



Why do zombies clean themselves? An initial test of the antimicrobial hypothesis in a parasite-host relationship

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Received: 7 December 2020 / Accepted: 20 January 2021 / Published online: 2 February 2021
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Abstract

The emerald jewel wasp *Ampulex compressa* attacks the cockroach *Periplaneta americana* which serves as the host of the parasite and injects venom directly into the cockroach's central nervous system. The cockroach responds by excessive grooming and cleaning its entire body with its legs and mandibles. The antimicrobial hypothesis suggests that self-grooming is adaptive for the parasite because it decreases ectoparasite and fungal infection rates from the cockroach's cuticle in order to prevent infection of the wasp's larvae. We demonstrated under laboratory conditions that microbial prevalence was not higher in stung cockroaches experimentally prevented from self-grooming than in self-groomed ones.

Keywords *Ampulex compressa* · Manipulation hypothesis · Parasitoid wasp · *Periplaneta americana*

Introduction

Based on the host manipulation hypothesis, numerous taxa of parasites can modify host behaviour and physiology thereby increasing the fitness of the parasite at the expense of the host (Moore 2002; Poulin 2010; Hughes et al. 2012; Poulin and Maure 2015). A typical example is an intracellular parasite *Toxoplasma gondii* inhibiting the anti-predator behaviour of its rodent host (Dass and Vyas 2014), or hairworms *Spinochordodes tellinii* (Camerano, 1888) inducing suicidal behaviour in bush crickets *Meconema thalassinum* (DeGeer, 1773) and allowing the adult hairworm to abandon the dying insect (Thomas et al. 2002). The acantocephalan *Pomphorhynchus laevis* (Zoega in Müller, 1776) increases the activity and light tolerance of its amphipod *Gammarus*

pulex (Linnaeus, 1758) host, thereby increasing its vulnerability to predation by the final hosts of the parasite (Cezilly et al. 2013) and parasitoid wasps *Dinocampus coccinellae* (Schrank, 1802) induce its spotted lady beetle (*Coleomegilla maculate*) (De Geer, 1775) host to protect parasitoid pupae from their natural enemies (Maure et al. 2011).

Although initial research provided certain evidence for host manipulation by parasites (Holmes and Bethel 1972; Moore 2002), considerable scepticism concerning this hypothesis exists (Poulin 2000; Moore and Gotelli 1990; Heil 2016). The altered behaviour of the host could be, for example, a non-adaptive side effect, or an adaptive response on the part of the host to the parasite infection (Poulin and Thomas 1999; Bakker et al. 2017). Furthermore, recent reviews emphasize that experimental manipulations of host traits are scarce, most evidence is correlative, and that theory outruns experimental studies (Poulin and Maure 2015; Bakker et al. 2017).

In the present study we investigated parasite-host interactions between the emerald jewel wasp *Ampulex compressa* (Fabricius, 1781) (Hymenoptera, Ampulicidae) and its host, the American cockroach *Periplaneta americana* (Linnaeus, 1758) (Blattidae). Upon encountering the cockroach, *A. compressa* attacks the cockroach which serves as host of the parasite (Libersat 2003). The wasp injects venom directly into the cockroach's subesophageal ganglion, in and around the central complex (Haspel et al. 2003) after which the cockroach engages in excessive grooming and thoroughly cleans its entire body with its legs and mandibles for roughly 30 min

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(Weisel-Eichler et al. 1999). The wasp then drags the stung cockroach into its burrow and lays a single egg on a mesothoracic leg of the host (Williams 1942). The stung cockroach serves as a living food supply for the development of the wasp's larva. It continues self-grooming, but this grooming is less intense than initial grooming emerging soon after the wasp's attack (P. Pecina, unpublished data). Specifically, we experimentally investigated whether the excessive grooming behaviour could serve as an effective strategy to remove fungi and other ectoparasites that are natural enemies from the cockroach's cuticle in order to prevent infection of the wasp's larvae. This is one of the adaptive hypotheses proposed by Libersat and Gal (2014). Our work is not a direct test of that hypothesis, because we focus only on bacteria and do not include multiple ecologically relevant pathogens such as fungi or ectoparasites, which are almost absent in lab-reared insects. We extended the prediction of this hypothesis that stung cockroaches experimentally prevented from self-grooming should have higher prevalence of microbial infection (the number of bacteria per host individual) on their cuticle than untreated cockroaches that groomed themselves.

Materials and methods

Experimental animals

Experimental *A. compressa* used in this study were third generation laboratory-reared descendants of individuals obtained from a commercially distributed population obtained in Terra-Expo, Germany, bought in 2018. The adults were reared in 29 × 30 × 20 cm plexiglass cages and provided with honey and water with sugar *ad libitum*. More specifically, 50 g of sugar (400 kcal/100 g) was diluted in 200 ml of water. Pine bark and pumice stones served as substrate. One female and one male were maintained in each breeding cage. Male and female cockroaches were reared together in 6 litre plastic containers with cardboard shelters, food (dog chow and apples) and water were provided *ad libitum*. All insects were maintained at room temperature (~24 °C and ~50 % humidity). The light/dark cycles were 10/14 hours. Adult male cockroaches (*P. americana*) were chosen for parasitization. Experiments were made between January–March 2019 in the plexiglass cages with an identical size and substrate as for breeding purposes. Each cage contained one plastic tube (SARSTEDT®, 50 ml, 114 × 28 mm) serving as the wasp's burrow.

Behavioural test

Wasps ($n = 15$) and cockroaches ($n = 45$) were randomly assigned to one of three treatments. A female wasp was placed inside the cage and allowed 30 min to acclimate. The experiment began after the cockroach entered the cage. After the

cockroach was stung by the wasp, it was removed from the cage, enwrapped with a sterile non-sticky cotton bandage and gently placed in the sterile plastic tube (identical in size to the tube serving as a burrow) to prevent self-grooming (Venom + Immobilization treatment, $n = 15$). We chose the amount of bandage to fill the space between the cockroach and the plastic tube to prevent cockroach movement. Stung cockroaches in the Venom + Intact treatment ($n = 15$) were removed from the cage and placed in the sterile plastic tube (50 ml). Control cockroaches ($n = 15$) were allowed to spend 5 minutes in the experimental cage in the absence of the wasp and then removed and placed in the sterile plastic tube. This time is comparable with the latency of attack in the treatments with a wasp (see below). All the experimental procedures were made with sterile gloves to minimize microbial contamination by hands. Mean body mass (M) and pronotum width (W) of cockroaches ($n = 45$) was $M = 0.86$ g (SE = 0.03) and $W = 9.59$ mm (SE = 0.03), respectively. After controlling for pronotum width, there were no significant differences in cockroach body mass across treatments (ANCOVA, $F_{2,41} = 0.14$, $p = 0.87$). The body mass of wasps was $M = 0.17$ g (SE = 0.003). Each wasp was used three times in total, but not more than once per day to prevent depletion of venom (Arvidson et al. 2018). Distribution of wasps across treatments was random (Pearson $\chi^2 = 28.0$, $p = 0.12$). We measured the latency time from when the cockroach was attacked and stung by the wasp (accuracy ± 1 min) and microbial prevalence on the second pair of cockroach legs (see below). All the experiments were made between 10:00–11:00 am under a natural photoperiod and standard temperature (~24 °C) and humidity (~50 %). The cockroaches were transported to a microbiology laboratory up to two hours after experiment. Simultaneous trials were visually isolated from each other by the placement of white paper between the experimental cages. Following the end of the trial, all the contents were removed from each cage, the wasps were returned to their housing containers, and the area was cleaned with water.

Determination of microbial prevalence

The second pair of cockroach legs, where the wasp lays its egg, was cut off and aseptically transferred to 0.5 ml of PBS buffer and immediately processed. A 1.5-ml tube containing 0.5 ml of PBS and a pair of legs was mixed by vortexing for 60 s. The bacterial suspension was then 10-fold serially diluted so that up to about 300 colony forming units (CFU) were found on the plate. After the dilutions were completed, 0.1 ml of all samples and dilutions were spread-plated on Mueller-Hinton broth agar plates that indicated total bacterial counts. The plates were then incubated for 24 h at 37 °C. After the incubation period, the plates were removed and the colony enumeration was performed. All the results are reported as a CFU/pair of legs.

Statistical analyses

The latency time from the beginning of the experiment to the stinging of the cockroach demonstrated the gamma distribution of the data. A Generalized Linear Mixed Model (GLMM) with treatment (Venom + Immobilization, Venom + Intact) as a fixed factor, the wasp's ID (random factor) and the latency time (dependent variable) was used for the data analysis. Microbial prevalence was first Box-Cox transformed in order to achieve normality and then was defined as a dependent variable in the GLMM. Treatment (Venom + Immobilization, Venom + Intact and Control) was defined as a fixed predictor and the wasp's ID was treated as a random factor. All statistical tests were performed with SPSS ver. 23. Raw data are available in the Electronic Supplementary material.

Results

The latency time for the Venom + Immobilization and Venom + Intact treatment ranged between 1 and 20 min. ($M = 5.9$ min., $SE = 0.9$, $n = 30$). There were no differences in the latency time between the Venom + Immobilization and Venom + Intact treatment (GLMM, $F_{1,28} = 1.60$, $p = 0.22$). There were significant differences in microbial prevalence across the three treatments (Venom + Immobilization and Venom + Intact, Control treatment) (GLMM, $F_{2,42} = 3.6$, $p = 0.036$). Specifically, cockroaches subjected to the wasp sting that were able to clean themselves showed a significantly higher level of microbial prevalence compared with untreated controls (Fig. 1). Other differences were not statistically significant. Inclusion of cockroach body and pronotum width to the model did not change results of statistical analyses. Our data suggest that the contact with wasp increase the amount of bacteria on the cockroach. To test this possibility, we pooled data on microbial prevalence from Venom + Immobilization and Venom + Intact treatment and compared them against the Control treatment. The results were significant (GLMM, $F_{1,43} = 5.59$, $p = 0.023$) suggesting that treated cockroaches showed a significantly higher level of microbial prevalence compared with untreated controls, whether they groom themselves or not. These results failed to support the antimicrobial hypothesis.

Discussion

This study demonstrated that excessive grooming by the stung American cockroach, a host of the emerald jewel wasp, is not an adaptive strategy to remove microbial infections from the cockroach's middle legs.

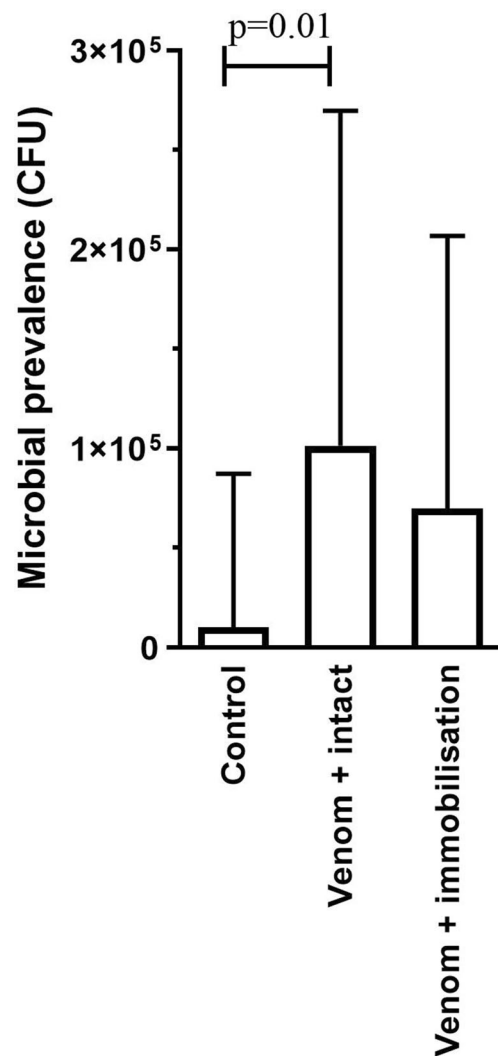


Fig. 1 Differences in microbial prevalence between treatments (means + 95% CI)

Sting-evoked grooming by the cockroach is induced by the injected venom (Weisel-Eichler et al. 1999; Weisel-Eichler and Libersat 2002). As far as we are aware, however, no study has examined yet the adaptive function of the stung cockroach's self-grooming. After *A. compressa* larva penetrates the cockroach's body, it impregnates the cockroach with oral secretions containing antimicrobials (Herzner et al. 2013) which could protect the larva against potentially harmful microbes. Libersat and Gal (2014) have suggested that self-grooming could similarly reduce microbial prevalence on the general surface of the cockroach and thus can interfere with larval development. In this sense, microbes can contaminate not only the larvae, but also the burrow where the cockroach is provisioned. This explanation makes sense as self-grooming in cockroaches helps with cleaning external chemosensory receptors (Böröczky et al. 2013; Zhukovskaya et al. 2013) which could, in theory, create a clean substrate for the wasp's egg. In contrast to this antimicrobial hypothesis, microbial prevalence was not higher in stung cockroaches which were

experimentally prevented from self-grooming. Interestingly, the lowest microbial prevalence was found among control cockroaches suggesting that wasp's combat with its prey increases the microbial prevalence on the cockroach's cuticle. It would seem that the stung cockroaches are particularly effective in cleaning their body parts close to the mouth parts, such as the first pair of legs. In contrast, the wasps prefer other, less cleaned body parts for oviposition where the eggs are less threatened by grooming by the mandibles.

Taking into account the possible adaptive function of the host's self-grooming, perhaps the wasp uses the cockroach's movement as a cue by which it is easily located and then transported to the burrow (Libersat and Gal 2014, but see Pecina et al. 2020). It needs to be pointed out, however, that lab-reared cockroaches in this study were presumably free from ectoparasites, eggs from other parasitoids, pathogenic microbes that would be found in nature, and most fungal pathogens. Thus, this study cannot rule out the possibility that the antimicrobial hypothesis could still be supported under more ecologically relevant conditions (e.g., if a broader range of parasites and fungi were considered), or if different measures of infection were quantified. Future research can experimentally expose cockroaches topically to fungal spore suspensions (e.g. generalist fungal entomopathogens: *Beauveria bassiana* or *Metarhizium anisopliae*) and quantify successful establishment of fungal infection in the cockroach following exposure (groomed vs. non-groomed individuals).

Another approach how to test the antimicrobial hypothesis is to make an experiment to show whether self-grooming improves parasite fitness. In particular, survival rate, developmental time and body condition of the jewel wasp that develops in the cockroach should be compared in the grooming versus non-grooming cockroach hosts. If the antimicrobial hypothesis works, then the successful completion of the wasp life cycle should be completed in groomed hosts more successfully than in non-groomed hosts.

Moreover, the stung cockroach grooms its entire body, not just its middle legs. This suggests that pathogens from other parts of the cockroach's body could damage the egg and/or larva until the larva penetrates into the cuticle to pupate, where it disinfects the cuticle internally. To address this possibility, pathogen prevalence throughout the cockroach's body, rather than only on the two legs where the wasp lays its egg needs to be examined. These possibilities require further research. Alternatively, induced self-grooming could be a non-adaptive response to the injected venom. A parallel phenomenon is exemplified by *Plasmodium*-infected mosquitoes (Koella et al. 1998). Their enhanced foraging activity does not need to be a result of manipulation by parasites but is instead likely to be compensation for the increased metabolic costs induced by the parasites (Heil 2016). Indeed, similar behavioural alterations were observed in mosquitoes that had been challenged with heat-killed *Escherichia coli* (Cator et al. 2012, 2013).

In conclusion, our results do not support the manipulation hypothesis in the case of the emerald jewel wasp / American cockroach system. The possibility that cockroach's self-grooming behaviour may be a pathological side effect of the wasp's venom cannot be rejected. Future research should examine the possibility that the wasps face a trade-off when choosing a host surface area for oviposition: they choose between clean but unsafe (due to host preening), and bacterium-contaminated but safe areas.

Acknowledgements We thank prof. Frederic Libersat and dr. Lajos Rózsa for valuable comments on an earlier draft of the manuscript. Two anonymous referees provided helpful comments on an earlier draft. David Livingstone improved the English. This research was supported by the scientific grant agency VEGA no. 2/0139/17, 1/007/21 and 2/0074/21.

Compliance with ethical standards

Experiments were performed in compliance with Slovakian laws.

Conflict of interest statement The authors declare they have no conflict of interests.

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