Testing the disturbance hiss of the Madagascar hissing Cockroach (\textit{Gromphadorhina portentosa}) as an anti-predatory response

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The display of the disturbance hiss by the Madagascar Hissing Cockroach (\textit{Gromphadorhina portentosa}) is considered to be an anti-predatory response despite there being little direct evidence linking the hiss with survival. Many studies have investigated the roles of the aggression and courtship hisses, displayed in this social species, but few have considered the role of the disturbance hiss. This study looked at the stimulus for the disturbance hiss response by placing unfamiliar individuals into different social contexts. A total of 10 male and 10 female \textit{G. portentosa} were kept separately before being placed into four different social situations for 5 min at a time. The individuals were placed into an established colony of all females, an established colony of all males and an established colony of mixed sex \textit{G. portentosa}. The subjects were also placed in the presence of a predator, an Emperor Scorpion (\textit{Pandinus imperator} Koch). All interactions and hisses were recorded both by video and on a sound-recording device. There was a highly significant difference in the setting in which the disturbance hiss was shown and a highly significant difference in the display rates of the disturbance hiss between the sexes in general, with most displays of the disturbance hiss being when introduced to a mixed sex colony. The findings suggest that the role of the disturbance hiss is not an anti-predatory response when presented with a predator of limited auditory senses. Further study into the behavioural ecology of this species is recommended to understand the range of anti-predatory responses used by this species when presented with different predators. It was also found that there is some social context for the display of the disturbance hiss which warrants further study.

Key words: hissing cockroach, \textit{Gromphadorhina portentosa}, disturbance hiss, anti-predator, behavioural ecology, startle response

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Introduction

The large, wingless cockroach \textit{Gromphadorhina portentosa} (Schaum) is a long-lived social arthropod with a lifespan of between 2 and 5 years which is endemic to the tropical lowland forests of Madagascar (Clark and Shanklin, 2013).

The species has the unique ability among insects of being able to communicate using modified spiracles on its abdomen (Nelson and Fraser, 1980). Most other invertebrates communicate by stridulation, rubbing body parts together. By forcing air through the respiratory spiracles, a hiss is emitted that serves as an auditory social signal. The hiss takes three recognized forms: aggression, courtship and disturbance (Nelson and Fraser, 1980; Clark and Moore, 1995a).

The aggression hiss is displayed by the male during agonistic encounters which form hierarchies within the colony (Clark and Moore, 1995b). The courtship hiss is again only displayed by males and is essential for successful mating (Clark and Moore, 1995a). It is thought that females may use the hiss to elicit preference in mating by choosing larger, more dominant males.
(Nelson and Fraser, 1980; Schal et al., 1984; Clark and Moore, 1995b). The third type of hiss, the disturbance hiss, is easily identifiable from the others as it is both the loudest in amplitude and shortest in frequency (Nelson and Fraser, 1980). This hiss is classified as an anti-predatory response and is displayed by all members of this species past the fourth instar (Clark and Moore, 1995a) (full maturity is reached upon the sixth instar at around 7 months). This particular hiss was also demonstrated in a study by Nelson and Fraser (1980) where hissing was reported upon the following stimuli: sudden onset of light, movement of shadows, vibration of substrate and handling. The hiss was also found to be more vigorous when in a Gecko’s mouth although how much of this was as a result of the mechanical pressure of the Geckos jaws around the cockroach was not described. Studies into the three social hisses have shown that there is a noticeable difference in the frequency and amplitude between the types of hiss, which makes hiss identification easier (Nelson and Fraser, 1980; Clark and Moore, 1995a).

Habituation of this species to not display the disturbance hiss is possible through regular handling by the same person (Davis and Heslop, 2004). It was also found that the cockroach was able to discriminate between handlers, suggesting that by habituating the cockroach to not display the disturbance hiss their study provided the first evidence of discrimination between humans by an insect species. The study by Davis and Heslop (2004) was the basis for a study carried out by this author in 2012 looking at the habituation of this species. The results (Supplemental Appendix S1) showed a large difference between male and female instances of the disturbance hiss, which is interesting when looking at other species of arthropod and their responses to predators. A study into anti-predatory response to chemical detection of a predator carried out by Williams et al. (2001) showed that in the spotted cucumber beetle (Diabrotica undecimpunctata Linea), the females were in fact more likely to display an anti-predatory response around a predator than males.

The role of the disturbance hiss was the main focus of this study as although it is classified as anti-predatory, there is little direct evidence linking the disturbance hiss with survival in G. portentosa (Schal et al., 1984). The studies by Davis and Heslop (2004) along with the author’s study in 2012 suggested that the hiss may not be an anti-predatory response. Thus, displays of this hiss in the presence of a predator and in social situations with conspecifics warrant further examination.

Method

The manipulative study introduced 20 novel unfamiliar subjects to a variety of social situations and predators for 5 min at a time with all instances of hiss recorded. The aim was to further establish that the difference in instances of disturbance hissing shown by male and female G. portentosa is shared across the entire species. The study also aimed to investigate the cause of the disturbance hiss and whether it should be quantified as an anti-predatory response.

A sample of 20 healthy adult individuals (10 male, 10 female) were purchased from Ebay.co.uk (seller zoocentre) prior to the study at the penultimate sixth instar to ensure that they were all of the same age. Differentiation between the sexes is relatively easy. After the fourth instar, males are larger and possess a large pair of horns on their pronotum as well as hairy antennae (Clark and Shanklin, 2013). These individuals were separated and placed in numbered plastic insect containers measuring 15 × 7 × 5 cm for ease of identification and handling. They were kept on beech chippings with egg crates for cover, free access to food (carrot, cucumber, Pedigree mixer original dog biscuits or Kellogg’s cornflakes) and water jelly.

Ideally, the cockroaches would have been isolated as nymphs and kept individually until maturity at 8–10 months (Clark and Moore, 1995a). This would have ensured that no subject had experienced social encounters previously. However, due to the time constraints of this study, this methodology was not possible and sub-adults were purchased.

Upon purchase, the 20 individuals were marked using a silver-coloured, non-water-soluble paint (Hagler and Jackson, 2001). This ensured easy identification from members of the established colonies, while also allowing easier monitoring under low light conditions. After marking the cockroaches, no handling took place other than to move the subject to and from the established colonies during the study. This reduced any negative effects such as habituation, which could arise from regular handling, as demonstrated by Davis and Heslop (2004).

The existing colonies consisted of 17 females with numerous young, 1 of 5 males and a mixed sex colony of 6 male and 6 female G. portentosa. In addition, the subjects were placed in the presence of a predator in the form of an Emperor Scorpion (P. imperator Koch). High densities of male cockroach lead to more aggression being displayed which poses risks to the health of the individuals. To reduce the risk of injury through aggression, the males in the existing colonies were kept in smaller groups of five and six throughout the study (Guerra and Mason, 2005).

The established colonies were kept in 8.75 l vivariums (Living world) on beech chippings with egg crates for cover, which were removed 1 h prior to the study each evening. This made the identification of the hisser simpler and ensured that all subjects were visible during each trial. Removing the cover 1 h prior to the trials also meant that disturbance of the colony was kept to a minimum at the introduction of the unfamiliar individual.

Pandinus imperator was kept in a 30 × 15 cm glass tank (Clear seal) on a mix of Exo terra plantation soil and Lucky Reptile humus brick at a humidity of 70–80% with two halved plant pots for shelter. Again, the plant pots were removed prior to each trial to enable easier monitoring of each subject.

All of the subjects were kept in a room under natural light:dark photoperiod conditions with a red light lamp.
turned on at 7 pm. Additional heating was provided via heat mats (ProRep 7 W), keeping a regular temperature of 26–28°C with ambient humidity. The 20 unfamiliar individuals were each tested over 4 days, being placed in one of the four treatments each evening at 9 pm under red light conditions to best replicate the natural nocturnal habits of the species involved without disturbing the subjects. Four subjects were used each night to ensure that the existing colonies were only used once per day, thus reducing the chance of confounding variables resulting from multiple introductions on one evening.

The subject was removed from its container and placed in the tank of an established colony or *P. imperator* for 5 min at a time. A 3-mm-thick plastic screen with 2.5 mm wide by 3.5 cm high vents along it separated the novel subject from the colony/predator for 1 min. This reduced the chance of hisses occurring due to interaction with the experimenter being included in the recording. Once the screen was removed the trial began, although the plastic remained in place throughout the trials using *P. imperator* to keep them separate and eliminate any actual predation. Here the vents in the sheet acted to keep *P. imperator* separate from the cockroach while not blocking any direct sensory detection by the cockroach.

Each trial was subject to continuous monitoring from a Panasonic SDR-S45 video camera with inbuilt dynamic stereo microphone and a sound meter from the mobile phone application (Smart Tools Co.). This method of monitoring helped to reduce any miscounts of hiss display by enabling later referral back to the interactions. Each interaction was recorded by Smart Tools, and the data were uploaded onto an Excel spreadsheet from which graphs were produced showing decibel level and the duration of each hiss (Fig. 1). The sound recordings were made from a consistent distance of 10 cm to ensure that the relative amplitude displayed during each trial was constant. Conformation of which hisses were produced by just the novel cockroach was carried out by looking at the contraction and telescoping of its abdomen (Clark and Moore, 1995a).

Initial recordings from the established colonies were made of the disturbance, aggression and courtship hisses (Fig. 1). Data collected during the trials were then compared with these recordings to verify the correct identification of the disturbance hiss.

The hypothesis was tested using the extended Kruskal–Wallis two-way ANOVA test for non-parametric data (Holmes et al., 2011) with the factors being disturbance hisses displayed and the setting in which the subject was placed. This was followed up by a post hoc multiple comparisons (Q) test.

### Results

Figure 2 shows the total number of disturbance hisses displayed by males and females in each social setting. Of the 20 subjects studied that showed the disturbance hiss in the presence of a predator, the median hiss rate was zero per encounter. However, the median hiss rate of the subjects that displayed the hiss when placed in mixed sex colonies was 0.5 per encounter.

The results (Table 1) showed a highly significant difference ($K = 12.48, P = 0.01$) in the setting in which the disturbance hiss was shown. There was no significant difference in the display rates of the disturbance hiss between the sexes in general. There was also no significant difference found in the interaction between the sex of the cockroach and the setting into which it was placed. The significant effect of the setting was found to be the result of a highly significant difference when females were placed in mixed colonies compared with males being placed in male colonies and with females placed with female colonies ($Q = 3.84, P = 0.01$) and when females were placed with mixed colonies compared with males being placed with female colonies ($Q = 3.35, P = 0.05$).
Subject number 20 showed a greater response than other females during the study. After the study was completed, it became apparent that the female was gravid so the analysis was carried out again with this individual removed. The representation in Figs. 3 and 4 shows the number of hisses displayed by males and females in each social setting without taking subject 20 into account due to her condition. Results of the test showed, as in the original analysis, a highly significant difference ($K = 12.66, P = 0.01$) in the setting in which the disturbance hiss was shown but no significant interaction between the sex of the cockroach and the setting into which it was placed. However, there was a highly significant difference ($K = 9.49, P = 0.01$) in the display rates of the disturbance hiss between the sexes in general.

**Table 1.** The number of *Gromphadorhina portentosa* displaying the disturbance hiss in various social settings with and without subject 20 which was found to be gravid with median hisses per encounter and inter-quartile ranges

| Test subjects               | Social settings |          |          |          |
|-----------------------------|-----------------|----------|----------|----------|
|                             | Male            | Female   | Mixed    | Predator |
| Males                       | 0               | 1        | 3        | 2        |
| Females                     | 3               | 0        | 7        | 1        |
| Females without subject 20  | 2               | 1        | 9        | 2        |
| Median hisses per encounter | 0               | 0        | 0.5      | 0        |
| Inter-quartile range of median hisses at 25% | 0        | 0        | 0        | 0        |
| Inter-quartile range of median hisses at 75%     | 0               | 0        | 3        | 0        |

**Discussion**

There was a highly significant difference in the setting in which the disturbance hiss was displayed. With half of the subjects displaying the disturbance hiss when placed in a mixed sex colony, the context in which the cockroach displayed the disturbance hiss was different to what would be expected. Only 10% of the subjects in this study displayed the disturbance hiss when placed with the predator. With females displaying the hiss in this situation, we are able to categorically discount this hiss being mistaken as the aggression response which is only displayed by males.

The study revealed a highly significant difference between the sexes in the display of the disturbance hiss. Females used the disturbance hiss almost exclusively when introduced to unfamiliar males, while males used other hiss responses when introduced to unfamiliar male cockroaches. The species lives in small, mixed sex colonies. Females are gregarious in their behaviour, and they live in groups without conflict (Nelson and Fraser, 1980). Males, however, have a non-linear social structure with varying levels of aggression, dominance and activity levels (Clark and Moore, 1995a). Males show territorial behaviour with the dominant male often defending a territory within the group. This territory is less than 1 m$^2$ and may simply be a piece of wood or raised ground from which a dominant male will chase any other invading male (Nelson and Fraser, 1980). It may be hypothesized that the females are using the disturbance hiss in mixed sex colonies to ward off unwanted attention from unfamiliar individuals. In all instances, the hiss was directed at a male cockroach of the established population which was either attempting to mate or asserting their dominance over the subject.

Pheromones play a crucial role in cockroach communication and the composition of released pheromones in relation to the disturbance hiss may be worth further investigation (Sueur and Aubin, 2006; Bell et al., 2007). Further studies may find that the pheromones accompanying the display of the disturbance hiss differ depending on its stimulus and whether it originates from a predator or contact with conspecifics.

There are many anti-predatory recognition and avoidance strategies utilized by invertebrates; the strategy employed varies from species to species (Dicke and Grostal, 2001). In the
cockroach, the most common anti-predatory response is to turn and flee, while there is some evidence of a few species using stridulation followed by the release of noxious chemicals (Dicke and Grostal, 2001). If the disturbance hiss of G. portentosa is indeed an effective anti-predatory response, then by not showing the hiss response the females are potentially at a higher risk from predators than the males that are more likely to display these auditory responses. One explanation could be that the hiss is a Batesian response in that the species is imitating a toxin-producing species. However, G. Portentosa has many natural predators across all orders: insects, rodents, reptiles, amphibians and mammals. As such there is no one animal that it could mimic to deter the wide variety of predators which each has different sensory abilities.

When the cockroaches were placed in the tank alongside the predator P. imperator, they moved around briefly waving their antennae, before stopping, placing the antennae laterally along the abdomen and then remaining stationary throughout the remainder of the trial. There can be no doubt that the subjects were aware of the presence of P. imperator, some remained next to the vented plastic with the scorpion directly on the other side becoming active and attempting to prey upon them. The disturbance hiss responses during this time were significantly less than in the social, non-predator settings. The scorpion relies on olfaction and basitarsal compound slit sensilla to detect its prey (Brownell, 1984). These mechanoreceptors are sensitive to vibration but not sound, so the disturbance hiss produced by G. portentosa may not have any startle effect on this predator but would in fact give away the cockroaches location. Instead, the freezing displayed in this study may offer an alternative anti-predatory response to predators with poor auditory abilities. More studies investigating whether the disturbance hiss is displayed towards other predatory species which have better auditory capabilities would enable us to elucidate the purpose of the disturbance hiss. The response may in fact be two different hisses used for different purposes which have individual pheromones associated with them. Alternatively, the hiss may be a single hiss that is used in multiple situations.

Threat detection by prey is essential to ensure the survival of an individual and any individual needs to learn quickly what constitutes a threat and what does not. It is most likely that mechanical and olfactory sensory cues are used by the cockroach and other arthropods (Comer and Baba, 2011). This was demonstrated to be the case in the spotted cucumber beetle (D. undecimpunctata Linneaus.), which was found to use both chemosensory and mechanical cues to help them recognize and differentiate between the most dangerous predators and other similar but less harmful species (Snyder and Wise, 2000).

Findings by Mishra and Meyer-Rochow (2008) suggest that vision is of lesser importance to the nocturnal scavenger G. portentosa than other senses and, as such, it is unlikely to be the sense used by this species for predator detection or in social situations. The study found that this species had fewer ommatidia than predatory insects such as praying mantis and non-predatory cockroach species Periplaneta americana but had wider facets that enable the eye to collect a greater amount of light at night when the species is most active.

Cockroaches however have excellent olfactory learning abilities and an olfactory memory with long retention rates for scent memories. These memories form quickly and are easily rewritten (Sakura and Mizunami, 2001). The subjects in this investigation may have detected the indirect sensory cues from P. imperator faeces indicating its presence. Chemical cues can be identified directly through taste, such as the detection of infochemicals from a known predator, excreta, exuviae, eggs and pheromones (Herberholz and Marquart, 2012). Another method of chemosensation used is that of indirect cues detected through olfaction such as alarm pheromones from injured conspecifics and any scent left on the ground by the predator (Dicke and Grostal, 2001; Herberholz and Marquart, 2012).

Megaloblatta blaberoides (Walker) uses mechanical sensory cues to detect predators with the detection of air movement through trichoid sensilla located on the cerci which could indicate the approach of a nearby predator (Schal et al., 1982). The discovery by Roeder (1963) demonstrated that cockroaches respond to the puff of wind on the cerci that could represent an approaching predator by making a swift turn followed by forward acceleration along preferred trajectories to escape. Structures such as the fine hairs on the body and antennae called trichoid sensilla could assist in detecting close field sound from conspecifics and predators through the movement of air, adding to the sensory ability of the cockroach (Sueur and Aubin, 2006). By placing the sensitive antennae along its abdomen and remaining still, the cockroaches may have then been waiting for a direct mechanoreceptory cue before acting any further.

Domenici et al. (2009) showed that P. americana has preferred escape trajectories in relation to the perceived threat indicated by puffs of air. This evolutionary response has enabled those with a quicker response to being startled to survive while those which linger do not. Active touch to the antennae can also result in this reaction if the object touched is unfamiliar or recognized as a predator (Comer and Baba, 2011). There is little direct evidence linking the disturbance hiss with survival in G. portentosa (Schal et al., 1982) and examples of the escape response displayed by other non-toxin-producing species such as P. americana (Schal et al., 1984; Domenici et al., 2009; Comer and Baba, 2011) are a more likely response to the indirect cue of a predator.

Finally, the behaviour of subject 20 in the study was very interesting and may shed some light on the reason for the display of the disturbance hiss. This female was the only female subject to display the disturbance hiss and was later found to be gravid, perhaps indicating that tolerance levels
are reduced when in this condition as the effectiveness of flight behaviour may become impaired in gravid females. This results in reduced locomotor performance leaving the female at a greater risk from predation (Pruitt and Troupe, 2010). As a result, the anti-predatory strategies of females of many species alter when gravid.

**Conclusion**

The results of this study allow us to challenge the hypothesis that the disturbance hiss is an anti-predatory response. Instead, there appears to be some social aspect to the disturbance hiss in that it is readily displayed in other situations and, most likely, as a warning resulting from unwanted direct contact with conspecifics. Further investigation into the use of the disturbance hiss by *G. portentosa* in social situations would help us to better understand the behavioural ecology of the species.

**Author biography**

I am a mature student and have just completed the Animal Biology degree at Worcester University as a top-up student after completing an HND at Pershore College in 2012. I currently work at Pershore College as both Lecturer in animal care and instructor technician. I designed the study as my final year study. I purchased the insects used, conducted the experiments, analysed the data performing all statistical analysis and wrote up the paper. I have sole responsibility for the study’s final content.

**Supplementary material**

Supplementary Material is available at BIOHOR online.

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