(4): 970-983.
- Brennan, P., Prum, R., McCracken, K., Sorenson, M., Wilson, R. and Birkhead, T. (2007). Coevolution of male and female genital morphology in waterfowl. *PLoS ONE*, 2(5): e418.
- Briskie, J. and Montgomerie, R. (1992). Sperm size and sperm competition in birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 247(1319): 89-95.
- Bulté, G., Chlebak, R., Dawson, J. and Blouin-Demers, G. (2018). Studying mate choice in the wild using 3D printed decoys and action cameras: a case of study of male choice in the northern map turtle. *Animal Behaviour*, 138: 141-143.
- Byrne, P., Simmons, L. and Dale Roberts, J. (2003). Sperm competition and the evolution of gamete morphology in frogs. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1528): 2079-2086.

- Calhim, S., Double, M., Margraf, N., Birkhead, T. and Cockburn, A. (2011). Maintenance of sperm variation in a highly promiscuous wild bird. *PLoS ONE*, 6(12): e28809.
- Calhim, S., Lampe, H., Slagsvold, T. and Birkhead, T. (2009). Selection on sperm morphology under relaxed sperm competition in a wild passerine bird. *Biology Letters*, 5(1): 58-61.
- Callander, S., Kahn, A., Maricic, T., Jennions, M. and Backwell, P. (2013). Weapons or mating signals? Claw shape and mate choice in a fiddler crab. *Behavioral Ecology and Sociobiology*, 67(7): 1163-1167.
- Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews*, 78: 575-595.
- Clark, D.C. (2010). Male mating success in the presence of a conspecific opponent in the Madagascar hissing cockroach, *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *Ethology*, 104(10): 877-888.
- Clark, D.C. and Moore, A.J. (1994). Genetic aspects of communication during male- male competition in the Madagascar hissing cockroach: Honest signalling of size. *Heredity*, 75: 198-205.
- Clark, D.C and Moore, A.J. (1995a). Social communication in the Madagascar hissing cockroach: features of male courtship hiss and a comparison of courtship and antagonistic hisses. *Behaviour*, 132(5-6): 401-417.
- Clark, D.C and Moore, A.J. (1995b). Variation and repeatability of male agonistic hiss characteristics and their relationship to social rank in *Gromphadorhina portentosa*. *Animal Behaviour* 50: 719-729.
- Clark, D., Macedonia, J., Rowe, J., Austin, M., Centurione, I. and Valle, C. (2019). Galápagos lava lizards (*Microlophus bivittatus*) respond dynamically to displays from interactive conspecific robots. *Behavioral Ecology and Sociobiology*, 73(10): e1-10.

Cooper, B. and Goller, F. (2004). Multimodal signals: Enhancement and constraint of song motor patterns by visual display. *Science*, 303(5657): 544-546.

Cuthill, I., Stevens, M., Sheppard, J., Maddocks, T., Pàrraga, A. and Troscianko, T. (2005). Disruptive colouration and background matching. *Nature*, 434(7029): 72-74.

Daniel, M., Koffinas, L. and Hughes, K. (2019). Habituation underpins preference for mates with novel phenotypes in the guppy. *Proceedings of the Royal Society B: Biological Sciences*, 286(1902): e20190435.

Darwin, C. (1859). *On the Origin of Species*. London: John Murray.

Dixon, M., Daar, J., Gunnarsson, K., Johnson, M. and Shayter, A. (2015). Stimulus preference and reinforcement effects of the Madagascar hissing cockroach (*Gromphodahina portentosa*): A case of reverse translational research. *The Psychological Record*, 66(1): 41-51.

Domingue, M., Csóka, G., Tóth, M., Véték, G., Péntzes, B., Mastro, V. and Baker, T. (2011). Field observations of visual attraction of three European oak buprestid beetles toward conspecific and heterospecific models. *Entomologia Experimentalis et Applicata*, 140(2): 112-121.

Domingue, M., Pulsifer, D., Lakhtakia, A., Berkebile, J., Steiner, K., Lelito, J., Hall, L. and Baker, T. (2014). Detecting emerald ash borers (*Agrilus planipennis*) using branch traps baited with 3D-printed beetle decoys. *Journal of Pest Science*, 88(2): 267-279.

Drijfhout, F., Kather, R. and Martin, S. (2009). *The role of cuticular hydrocarbons in insects*. In: Zhang, W. and Liu, H. (eds) *Behavioral and chemical ecology*, 91-114.

Durrant, K.L., Skicko, I.M., Sturrock, C. and Mowles, S.L. (2016). Comparative morphological trade-offs between pre- and post-copulatory sexual selection in giant hissing cockroaches (Tribe: *Gromphadorhini*). *Scientific Reports*, 6: e36755.

- Dzieweczynski, T. and Leopard, A. (2010). The effects of stimulus type on consistency of responses to conflicting stimuli in Siamese fighting fish. *Behavioural Processes*, 85(2): 83-89.
- Eady, P., Hamilton, L. and Lyons, R. (2007). Copulation, genital damage and early death in *Callosobruchus maculatus*. *Proceedings of the Royal Society B: Biological Sciences*, 274(1607): 247-252.
- Eberhard, W. (1996). *Female control: Sexual selection by cryptic female choice*. USA: Princeton University Press
- Eckelbarger, K. (1984). Ultrastructure of spermatogenesis in the reef-building polychaete *Phragmatopoma lapidosa* (Sabellariidae) with special reference to acrosome morphogenesis. *Journal of Ultrastructure Research*, 89(2): 146-164.
- Edward, D., Stockley, P. and Hosken, D. (2015). Sexual conflict and sperm competition. *Cold Spring Harbor Perspectives in Biology*, 7(4): a017707.
- Elias, D., Kasumovic, M., Punzalan, D., Andrade, M. and Mason, A. (2008). Assessment during aggressive contests between male jumping spiders. *Animal Behaviour*, 76(3): 901-910.
- Emlen, D. (2008) The evolution of animal weaponry. *Annual Review of Ecology, Evolution Systematics*, 39: 387-413.
- Evans, M. and Hatchwell, B. (1992). An experimental study of male adornment in the scarlet-tufted malachite sunbird: II. The role of the elongated tail in mate choice and experimental evidence for a handicap. *Behavioral Ecology and Sociobiology*, 29(6): 421-427.
- Familiari, G., Heyn, R., Relucenti, M. and Sathananthan, H. (2007). Structural changes of the zona pellucida during fertilization and embryo development. *Frontiers in Bioscience*, 13(17): 6730- 6751.

- Fan, C., Yang, E. and Tso, I. (2009). Hunting efficiency and predation risk shapes the colour-associated foraging traits of a predator. *Behavioral Ecology*, 20(4): 808-816.
- Ferrante, M., Barone, G. and Lövei, G. (2017). The carabid *Pterostichus melanarius* uses chemical cues for opportunistic predation and saprophagy but not for finding healthy prey. *BioControl*, 62(6): 741-747.
- Fisher, R.A. (1915). The evolution of sexual preference. *Eugenics Review*, 7: 184–192.
- Fisher, R.A. (1958). The genetical theory of natural selection. 2nd eds. New York: HardPress Publishing.
- Fitzpatrick, J., Montgomerie, R., Desjardins, J., Stiver, K., Kolm, N. and Balshine, S. (2009). Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proceedings of the National Academy of Sciences*, 106(4): 1128-1132.
- Forsgren, E., Karlsson, A. and Kvarnemo, C. (1996). Female sand gobies gain direct benefits by choosing males with eggs in their nests. *Behavioral Ecology and Sociobiology*, 39(2): 91-96.
- Franklin, A., Marshall, N. and Lewis, S. (2016). Multimodal signals: Ultraviolet reflectance and chemical cues in stomatopod agonistic encounters. *Royal Society Open Science*, 3(8): e160329.
- Frantsevich, L. and Mokrushov, P. (1983). Visual stimuli releasing attack of a territorial male in *Sympetrum* (Anisoptera: Libellulidae). *Odonatologica*, 13(3): 335-350.
- Frith, D. and Frith, C. (1987). Courtship display and mating of the superb bird of paradise *Lophorina superb*. *Austral Ornithology*, 88(3): 183-188.
- Firman, R. and Simmons, L. (2009). Experimental evolution of sperm quality via postcopulatory sexual selection in house mice. *Evolution*, 64(5): 1245-56.

Frohnwieser, A., Pike, T., Murry, J. and Wilkinson, A. (2019). Perception of artificial conspecifics by bearded dragons (*Pogona vitticeps*). *Integrative Zoology*, 14(2), pp.214-222.

Frazer, J. and Nelson, M.C. (1984) Communication in the courtship of a Madagascan hissing cockroach. I. Normal courtship. *Animal Behaviour* 32(1): 194-203.

Gage, M. and Freckleton, R. (2003). Relative testis size and sperm morphometry across mammals: No evidence for an association between sperm competition and sperm length. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1515): 625-632.

Gerhardt, H., Schmitt, C., Betz, O., Albert, K. and Lammerhofer, M. (2015). Contact solid-phase microextraction with uncoated glass and polydimethylsiloxane-coated fibers versus solvent sampling for the determination of hydrocarbons in adhesion secretions of Madagascar hissing cockroaches *Gromphadorrhina portentosa* (Blattodea) by gas chromatography-mass spectrometry. *Journal of Chromatography*, 1388: 24-35.

Godwin, J., Vasudeva, R., Michalczyk, Ł., Martin, O., Lumley, A., Chapman, T. and Gage, M. (2017). Experimental evolution reveals that sperm competition intensity selects for longer, more costly sperm. *Evolution Letters*, 1(2): 102-113.

Gomendio, M. and Roldan, E. (1991). Sperm competition influences sperm size in mammals. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 243(1308): 181-185.

Gomendio, M. and Roldan, E. (2008). Implications of diversity in sperm size and function for sperm competition and fertility. *The International Journal of Developmental Biology*, 52(5-6): 439-447.

Gómez-Montoto, L., Magaña, C., Tourmente, M., Martín-Coello, J., Crespo, C., Luque-Larena, J., Gomendio, M. and Roldan, E. (2011). Sperm competition, sperm numbers and sperm quality in Muroid rodents. *PLoS ONE*, 6(3): e18173.

- Goodman, B., Hudson, S., Isaac, J. and Schwarzkopf, L. (2008). The evolution of body shape in response to habitat: is reproductive output reduced in flat lizards? *Evolution*, 63(5): 1279-1291.
- Gorb, S. (1998) Visual cues in mate recognition by males of the damselfly, *Coenagrion puella* (L.) (Odonata: Coenagrionidae). *Journal of insect behaviour*, 11(1): 73-92.
- Gray, D. (1997). Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour*, 54(6): 1553-1562.
- Gross, M. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology and Evolution*, 11(2): 92-98.
- Guilford, T. and Dawkins, M. (1995). What are conventional signals? *Animal Behaviour*, 49(6): 1689-1695.
- Gwynne, D. (1982). Mate selection by female katydids (Orthoptera: Tettigoniidae, *Conocephalus nigropleurum*). *Animal Behaviour*, 30(3): 734-738.
- Haley, S. and Wessel, G. (2004). Regulated proteolysis by cortical granule serine protease 1 at fertilization. *Molecular Biology of the Cell*, 15(5): 2084-2092.
- Hall, M., Kingma, S. and Peters, A. (2013). Male songbird indicates body size with low-pitched advertising songs. *PLoS ONE*, 8(2): e56717.
- Hamilton, J., Wada-Katsumata, A. and Schal, C. (2019). Role of cuticular hydrocarbons in German cockroach (Blattodea: Ectobiidae) Aggregation Behavior. *Environmental Entomology*, 48(3): 546-553.
- Hayakawa, Y. (2007). Parasperm: Morphological and functional studies on nonfertile sperm. *Ichthyological Research*, 54(3): 317-317.
- Hayakawa, Y., Akiyama, R. Munehara, H. and Komaru, A. (2002). Dimorphic sperm influence semen distribution in a non-copulatory sculpin *Hemilepidotus gilberti*. *Environmental Biology of Fishes*, 65(3): 311-317.

- Hebets, E. and Papaj, D. (2004). Complex signal function: developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, 57(3): 197-214.
- Hedrick, A. (1988). Female choice and the heritability of attractive male traits: An empirical study. *The American Naturalist*, 132(2): 267-276.
- Heinen-Kay, J., Morris, K., Ryan, N., Byerley, S., Venezia, R., Peterson, M. and Langerhans, R. (2014). A trade-off between natural and sexual selection underlies diversification of a sexual signal. *Behavioral Ecology*, 26(2): 533-542.
- Helfenstein, F., Podelvin, M. and Richner, H. (2010). Sperm morphology, swimming velocity, and longevity in the house sparrow *Passer domesticus*. *Behavioral Ecology and Sociobiology*, 64(4): 557-565.
- Hermosell, I., Laskemoen, T., Rowe, M., Møller, A., Mousseau, T., Albrecht, T. and Lifjeld, J. (2013). Patterns of sperm damage in Chernobyl passerine birds suggest a trade-off between sperm length and integrity. *Biology Letters*, 9(5): e20130530.
- Higginson, D., Miller, K., Segreaves, K. and Pitnick, S. (2012). Convergence, recurrence and diversification of complex sperm traits in diving beetles (Dytiscidae). *Evolution*, 66(5): 1650-1661.
- Hoffmann, A. (2012). Evolutionary limits and constraints. In J. Losos, eds. *The Princeton Guide to Evolution*. USA: Princeton University Press. 247–52.
- Holldobler, B. (1995). The chemistry of social regulation: multicomponent signals in ant societies. *Proceedings of the National Academy of Sciences*, 92(1): 19-22.
- Holman, L. and Snook, R. (2008). A sterile sperm caste protects brother fertile sperm from female-mediated death in *Drosophila pseudoobscura*. *Current Biology*, 18(4): 292-296.
- Hosken, D. and Ward, P. (2001). Experimental evidence for testis size evolution via sperm competition. *Ecology Letters*, 4(1): 10-13.

- Hunsinger, E., Root-Gutteridge, H., Cusano, D.A. and Parks, S.E. (2017). A description of defensive hiss types in the flat horned hissing cockroach (*Aeluropoda insignis*). *The International Journal of Animal Sound and its Recording*, 27(3): 261-271.
- Huyghe, K., Vanhooydonck, B., Herrel, A., Tadić, Z. and Van Damme, R. (2012). Female lizards ignore the sweet scent of success: Male characteristics implicated in female mate preference. *Zoology*, 115(4): 217-222.
- Igic, B., Nunez, V., Voss, H., Croston, R., Aidala, Z., López, A., Van Tatenhove, A., Holford, M., Shawkey, M. and Hauber, M. (2015). Using 3D printed eggs to examine the egg-rejection behaviour of wild birds. *PeerJ*, 3: e965.
- Iglesias, P. and Hasson, E. (2017). The role of courtship song in female mate choice in South American *Cactophilic Drosophila*. *PLoS ONE*, 12(5): e0176119.
- Immler, S. and Birkhead, T. (2006). Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 274(1609): 561-568.
- Immler, S., Calhim, S. and Birkhead, T. (2008). Increased postcopulatory sexual selection reduces the intramale variation in sperm design. *Evolution*, 62(6): 1538-1543.
- Immler, S., Pitnick, S., Parker, G., Durrant, K., Lupold, S., Calhim, S. and Birkhead, T. (2011). Resolving variation in the reproductive trade-off between sperm size and number. *Proceedings of the National Academy of Sciences*, 108(13): 5325-5330.
- Immler, S., Saint-Jalme, M., Lesobre, L., Sorci, G., Roman, Y. And Birkhead, T. (2007). The evolution of sperm morphometry in pheasants. *Journal of Evolutionary Biology*, 20(3): 1008-1014.

Inui, Y., Shimizu-kaya, U., Okubo, T., Yamsaki, E. and Itioka, T. (2015). Various Chemical Strategies to Deceive Ants in Three *Arhopala* Species (Lepidoptera: Lycaenidae) Exploiting *Macaranga* Myrmecophytes. *PLoS ONE*, 10(4): e0120652.

Inward, D., Beccaloni, G. and Eggleton, P. (2007). Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters*, 3(3): 331-335.

Iyengar, V. and Starks, B. (2008). Sexual selection in harems: male competition plays a larger role than female choice in an amphipod. *Behavioral Ecology*, 19(3): 642-649.

Jaffe, L., Gould-Somero, M. and Holland, L. (1982). Studies of the mechanism of the electrical polyspermy block using voltage clamp during cross-species fertilization. *The Journal of Cell Biology*, 92(3): 616-621.

Johnson, D. and Briskie, J. (1999). Sperm competition and sperm length in shorebirds. *The Condor*, 101(4): 848-854.

Johnson, D., Monro, K. and Marshall, D. (2012). The maintenance of sperm variability: Context-dependent selection on sperm morphology in a broadcast spawning invertebrate. *Evolution*, 67(5): 1383-1395.

Johnstone, R. (1996). Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 351(1337): 329-338.

Johnstone, R. A. (1997). The Evolution of Animal Signals. In: J. Krebs and N. Davies, eds. *Behavioural Ecology: An Evolutionary Approach*. Oxford: Blackwell Science, 155-178.

Kambhampati, S. and Smith, P. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology*, 4(4): 233-236.

- Katsuki, M. and Lewis, Z. (2015). A trade-off between pre- and post-copulatory sexual selection in a bean beetle. *Behavioral Ecology and Sociobiology*, 69(10): 1597-1602.
- Katsuki, M., Yokoi, T., Funakoshi, K. and Oota, N. (2014). Enlarged hind legs and sexual behavior with male-male interaction in *Sagra femorata* (Coleoptera: Chrysomelidae). *Entomological News*, 124(3): 211-220.
- Kelly, C. (2006). Fighting for harems: assessment strategies during male–male contests in the sexually dimorphic Wellington tree weta. *Animal Behaviour*, 72(3): 727-736.
- Keyser, A. and Hill, G. (2000). Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behavioral Ecology*, 11(2): 202-209.
- Klein, B., Stein, J. and Taylor, R. (2012). Robots in the service of animal behaviour. *Communicative and Integrative Biology*, 5(5): 466-472
- Kleven, O., Laskemoen, T., Fossøy, F., Robertson, R. and Lifjeld, J. (2008). Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. *Evolution*, 62(2): 494-499.
- Knapp, R. and Warner, R. (1991). Male parental care and female choice in the bicolour damselfish, *Stegastes partitus*: bigger is not always better. *Animal Behaviour*, 41(5): 747-756.
- Kodric-Brown, A. and Brown, J. (1984). Truth in advertising: The kinds of traits favoured by sexual selection. *The American Naturalist*, 124(3): 309-323.
- Kodric-Brown, A. and Brown, J. (1987). Anisogamy, sexual selection, and the evolution and maintenance of sex. *Evolutionary Ecology*, 1(2): 95-105.
- Kondracki, S., Iwanina, M., Wysokińska, A. and Huszno, M. (2012). Comparative analysis of Duroc and Pietrain boar sperm morphology. *Acta Veterinaria Brno*, 81(2): 195-199.

- KonDo, Y., Naka, H. and Tsuchida, K. (2012). Pheromones and body coloration affect mate recognition in the Japanese nine-spotted moth *Amata fortunei* (Lepidoptera: Arctiidae). *Journal of Ethology*, 30(2): 301-308.
- Kozak, E. and Uetz, G. (2016). Cross-modal integration of multimodal courtship signals in a wolf spider. *Animal Cognition*, 19(6): 1173-1181.
- Lailvaux, S., Hathway, J., Pomfret, J. and Knell, R. (2005). Horn size predicts physical performance in the beetle *Euoniticellus intermedius* (Coleoptera: Scarabaeidae). *Functional Ecology*, 19(4): 632-639.
- Laurinyecz, B., Vedelek, V., Kovács, A., Szilasi, K., Lipinski, Z., Slezák, C., Darula, Z., Juhász, G. and Sinka, R. (2019). Sperm-Leucylaminopeptidases are required for male fertility as structural components of mitochondrial paracrystalline material in *Drosophila melanogaster* sperm. *PLOS Genetics*, 15(2): e1007987.
- Leibensperger, L.B., Traniello, J.F.A. and Fraser, J.M. (1985). Olfactory cues used by female *Gromphadorhina portentosa* (Dictyoptera: Blaberidae) during mate choice. *Annals of the Entomological Society of America*, 78(5): 629-634.
- Lihoreau, M. and Rivault, C. (2008). Kin recognition via cuticular hydrocarbons shapes cockroach social life. *Behavioral Ecology*, 20(1): 46-53.
- Lindström, K. and Lundström, J. (2000). Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. *Behavioral Ecology and Sociobiology*, 48(1): 44-51.
- Lisboa, C., Bajer, K., Pessoa, D., Huber, M. and Costa, G. (2017). Female Brazilian whiptail lizards (*Cnemidophorus ocellifer*) prefer males with high ultraviolet ornament reflectance. *Behavioural Processes*, 142: 33-39.
- Locatello, L., Pizzolon, M. and Rasotto, M. (2012). One trait, many signals: Different information on male quality is enclosed within the same trait in a blenny fish. *Naturwissenschaften*, 99(10): 863-867.

- Locatello, L., Poli, F. and Rasotto, M. (2015). Context-dependent evaluation of prospective mates in a fish. *Behavioral Ecology and Sociobiology*, 69(7): 1119-1126.
- Logue, D.M., Mishra, S., McCaffrey, D., Ball, D. and Cade, W.H. (2009). A behavioural syndrome linking courtship behaviour towards males and females predicts reproductive success from a single mating in the hissing cockroach, *Gromphadorhina portentosa*. *Behavioural Ecology*, 20: 781-788.
- Luna, G., Hödl, W. and Amézquita, A. (2010). Colour, size and movement as visual subcomponents in multimodal communication by the frog *Allobates femoralis*. *Animal Behaviour*, 79(3): 739-745.
- Lüpold, S., Calhim, S., Immler, S. and Birkhead, T. (2009). Sperm morphology and sperm velocity in passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 276(1659): 1175-1181.
- Lüpold, S., Tomkins, J., Simmons, L. and Fitzpatrick, J. (2014). Female monopolization mediates the relationship between pre- and postcopulatory sexual traits. *Nature Communications*, 5(3184).
- Malo, A., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A. and Roldan, E. (2006). Sperm design and sperm function. *Biology Letters*, 2(2): 246-249.
- Mangels, R., Tsung, K., Kwan, K. and Dean, M. (2016). Copulatory plugs inhibit the reproductive success of rival males. *Journal of Evolutionary Biology*, 29(11): 2289-2296.
- McCullough, E., Ledger, K., O'Brien, D. and Emlen, D. (2015). Variation in the allometry of exaggerated rhinoceros beetle horns. *Animal Behaviour*, 109: 133-140.
- McCullough, E. and Simmons, L. (2016). Selection on male physical performance during male–male competition and female choice. *Behavioral Ecology*, 27(5): 1288-1295.

McDermott, D.R., Chips, M.J., McGuirk, M., Armagost, F., DiRienzo, N. and Pruitt, J.N. (2014). Boldness is influenced by sublethal interactions with predators and is associated with successful harem infiltration in Madagascar hissing cockroaches. *Behavioural Ecology and Sociobiology*, 68: 425-435.

Miller CW. (2013). Sexual selection: male-male competition. In J. Losos, eds. *The Princeton Guide to Evolution*. USA: Princeton University Press. 641–46.

Mishra, M. and Meyer-Rochow, V. (2008). Fine structural description of the compound eye of the Madagascar ‘hissing cockroach’ *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *Insect Science*, 15(2): 179-192.

Moczek, A. and Emlen, D. (2000). Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Animal Behaviour*, 59(2): 459-466.

Modig, A. (1996). Effects of body size and harem size on male reproductive behaviour in the southern elephant seal. *Animal behaviour*, 51(6): 1295-1306.

Moore, A. (1990). The inheritance of social dominance, mating behaviour and attractiveness to mates in male *Nauphoeta cinerea*. *Animal Behaviour*, 39(2): 388-397.

Moore, P., Harris, W., Montrose, V., Levin, D. and Moore, A. (2004). Constraints on evolution and postcopulatory sexual selection: Trade-offs among ejaculate characteristics. *Evolution*, 58(8): 1773-1780.

Moore, J., Obbard, D., Reuter, C., West, S. and Cook, J. (2009). Male morphology and dishonest signalling in a fig wasp. *Animal Behaviour*, 78(1): 147-153.

Morina, D., Demarais, S., Strickland, B. and Larson, J. (2018). While males fight, females choose: Male phenotypic quality informs female mate choice in mammals. *Animal Behaviour*, 138: 69-74.

Mossman, J., Slate, J., Humphries, S. and Birkhead, T. (2009). Sperm morphology and velocity are genetically codetermined in the zebra finch. *Evolution*, 63(10): 2730-2737.

Mountjoy, D. and Lemon, R. (1996). Female choice for complex song in the European starling: a field experiment. *Behavioral Ecology and Sociobiology*, 38(1): 65-71.

Muff, S., Signer, J. and Fieberg, J. (2019). Accounting for individual-specific variation in habitat-selection studies: Efficient estimation of mixed-effects models using Bayesian or frequentist computation. *Journal of Animal Ecology*, doi.org/10.1111/1365-2656.13087.

Murphy, C. (1998). Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution*, 52(1): 8-18.

Naretto, S., Cardozo, G., Blengini, C. and Chiaraviglio, M. (2013). Sexual selection and dynamics of jaw muscle in *Tupinambis* lizards. *Evolutionary Biology*, 41(2): 192-200.

Nessler, S., Uhl, G. and Schneider, J. (2007). Genital damage in the orb-web spider *Argiope bruennichi* (Araneae: Araneidae) increases paternity success. *Behavioural Ecology*, 18: 174-181.

Oneal, E., Connallon, T. and Knowles, L. (2007). Conflict between direct and indirect benefits of female choice in desert *Drosophila*. *Biology Letters*, 3(1): 29-32.

Pai, A. and Yan, G. (2002). Female mate choice in relation to heterozygosity in *Tribolium castaneum*. *Journal of Evolutionary Biology*, 15(6): 1076-1082.

Painting, C. and Holwell, G. (2013). Exaggerated trait allometry, compensation and trade-offs in the New Zealand giraffe weevil (*Lasioryhynchus barbicornis*). *PLoS ONE*, 8(11): e82467.

Parrish, M. and Fowler, H. (1983). Contrasting foraging related behaviours in two sympatric wasps (*Vespula maculifrons* and *V. germanica*). *Ecological Entomology*, 8(2): 185-190.

Parker, G. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45(4): 525-567.

Parker, G. (1982). Why are there so many tiny sperm? sperm competition and the maintenance of two sexes. *Journal of Theoretical Biology*, 96(2): 281–94.

Parker, G., Ball, M. A., Stockley, P. and Gage, M. J. G. (1997). Sperm competition games: A prospective analysis of risk assessment. *Proceedings of the Royal Society of London B: Biological Sciences*, 264(1389): 1793–1802.

Parker, G., Lessells, C. and Simmons, L. (2013). Sperm competition games: a general model for precopulatory male-male competition. *Evolution*, 67(1): 95-109.

Parker, G. and Simmons, L. (2000). Optimal copula duration in yellow dung flies: Ejaculatory duct dimensions and size-dependent sperm displacement. *Evolution*, 54(3): 924.

Pfefferle, D. and Fischer, J. (2006). Sounds and size: identification of acoustic variables that reflect body size in hamadryas baboons, *Papio hamadryas*. *Animal Behaviour*, 72(1): 43-51.

Pitnick, S., Brown, W. and Miller, G. (2001). Evolution of female remating behaviour following experimental removal of sexual selection. *Proceedings of the Royal Society B: Biological Sciences*, 268(1467): 557-563.

Pitnick, S. and Markow, T. (1994). Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. *Proceedings of the National Academy of Sciences*, 91(20): 9277-9281.

Pizzari, T. and Birkhead, T. (2000). Female feral fowl eject sperm of subdominant males. *Nature*, 405(6788): 787-789.

- Primakoff, P. and Myles, D. (2002). Penetration, adhesion, and fusion in mammalian sperm-egg interaction. *Science*, 296(5576): 2183-2185.
- Preininger, D., Boeckle, M., Freudmann, A., Starnberger, I., Sztatecsny, M. and Hödl, W. (2013). Multimodal signaling in the small torrent frog (*Micrixalus saxicola*) in a complex acoustic environment. *Behavioral Ecology and Sociobiology*, 67(9): 1449-1456.
- Prokop, Z., Michalczyk, Ł., Drobniak, S., Herdegen, M. and Radwan, J. (2012). Meta-analysis suggests choosy females get sexy sons more than “good genes”. *Evolution*, 66(9): 2665-2673.
- Rebora, M., Piersanti, S. and Gaino, E. (2004). Visual and mechanical cues used for prey detection by the larva of *Libellula depressa* (Odonata Libellulidae). *Ethology Ecology and Evolution*, 16(2): 133-144.
- Reber, S., Janisch, J., Torregrosa, K., Darlington, J., Vliet, K. and Fitch, W. (2017). Formants provide honest acoustic cues to body size in American alligators. *Scientific Reports*, 7(1): e1816.
- Rebora, M., Piersanti, S. and Gaino, E. (2004). Visual and mechanical cues used for prey detection by the larva of *Libellula depressa* (Odonata Libellulidae). *Ethology Ecology and Evolution*, 16(2): 133-144.
- Rodríguez, R., Araya-Salas, M., Gray, D., Reichert, M., Symes, L., Wilkins, M., Safran, R. and Höbel, G. (2014). How acoustic signals scale with individual body size: common trends across diverse taxa. *Behavioral Ecology*, 26(1): 168-177.
- Ronald, K., Fernández-Juricic, E. and Lucas, J. (2018). Mate choice in the eye and ear of the beholder? Female multimodal sensory configuration influences her preferences. *Proceedings of the Royal Society B: Biological Sciences*, 285(1878): e20180713.

- Ronald, K., Zeng, R., White, D., Fernández-Juricic, E. and Lucas, J. (2017). What makes a multimodal signal attractive? A preference function approach. *Behavioral Ecology*, 28(3): 677-687.
- Rouz, E., Sreng, L., Provost, E., Roux, M. and Clement J. (2002). Cuticular hydrocarbon profiles of dominant versus subordinate male *Nauphoeta cinerea* cockroaches. *Journal of Chemical Ecology*, 28(6): 1221-1235.
- Roux, O., Martin, J., Ghomsi, N. and Dejean, A. (2009). A non-lethal water-based removal-reapplication technique for behavioral analysis of cuticular compounds of ants. *Journal of Chemical Ecology*, 35(8): 904-912.
- Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, 58(5): 921-931.
- Rowe, M., Laskemoen, T., Johnsen, A. and Lifjeld, J. (2012). Evolution of sperm structure and energetics in passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 280(1753): e20122616.
- Ruther, J., Sieben, S. and Schricker, B. (2002). Nestmate recognition in social wasps: manipulation of hydrocarbon profiles induces aggression in the European hornet. *Naturwissenschaften*, 89(3): 111-114.
- Rutowski, R., McCoy, L. and Demlong, M. (2001). Visual mate detection in a territorial male butterfly (*Asterocampa leilia*): Effects of distance and perch location. *Behaviour*, 138(1): 31-43.
- Schal, C., Gautier, J.Y. and Bell, W.J. (1984). Behavioural ecology of cockroaches. *Biology Reviews*, 59: 209-254 .
- Schnell, A., Smith, C., Hanlon, R. and Harcourt, R. (2015). Giant Australian cuttlefish use mutual assessment to resolve male-male contests. *Animal Behaviour*, 107: 31-40.
- Schulte-Hostedde, A. and Millar, J. (2004). Intraspecific variation of testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for

sperm competition and reproductive success. *Behavioral Ecology and Sociobiology*, 55(3): 272-277.

Scordato, E., Symes, L., Mendelson, T. and Safran, R. (2014). The role of ecology in speciation by sexual selection: A systematic empirical review. *Journal of Heredity*, 105(1): 782-794.

Sefc, K., Mattersdorfer, K., Ziegelbecker, A., Neuhöttler, N., Steiner, O., Goessler, W. and Kobl Müller, S. (2017). Shifting barriers and phenotypic diversification by hybridisation. *Ecology Letters*, 20(5): 651-662.

Servedio, M. and Boughman, J. (2017). The role of sexual selection in local adaptation and speciation. *Annual Review of Ecology, Evolution, and Systematics*, 48(1): 85-109.

Sentenska, L., Pekàr, S., Michalik, P. and Uhl, G. (2015). Female control of mate plugging in a female-cannibalistic spider (*Micaria sociabilis*). *BMC Evolutionary Biology*. 15(1): 18.

Shahaney, D., Fisk, F. and Parrish, W. (1972). Ultrastructure of spermatozoa of the cockroach, *Pycnoscelus indicus (fabricius)* (Dictyoptera: Blattarta: Blaberidae). *International Journal of Insect Morphology and Embryology*, 1(2): 121-131.

Shutler, D. (2011). Sexual selection: When to expect trade-offs. *Biology Letters*, 7(1): 101-104.

Snook, R. and Karr, T. (1998). Only long sperm are fertilization-competent in six sperm-heteromorphic *Drosophila* species. *Current Biology*, 8(5): 291-294.

Somjee, U., Woods, H., Duell, M. and Miller, C. (2018). The hidden cost of sexually selected traits: the metabolic expense of maintaining a sexually selected weapon. *Proceedings of the Royal Society B: Biological Sciences*, 285(1891): e20181685.

- Stange, N., Page, R., Ryan, M. and Taylor, R. (2017). Interactions between complex multisensory signal components result in unexpected mate choice responses. *Animal Behaviour*, 134: 239-247.
- Stewart, J., Moore, M., Forshee, J., Boyles, K., Harmon, M., Bruck, J. and French, D. (2017). 3D-printed fish models for testing guppy mate choice. In K McMahon, eds. *Proceedings of the 38th Conference of the Association for Biology Laboratory Education (ABLE)*, Houston. 38.
- Stutt, A. and Siva-Jothy, M. (2001). Traumatic insemination and sexual conflict in the bed bug *Cimex lectularius*. *Proceedings of the National Academy of Sciences*, 98(10): 5683-5687.
- Sueur, J. and Aubin, T. (2006). When males whistle at females: complex FM acoustic signals in cockroaches. *Naturwissenschaften*, 93: 500-505.
- Supriya, K., Price, T. and Rowe, M. (2018). Positive correlations between pre- and post-copulatory sexual traits in warblers. *Journal of Avian Biology*, 49(5): doi.org/10.1111/jav.01694.
- Tinbergen, N. (1951). *The Study of Instinct*. Oxford: Clarendon Press.
- Tinbergen, N. (1952). The curious behavior of the stickleback. *Scientific American*, 187: 22-26.
- Tokarz, R. (1985). Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Animal Behaviour*, 33(3): 746-753.
- Tomkins, J., Hazel, W., Penrose, M., Radwan, J. and LeBas, N. (2011). Habitat complexity drives experimental evolution of a conditionally expressed secondary sexual trait. *Current Biology*, 21(7): 569-573.
- Tourmente, M., Rowe, M., González-Barroso, M., Rial, E., Gomendio, M. and Roldan, E. (2013). Postcopulatory sexual selection increases ATP content in rodent spermatozoa. *Evolution*, 67(6): 1838-1846.

- Trivers R. L. (1972). Parental investment and sexual selection. In B. Campbell, eds. *Sexual selection and the descent of man*. Chicago: Aldine. 136–179.
- Tseng, M. and Rowe, L. (1999). Sexual dimorphism and allometry in the giant water strider *Gigantometra gigas*. *Canadian Journal of Zoology*, 77(6): 923-929.
- Tuni, C., Han, C. and Dingemanse, N. (2018). Multiple biological mechanisms result in correlations between pre- and post-mating traits that differ among versus within individuals and genotypes. *Proceedings of the Royal Society B: Biological Sciences*, 285(1885): e20180951.
- Turillazzi, S., Sledge, M. and Moneti, G. (2010). Use of a simple method for sampling cuticular hydrocarbons from live social wasps. *Ethology Ecology and Evolution*. 10(3): 293-297.
- Uetz, G., Roberts, J. and Taylor, P. (2009). Multimodal communication and mate choice in wolf spiders: Female response to multimodal versus unimodal signals. *Animal Behaviour*, 78(2): 299-305.
- Vanpé, C., Kjellander, P., Galan, M., Cosson, J., Aulagnier, S., Liberg, O. and Hewison, A. (2008). Mating system, sexual dimorphism, and the opportunity for sexual selection in a territorial ungulate. *Behavioral Ecology*, 19(2): 309-316.
- Vielle, A., Callemeyn-Torre, N., Gimond, C., Pouillet, N., Gray, J., Cutter, A. and Braendle, C. (2016). Convergent evolution of sperm gigantism and the developmental origins of sperm size variability in *Caenorhabditis* nematodes. *Evolution*, 70(11): 2485-2503.
- Vladić, T. and Järvi, T. (2001). Sperm quality in the alternative reproductive tactics of Atlantic salmon: The importance of the loaded raffle mechanism. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1483): 2375-2381.

- Voituron, Y., Brepson, L., Richardson, C., Joly, P. and Lengagne, T. (2012). Energetics of calling in the male treefrog *Hyla arborea*: When being large means being sexy at low cost. *Behaviour*, 149(7): 775-793.
- Voje, K. (2016). Scaling of morphological characters across trait type, sex, and environment. *The American Naturalist*, 187(1): 89-98.
- Waage, J. (1979). Dual function of the damselfly penis: Sperm removal and transfer. *Science*, 203(4383): 916-918.
- Ward, A. and Mehner, T. (2010). Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. *Behavioral Ecology*, 21(6): 1315-1320.
- Weatherhead, P. and Robertson, R. (1979). Offspring quality and the polygyny threshold: The sexy son hypothesis. *The American Naturalist*, 113(2): 201-208.
- Weaver, R., Koch, R. and Hill, G. (2017). What maintains signal honesty in animal colour displays used in mate choice?. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1724): e20160343.
- Welch, A., Semlitsch, R. and Gerhardt, H. (1998). Call duration as an indicator of genetic quality in male gray tree frogs. *Science*, 280(5371): 1928-1930.
- Whittingham, L., Freeman-Gallant, C., Taff, C. and Dunn, P. (2015). Different ornaments signal male health and MHC variation in two populations of a warbler. *Molecular Ecology*, 24(7): 1584-1595.
- Wilkinson, G. and Reillo, P. (1994). Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 255(1342): 1-6.
- Williams, P., Day, T. and Cameron, E. (2005). The evolution of sperm-allocation strategies and the degree of sperm competition. *Evolution*, 59(3): 492-499.

- Wilkins, M., Shizuka, D., Joseph, M., Hubbard, J. and Safran, R. (2015). Multimodal signalling in the North American barn swallow: a phenotype network approach. *Proceedings of the Royal Society B: Biological Sciences*, 282(1816): e20151574.
- Wofford, S., Earley, R. and Moore, P. (2015). Evidence for assessment disappears in mixed-sex contests of the crayfish, *Orconectes virilis*. *Behaviour*, 152(7-8): 995-1018.
- Wong, B. and Candolin, U. (2005). How is female mate choice affected by male competition? *Biological Reviews*, 80(4): 559-571.
- Woodhead, A. (1985). Sperm mixing in the cockroach *Diploptera punctata*. *Evolution*, 39(1): 159-164.
- Yang, L. and Wang, Q. (2004). Precopulation sexual selection in *Nysius huttoni* white (Heteroptera: Lygaeidae) in relation to morphometric traits. *Journal of Insect Behavior*, 17(5): 695-707.
- Zagatti, P. and Castel, Y. (1987) Courtship behaviour of the false codling moth, *Cryptophlebia leucotreta* (Meyrick): Androconial display and mating success. (Lepidoptera, Tortricidae). *Annales- Societe Entomologique de France*. 23(2): 113-123.
- Zahavi, A. (1975). Mate selection: A selection for a handicap. *Journal of Theoretical Biology*, 53(1): 205-214.
- Zhang, L., Yang, P., Bian, X., Zhang, Q., Ullah, S., Waqas, Y., Chen, X., Liu, Y., Chen, W., Le, Y., Chen, B., Wang, S. and Chen, Q. (2016). Modification of sperm morphology during long-term sperm storage in the reproductive tract of the Chinese soft-shelled turtle, *Pelodiscus sinensis*. *Scientific Reports*, 5: e16096.
- Zhu, B., Wang, J., Zhao, L., Sun, Z., Brauth, S., Tang, Y. and Cui, J. (2016). Bigger is not always better: females prefer males of mean body size in *Philautus odontotarsus*. *PLoS ONE*, 11(2): e0149879.

Appendix

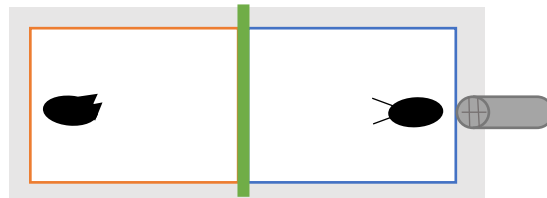


Figure. A Diagram of pilot trail arena. The live cockroach is placed in the blue area and the model in the orange area, the sides alternate randomly to avoid any bias. The barrier highlighted in green is removed once trail commences. Sound recorder stays on the right in front of a mesh covered hole.

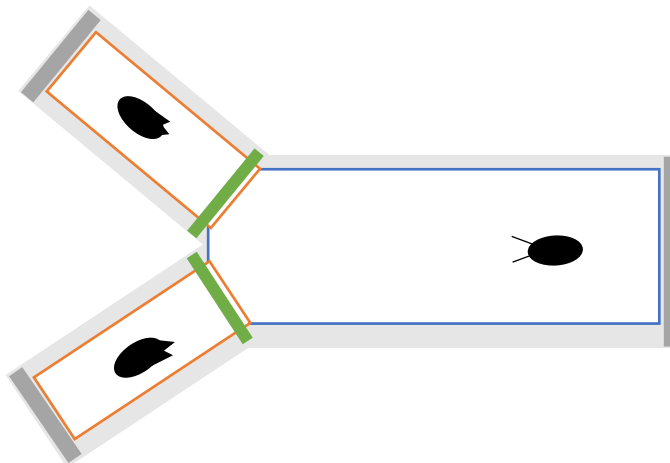


Figure. B Diagram of Y- maize. The live cockroach is placed in the blue area and the two models in the orange areas. The barriers highlighted in green are removed once trail commences.