Getting the Most Out of Your Zombie: Abdominal Sensors and Neural Manipulations Help Jewel Wasps Find the Roach’s Weak Spot

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Abstract
The parasitoid emerald jewel wasp (Ampulex compressa) subdues the American cockroach (Periplaneta americana) with a sting to the 1st thoracic ganglion, followed by a sting to the roach’s brain, causing long-term pacification. The wasp then leads the cockroach to a hole where it lays an egg on the roach middle leg before barricading the entrance and departing. Although many aspects of the wasp’s initial attack have been investigated, few studies have detailed the egg-laying process and the subsequent fate of the larvae. Here I show that larval survival depends on precise egg positioning on the cockroach by the female wasp. Ablation of sensory hairs on the wasp’s abdomen resulted in mislaid eggs, which seldom survived. In addition, the cockroach femur may block the oviposition site. The wasp contended with this challenge with a newly discovered suite of stings, 3 directed into the 2nd thoracic ganglion which resulted in extension of the femur, thus exposing the oviposition site and removing a potential barrier to the wasp’s successful reproduction. When the femur was glued in place, the wasp stung the cockroach over 100 times, in an apparent fixed action pattern triggered by the obscured oviposition target. These findings highlight the importance of proper egg placement by the wasp, and reveal sensors and new neural manipulations that facilitate the process.

Introduction
The epic battle between the jewel wasp and the American cockroach (Periplaneta americana) was first described in detail by the entomologist Francis Williams in 1942 [Williams, 1942], and since that time, the wasp has become one of the most famous and well-studied parasitoids. It has been the subject of many investigations [i.e., Williams, 1942; Piek et al., 1984; Veltman and Wilhelm, 1991; Richardson and Paul, 1993; Fouad et al., 1994; Weisel-Eichler et al., 1999; Haspel and Libersat, 2003; Haspel et al., 2003; Gal et al., 2005; Banks and Adams, 2012; Moore et al., 2018; Arvidson et al., 2016, 2019; Kaiser et al., 2019], and it holds a prominent place in the emerging field of neuroparasitology [Hughes and Libersat, 2018]. Moreover, the model system is covered in many biology classes and is often featured...
in popular science accounts because it captures the public’s imagination. This stems from the wasp’s astonishing reproductive strategy. To secure food for each of its young, the wasp must win a pitched battle with a much larger cockroach, ultimately pacifying the roach with a venom cocktail injected directly into the brain. The wasp’s venom is said to “zombify the roach.”

To provide a context for the present study, it helps to recount the stages of the wasp’s attack. The struggle between these 2 contestants begins when a stalking jewel wasp discovers and approaches a cockroach. If the roach is vigilant it may put up a successful defense [Williams, 1942; Fox et al., 2009; Catania, 2018] by standing tall (stilt-standing), thus increasing the distance between its body and the wasp. This posture allows the roach to use its mechanosensitive and barbed legs as a roadblock of sorts. The wasp must leap past this barrier to reach the cockroach, often contacting the legs or antennae in the process and thus activating the roach’s fastest escape response [Comer and Dowd, 1993; Comer et al., 1994; Ye et al., 2003]. At the same time, stilt-standing allows the roach to kick at the wasp with its powerful, spiny hind legs. Most wasps are deterred after receiving multiple kicks to the head [Catania, 2018].

But if the cockroach does not defend itself, or if the defense fails, the lunging wasp will grasp the roach’s protective shield (the pronotum) just behind the head. Although the roach flails and struggles to throw the wasp off, within half a second the wasp can usually direct its sting through the soft membranes below the pronotum and ultimately into the roach’s first thoracic ganglion where venom is delivered. The result is temporary paralysis of the roach’s front legs [Haspel and Libersat, 2003]. With the roach thus hobbled and unable to defend its head, the wasp then directs its sting through the soft tissue of the throat and into the brain.

This second sting results in a long-term pacification of the cockroach. The paralysis of the front legs wears off, and the cockroach is able to walk, run, or even fly if properly stimulated. And yet it does not attempt to escape as the wasp spends the next 30 min or more searching for a nearby hole. When the wasp returns, she then severs both of the roach’s sensitive antennae and takes a blood meal from each bleeding stump. Next she pulls her compliant host and transporting it to the crypt. This phase of the wasp’s behavior required a detailed examination of the egg-laying process, which in turn required determining where the larvae are able to feed, and then tracking their survival for different egg positions. It was in the course of these observations of oviposition that the additional suite of stings was discovered – the stings are covered in part 2 of the Results.

When all of these results and observations are combined, a new picture emerges regarding the challenges facing a wasp during the reproductive process. As described above, the wasp’s first challenge is securing the host and transporting it to the crypt. This phase of the wasp’s behavior includes stings 1 and 2 and has been extensively studied in previous investigations.

The second challenge is securing the egg in a precise location that will allow the nearly helpless larva to penetrate the cockroach cuticle. This component of the wasp’s behavior has been largely overlooked, and yet it is crucial; minor mistakes by the wasp are often fatal for the larva. Here I provide evidence that this second challenge – proper egg placement – is overcome using the sensory hairs on the wasp’s abdomen to search for the proper location, and that the newly reported stings facilitate the search phase and subsequent egg-laying process. These findings show the jewel wasp’s behavior to be more complex, and its attack more sophisticated, than previously reported.
Materials and Methods

See supplementary materials for full-length methods (for all suppl. material, see www.karger.com/doi/10.1159/000511548). Emerald jewel wasps (Ampulex compressa, Hymenoptera: Ampulicidae) were obtained from Hawaii and maintained and bred in an approved insect containment facility (facility 3226) under United States Department of Agriculture permit P526P-17-01368. Lights were cycled on a 12-h/12-h light–dark regimen. Room temperature was approximately 23 °C. Supplementary lighting raised the temperature of the BioQuip dorms to 25 °C. Parasitized cockroaches were transferred to ventilated 50-ml tubes (Celltreat 229479 Bio-Reaction Tube, Daigger Scientific Inc.) and placed into a separate Plexiglas chamber. Supplementary radiant heat lamps and a water reaction tube (Daigger Scientific Inc.) and placed into a separate BioQuip dorm. Parasitized cockroaches were anesthetized with CO₂, fixed in 4% paraformaldehyde, dehydrated through alcohols, critical point-dried using CO₂ in an E3000 drier (Quorum Technologies), sputter-coated with gold, and viewed in a Tescan Vega II microscope (Tescan). Wasp abdominal hairs were trimmed off using a straight, 6-mm cutting edge obsidian scalpel (Fine Science Tools item 10110-01). For manipulation of cockroaches, animals were first anesthetized with CO₂, before leg removal or for partial removal of the basisternum (Fig. 12). For the trials in which the 2nd femur was glued in position, cyanoacrylate was sparingly applied to the area around the trochanter (in anesthetized roaches) and then a tiny amount of cyanoacrylate accelerator (Bob Smith Industries Inc.) was applied from a capillary tube to ensure rapid solidification. Video for supplementary movies was finalized with legends and pointers in Adobe Premier Pro CC 2018.

Results

Part 1: Wasp Sensory Hairs, Oviposition Behavior, and Consequences for Larvae

Larval Feeding Sites

Little is known about the precise location of the egg or the behavior of the larva after it hatches, and this in turn has left many open questions. How stereotyped is egg location? How many options does the newly hatched larva have? Can it feed at any location? Could it survive on the front or back legs, rather than the central pair? In order to begin to address these questions, the feeding location of 100 successfully feeding wasp larvae was observed and photographed.

Figure 1 provides a summary of the results (with left-side cases flipped to the right-side orientation). As reported in previous studies, under normal conditions, all eggs were laid on the coxa of either the left or right middle leg (e.g., Fig. 1b). Eggs were positioned such that, once hatched, the head of the larvae would emerge from the upper (blunt) end of the egg, close to 2 soft membranes at the joint between the coxa and body of the roach.

These membranes were described by Williams [1942] and labeled as “A” and “B” from bottom to top, respectively. In the present study, for 100% of 100 successful larvae that were tracked, feeding was initiated at location “A” – the large membrane between the coxa and the trochantin (Fig. 1c, d). For the most part (in 89% of the cases) the membrane was pierced in the upper quarter (note that for these, and all subsequent data, the long axis of this membrane is used to set the y axis of measurements and plotted positions).

A number of observations and additional experiments suggest this is an obligatory feeding point (and subsequent entry point) for the developing larvae. For example, in the course of maintaining a wasp colony for over 5 years, the author has never observed a larva successfully feed at any other site on the cockroach. More importantly (as will be subsequently illustrated) larvae that attempted to feed at
Fig. 1. An overview of egg placement and larval feeding sites in the emerald jewel wasp (Ampulex compressa).

**a** A wasp holds the pronotum while bending its abdomen forward to deliver a sting to the brain of an adult cockroach. This view shows the exposed coxa of the roach’s middle leg, where the wasp will later deposit an egg.

**b** The coxa with an attached egg close to 2 soft membranes (A and B) at the joint between the coxa and the thorax.

**c** The hatched larva has broken through the membrane at “A” for a blood meal.

**d** Summary of 100 feeding sites and their relative position on the membrane. Note that for these and other data in the study, left-side cases were reflected to the right-side orientation for consistency of presentation and data analysis. In contrast to the suggestion of Williams [1942] there was no evidence that larvae feed at the upper membrane at “B”.

Color version available online
other locations, including at the membrane labeled “B” (Fig. 1) inevitably perished (membrane “B” is located between the cockroach meron and the epimeron). From hereafter, the target membrane at “A” (Fig. 1) will be referred to as the “trochantinal membrane.” Note that the data described above for feeding sites (Fig. 1c, d) do not address failed eggs and larvae. Failure to feed can result from a myriad of reasons, as will be addressed in a later section.

**Egg Position**

Having established the top of the trochantinal membrane as the primary target of the wasp’s larvae, the next parameter measured was the relative location of eggs laid by the wasps. This was determined for a single trial (1 egg) for each of 25 wasps, as measured immediately following egg deposition. For these data adult female cockroaches were used as hosts. Adult cockroaches often successfully defend themselves by kicking with their hind legs, thereby deterring the wasp’s attack [Fox et al., 2009; Catania, 2018]. Therefore, for this first part of the study, the hind legs were removed to facilitate host handling.

To plot the mean egg position from the 25 cases, both a generic adult female wasp second leg, and a generic egg, were illustrated (see Methods). The x and y coordinates for egg position were centered on the top middle of the trochantinal membrane, with the long axis used to fix the y axis, and the top center of the membrane used for the center point of the x axis. The angle of the egg was plotted using the vertical (y axis) to indicate zero degrees, with clockwise angles designated as positive. Figure 2 shows the resulting mean egg position and angle, along with standard deviations for the position of the top center of the egg and the angle of the egg (see Methods). For the conditions of this study, the (mean) top center of the egg was in the left upper quadrant, 139 μm to the left (x coordinate) and 130 μm above (y coordinate) the top center of the trochantinal membrane. The mean angle of the egg was 21.4° clockwise from the y axis. Note that the mean egg position places the tip of the egg remarkably close to the most common target of the larvae.

**The Egg-Laying Process and Candidate Sensors**

Given the consistent oviposition site in an apparently favorable location for the larvae, the next phase of this study was aimed at determining how the wasp identifies the appropriate location for the egg. Previous investiga-
tors have noted that female wasps explore the cockroach leg with the tip of their abdomen [Gnatzy et al., 2018], and Veltman and Wilhelm [1991] suggest the hairs at the tip of the abdomen “aid in this purpose.”

To document the possible use of the hairs, and the general exploratory behavior of the wasps, a Wild M400 microscope with an attached video camera was inverted. Clear-bottomed Plexiglas chambers, suitable for host entombment, were attached to each of the female wasp cages via a short tunnel. The cage could then be positioned such that the chamber was above the microscope to allow for high magnification imaging from below the cockroach (Fig. 3a). The observations using this paradigm confirmed the fact that ovipositing female wasps always make an extensive and stereotyped investigation of the cockroach leg (coxa) using the very tip of the abdomen, suggesting the associated hairs play an important role in the process (online suppl. movie S1). In addition to these hairs, the sting sheath or the stinger were sometimes extended and made contact with the coxa – at the same time as the hairs. However, the position and use of these other structures during the exploratory process were variable across trials and across individual wasps (and in many cases never made contact). In contrast, the hairs at the tip of the abdomen were in nearly constant contact with the coxa during the exploratory phase, which lasts between 1 and 2 min (see text). Trace was made from video and then overlaid on generic coxa. C Scanning electron micrograph of the tip of the wasp’s abdomen showing the hairs (arrow) that are in nearly constant contact with the roach’s leg during the exploratory phase. D Scanning electron micrograph of the inner side of the wasp’s abdomen after removing a layer of (inner) cuticle. A dense network of nerve fibers supplies the abdominal hairs (arrow).

Fig. 3. Abdominal movements and use of sensory hairs during oviposition. A Image captured from video showing the tip of the wasp’s abdomen pressed to the coxa during the exploratory phase prior to egg deposition. The red arrow indicates the location of prominent hairs in contact with the coxa. B The red lines indicate the movements of the tip of the abdomen during the exploratory phase, which lasts between 1 and 2 min (see text). Trace was made and then overlaid on generic coxa. C Scanning electron micrograph of the tip of the wasp’s abdomen showing the hairs (arrow) that are in nearly constant contact with the roach’s leg during the exploratory phase. D Scanning electron micrograph of the inner side of the wasp’s abdomen after removing a layer of (inner) cuticle. A dense network of nerve fibers supplies the abdominal hairs (arrow).
specimens was hemisected, and the internal side of the sclerite was carefully exposed for viewing. This process revealed a dense network of nerve fibers supplying the abdominal hairs (arrow, Fig. 3d), further suggesting their importance as sensors during oviposition (high magnification scanning, not illustrated, did not reveal pores that would suggest a chemosensory role for the hairs).

Ablation of Sensory Hairs and Effect on Egg Laying

Having established the abdominal hairs as candidate sensors that may play an important role in identifying the proper location for egg placement, the next experiment was to remove these hairs from numerous specimens and examine the subsequent effect on egg laying. First, the position of eggs laid by 10 different wasps was documented. Next, wasps were briefly CO$_2$-anesthetized, and the hairs were gently trimmed (Fig. 4) from the abdomen using an obsidian scalpel (see Methods). In addition, 5 wasps were CO$_2$-anesthetized, and the abdominal hairs were gently stroked with the blunt end of the scalpel to serve as controls. In the case of anesthesia alone, the eggs from a single trial both before and after anesthesia were all within 2 standard deviations (SDs) of the mean egg location and angle (as illustrated in Fig. 2). However, in the case of wasps that had their sensory hairs trimmed from the abdomen there was a marked effect.

Figure 5 illustrates the results. For the pre-trim condition, the 10 eggs laid by the 10 wasps were all within 2 SDs of both the normal mean egg location and the normal mean egg angle (normal as documented for eggs laid on adult female roaches, see Fig. 2). All of these eggs hatched, and the larvae later successfully pierced the trochantinal membrane to feed. In contrast, after the hairs had been trimmed from the tip of the abdomen, 8 out of the 10 eggs were more than 2 SDs distant from the normal location, and 4 of the 10 eggs were laid at an angle that was more than 2 SDs from the normal egg angle (Fig. 5). Importantly, only 3 out of the 10 eggs from the post-trim condition were successful, with the larvae surviving and piercing the trochantinal membrane to feed (illustrated with green fill, but red outline, Fig. 5).

To further document the effect of hair trimming, additional egg-laying behavior was tracked for a number of the experimental wasps. For example, Figure 6 shows the subsequent oviposition sites from wasps A and F. Importantly, none of these eggs produced successful larvae that were able to pierce the membrane (online suppl. movie S2 shows 5 examples of abnormal oviposition after the sensory hairs had been trimmed off).

These results, along with additional cases not illustrated, amply demonstrated the importance of the abdominal hairs during oviposition. But the results also raised an additional question. Namely, why did the eggs laid in abnormal positions fail? The paradigm described above provided a unique opportunity to address this question. There are 2 essential requirements for larval survival: (1) first, the egg must remain attached to the cockroach until the larva hatches in 2–3 days, and (2) the hatched larva must find and pierce a soft membrane to obtain a continuous blood meal while further developing. These 2 facets of larval survival are addressed below.

Egg Failure

The female wasp attaches the egg to the cockroach with an efficient biological glue that is emitted along with the egg [Gnatzy et al., 2018]. However, the cockroach...
coxa is a complex surface offering limited options for secure adhesion. The large smooth central portion of the coxa (the coxal plate), as outlined in the figures, is the primary site of adhesion between the egg and the cuticle (arrow, Fig. 1b). Typically, only the caudal end of the egg is adhered, whereas the upper portion of the egg projects past a ridge and over a depression at the top front of the coxa. This normal egg position may be the result of a “topographical constraint” on the wasp’s choices. The adhesion point seems to be a compromise between the need for at least part of a smooth surface (the coxal plate) on which to glue the cylindrical egg and the need for the tip of the egg to be near the trochantinal membrane.

Fig. 5. Trimming the abdominal hairs at the tip of the wasp’s abdomen resulted in abnormal egg placement. Larva from green filled eggs survived to feed. Green outline indicates pre-trim condition. Red outline indicates post-trim condition. Larvae of only 3 out of the 10 post-trim eggs survived to pierce the membrane and take a blood meal (as indicated by a green-filled egg with a red outline). Larvae from red-filled eggs died without feeding (including cases where the egg was dislodged prior to hatching). For these data, the long axis of the target (trochantinal) membrane was used to establish the y axis, and the top of the trochantinal membrane was used to establish the location of the x axis. The top center of the egg was used as the reference point for plotting egg positions, whereas the long, central axis of the egg was used to determine egg angles (see Fig. 2 and Methods for details). The dotted line represents 2 SDs for the previously determined, normal mean egg position (Fig. 2). The gray triangle on the upper left of each coxa represents 2 SDs from the mean (normal) egg angle. All of the pre-trim trials fell within 2 SDs of the mean normal egg position and angle, and all of the larvae hatched and pierced the membrane to feed. After hair trimming, 8 out of 10 eggs were laid more than 2 SDs from the mean normal egg position, and 4 of the 10 eggs were laid at an angle that was more than 2 SDs from the normal egg angle.

These aforementioned considerations underscore a problem with all eggs that are laid too far rostrally or dorsally on the coxa (i.e., having displacements in the positive x and y coordinate system as shown in Fig. 2). The first, post-trimming eggs shown in Figure 5 for wasps C, D, E, and I were laid in such an abnormal position. These eggs were precariously glued along the top of a cuticular ridge at the front of the coxa. As a result, such eggs had only a fraction of the normal adhesive connection of a typical egg (e.g., Fig. 7a, b), and it was common for them to fall off (or be knocked off), as evidenced by finding the egg on the floor of the chamber in the first 24 h after oviposition.
There is also an obvious, but different, problem with eggs that are laid too far in the opposite direction – caudally on the coxal plate (e.g., wasp G in Fig. 5). Namely, the flat portion of the coxa is the area into which the femur may be retracted during the natural behavior of the cockroach. Although the zombified cockroach is markedly less active than an unstung cockroach, it is nevertheless common for the cockroach to move about within the chamber over the course of the first 48 h. If the cockroach retracts the femur sufficiently while moving, it will contact, push, and potentially dislodge eggs that are placed caudally on the coxa. Similarly, eggs that are simply rotated further clockwise from the normal position will necessarily have their caudal end projecting caudally on the coxa, also in a position to be pushed by the retracted femur.

Figure 7c–e shows images of different femur positions from an anesthetized cockroach (see also online suppl. movies for natural femur retracted – flexion – positions). These images underscore the potential problems for eggs laid in a myriad of abnormal positions and angles. Moreover, superposition of the previously determined average egg position onto the image of a retracted femur (Fig. 7e) reveals a remarkable congruence between the angle of the femur’s leading edge and the (average) angle of the long axis of the egg.

Failure of Larvae

Egg failure seemed most directly related to mechanical considerations of adhesion and egg position relative to the “moving parts” of the cockroach leg. In contrast, survival after hatching depends critically on the behavioral abilities, and limitations, of the larvae. Many eggs that were laid in unusual positions (i.e., more than 2 SDs from normal egg mean position) remained attached until hatching, and thus the behavior and fate of the larvae could be observed. The 5 cases illustrated in Figure 8 and described below include hatched eggs from wasps A–J (Fig. 5, 6). However, additional experimental cases were produced by removing the hairs from additional wasps (not illustrated). In total 12 larvae from eggs laid in unusual positions on the 2nd leg were tracked. None survived, and yet the observations provided many new insights, as described below.

Figure 8a illustrates an example from wasp D, with the egg laid in an unusually rostral and dorsal location. Despite the larva’s head having emerged within 500 μm of the most commonly pierced portion of the trochantinal membrane, this larva was unable to find the target. Importantly, this larva seemed doomed from the outset, because there is no evidence that a larva can move backwards. Therefore, it seems likely that, in general, larvae that hatch with their head projecting past the membrane are unlikely to obtain a blood meal and survive.
Figure 8b provides a different, but equally important example because this larva hatched with its head positioned directly over the second, candidate membrane (the membrane between the meron and the epimeron). This corresponded to Williams’s membrane “B” [Williams, 1942]. The larva was tracked (periodically) for 24 h. It spent the entire time trying to chew through the membrane and ultimately died in that location (inset Fig. 8b).

Figure 8c and d provides examples of larvae that hatched distant from the trochantinal membrane. Both of these larvae remained on the coxa, attempting to chew through the cuticle while periodically exploring to either side, and both ultimately died in place within 24 h. Finally, Figure 8e provides an example of a larva that moved substantially over the course of 2.5 h. Although it hatched relatively close to the lower portion of the trochantinal membrane, it subsequently moved further away in a counterclockwise direction until becoming lodged at a ridge near the joint with the femur where it remained and died (last panel, Fig. 8e).

What about eggs laid on the first or third pair of legs? Although this was never observed under natural conditions, the possibility is of theoretical interest as it may illuminate evolutionary constraints upon wasp behavior. This was briefly addressed by either damaging the smooth surface of the coxa on the 2nd legs, or by removing the 2nd legs entirely from cockroaches prior to the final molt. Under these experimental conditions, normal wasps laid...
one egg in the analogous coxal position of the front leg, and 3 eggs in the analogous coxal position of the 3rd leg. Despite there being only one case to consider for the front leg position, observation of the subsequently hatched larva shows this location is untenable for larval survival. This conclusion follows from the very different anatomical arrangement of the front leg and corresponding trochantinal membrane (as compared to the 2nd leg) which leaves a large gap between the larva’s head (red arrow, Fig. 9b) and the target (green arrow, Fig. 9b). Once extended partway over this gap, the larva is unable to move because no contact can be made to any part of the cuticle with the front portion of its body. In the case of eggs laid on the 3rd pair of legs – none of the larvae survived, although the reasons for failure were not obvious because the larvae were missing or dead within 4 days of oviposition.

All of the results described above illustrate a critical role for female wasp behavior, through use of sensory hairs on the abdomen, in determining the proper location for oviposition. This process takes between 1 and 2 min once it has been initiated during which time the wasp makes an extensive investigation of the coxa (Fig. 3b) before choosing the appropriate location (for 20 oviposition trials the mean time was 89 s from first contact to coxa to completion of oviposition, the SD was 26 s, see Methods). This requirement, in turn, raises a new challenge for the wasp to overcome, as outlined in the next section.
Part 2: A Different Challenge to Oviposition and Its Solution

Figure 10a shows the ventral side of a cockroach as the female wasp begins the next stage of the egg-laying process (the wasp’s abdomen is probing the underside of the roach – arrowheads). However, the scene highlights an obvious problem for this wasp – namely, the roach’s femur is blocking access to the oviposition site on the coxa. As will be illustrated below, the solution to the wasp’s dilemma is located close to the coxa, as indicated in Figure 10a.

The roach’s 2nd thoracic ganglion is positioned under the caudal portion of a midline sclerite – the basisternum. The second thoracic ganglion (part of the ventral nerve cord – Fig. 10b) contains the cell bodies of the motor neurons that control the 2nd pair of legs (Fig. 10c). Notice that the abdomen of the wasp in Figure 10a (arrowheads) is not exploring the coxa of the second leg, as would be expected for oviposition. Rather, it is extended behind the coxa of the first leg and toward the basisternum of the mesothorax (middle of the thorax). In fact, the wasp is delivering a sting below the mesothoracic basisternum, directed toward the second thoracic ganglion. Immediately after the sting (Fig. 10d), the cockroach extended its femur to uncover the target coxa and the wasp began the stereotyped exploratory behavior required for oviposition.

Detailed review and observation from high-magnification video of over 100 oviposition trials from dozens of wasps show that all wasps sting (part of the ventral nerve cord – Fig. 10b) contains the cell bodies of the motor neurons that control the 2nd pair of legs (Fig. 10c). Notice that the abdomen of the wasp in Figure 10a (arrowheads) is not exploring the coxa of the second leg, as would be expected for oviposition. Rather, it is extended behind the coxa of the first leg and toward the basisternum of the mesothorax (middle of the thorax). In fact, the wasp is delivering a sting below the mesothoracic basisternum, directed toward the second thoracic ganglion. Immediately after the sting (Fig. 10d), the cockroach extended its femur to uncover the target coxa and the wasp began the stereotyped exploratory behavior required for oviposition.

Detailed review and observation from high-magnification video of over 100 oviposition trials from dozens of wasps show that all wasps sting under the basisternum, followed by 1 sting to the front leg in 17 cases (in the remaining 3 cases, twice there were 2 stings under the basisternum followed by a sting to the base of the front leg, and once there was a single sting under the basisternum followed by a sting to the base of the front leg).

The separate stings below the basisternum were obvious because the stinger and sting sheath were entirely retracted between each sting. In addition, a fourth sting, in a different location, was also made 100% of the time by every wasp prior to oviposition. Although the stinger was often seen below the basisternum as evidence of stings, they are most clearly visualized by removing the cockroach front legs (e.g., Fig. 11b, and online suppl. movie S3).

The 4th, newly discovered sting was made at the base of the first leg, in a manner and location that resembled the very first sting made by wasps during the initial attack on the cockroach. Figure 11a illustrates and summarizes these newly discovered stings. Figure 11b shows frames captured from video during the 3 separate stings under the basisternum (4th sting not illustrated, but see online suppl. movies S3 and S4).

Given that these new observations greatly expand the “sting repertoire” of the emerald jewel wasp, it seems that a nomenclature of stings is necessary in order to avoid confusion. Therefore, I will hereafter refer to the previously well-documented 1st and 2nd stings (to the 1st thoracic ganglion and brain, respectively) as “sting 1” and “sting 2.” To these I will add stings “3, 4, and 5” which are made under the mesothoracic basisternum and “sting 6” which is made at the base of the front leg just prior to the onset of the search behavior that leads to oviposition on the coxa.

Although the function of sting 6 (at the base of the front leg) was not clear, the result of stings 3–5 (under the basisternum, followed by 1 sting to the front leg) in 17 cases (in the remaining 3 cases, twice there were 2 stings under the basisternum followed by a sting to the base of the front leg, and once there was a single sting under the basisternum followed by a sting to the base of the front leg).
Fig. 10. Cockroach leg positions present a potential challenge to oviposition. a In this case, the coxa of both middle legs is blocking access to the oviposition site. The wasp’s abdomen can be seen on the upper right (arrowheads). The wasp is not attempting to explore the coxa of the leg. Rather (as will be subsequently shown, e.g., Fig. 11), it is extending its stinger under the basisternum and toward the 2nd thoracic ganglion (dotted line). b Schematic of the cockroach ventral nerve cord showing the 2nd thoracic ganglion (T2). c Schematic of the 2nd thoracic ganglion and the 2 motor neurons that control the muscles that cause femur extension for the right leg. These send axons through the fifth nerve. d After the wasp has stung, the cockroach extends its femur and the wasp can explore the coxa to find the appropriate subsection for oviposition.
basisternum) could be readily observed, and the utility of these stings seemed obvious. Figure 11c summarizes the result of these stings from 10 trials for 10 different cockroaches. The angle between the coxa and the femur was measured (from the high-magnification video) at 3 time points: 30 s prior to the stings, at the initiation of the stings, and 30 s after the stings under the basisternum (note that left-side stings were flipped to the right side for consistency of data presentation and for uniform analysis – but wasps may sting to either side of the cockroach; under normal circumstances stings 3–6 were all made on the same side – the side chosen for oviposition). The angle between the coxa and the femur was measured for the middle and hind legs at each time point (the front legs are situated at a position that such measurements are difficult). An ANOVA (made independently for the mesothoracic [middle] legs and the metathoracic [hind] legs) indicates that the femur of the mesothoracic leg on the side of the sting has a significantly (p < 0.001) greater angle (mean of 50° greater) after the stings, whereas the other...
legs were not significantly different from the pre-sting condition.

In short, the stings under the mesothoracic basisternum and toward the ganglion that controls the target leg were usually followed by a subsequent leg movement that was favorable for oviposition (the same result was obvious in many of the previous trials examining the function of sensory hairs; however, because the roach’s hind legs were removed for those trials, those trials were not useful for comparative analysis of leg movements before and after stings). In some instances, both middle legs extended after the sting (online suppl. movie S4). More often, only the leg on the side from which the sting was delivered was affected.

Direct Evidence for Stings into the Ganglion
The observed leg movement, and its short latency relative to the stings, suggested the wasp might be stinging the cockroach directly into the 2nd thoracic ganglion. This possibility was strengthened by previous results.
[Haspel and Libersat, 2003] that have shown the wasp’s first sting (sting 1, preceding the brain sting) is made directly into the first thoracic ganglion. Previous studies made use of radioactive venom to localize the wasp’s injection site, as the early, pitched battle between the wasp and the cockroach makes for a difficult experimental preparation. In the present case, however, the wasp is stinging a pacified cockroach in a restricted location that can be readily recorded on high-magnification video.

This circumstance was leveraged by creating a surgical “window” through the cockroach cuticle, thereby exposing the ventral side of the 2nd thoracic ganglion, before offering the cockroach to a wasp. Specifically, a caudal portion of the basisternum was removed, while leaving the front portion of the basisternum intact (the front of the basisternum is an obvious landmark for insertion of the wasp’s stinger). As a result of this manipulation, it was possible to directly view (and record) the wasp’s stinger as it reached its target. The target was indeed the 2nd thoracic ganglion (Fig. 12). Moreover, in an impressive feat of marksmanship, the wasps stung into the side of the ganglion that controls the leg on the same side as oviposition. This latter determination was possible, because the tracheae on the ventral surface of the ganglion form a distinctive pattern on the left and right sides, thus delineating the center. Online supplementary movie S5, clip 1, shows a sting directly into the right side of the 2nd thoracic ganglion. The right side of the ganglion contains the motor neurons controlling the femur on the right side [Pearson and Iles, 1970; Iles and Pearson, 1971; Whittington, 1979; Denburg, 1982] – which has the potential to block the coxa where oviposition will later occur. Figure 12e shows the sting into the ganglion for a second case, this time into the left side of the ganglion (clip 2, online suppl. movie S5) also into the side of the ganglion that corresponds to the side of later oviposition.

Similar attempts to directly view the central target of sting 6 were not successful. One of the major impediments was the normal position of the front legs, the coxa of which were usually held tightly over the area into which sting 6 is directed. Additionally, unlike stings 3–5 which were made superficially below the basisternum, sting 6 was made more deeply, into thick connective tissue at the base of the front leg, making it challenging to see the stinger. However, in one case the stinger was briefly observed under the connective tissue at the base of the front leg, moving in the general direction of the 1st thoracic ganglion (online suppl. movie S6). Figure 13 summarizes the locations of the newly observed stings.

Additional Stings
After sting 6, wasps typically followed one of 2 different behavior patterns: (1) immediate transition to searching for the oviposition site, followed by oviposition, or

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**Fig. 13.** The approximate location of stings and their central targets in the cockroach.

- **a** Red arrows indicate approximately where the stinger enters the cockroach for each sting. The stings may be made on either side of the roach. However, stings 1 and 2 are generally both made from the side on which the wasp initially captures and holds the cockroach. Stings 3–6 are all made on the same side, but either side may be chosen. In the course of this study, individual wasps were observed to alternatively sting different roaches on different sides (e.g., they did not exhibit a fixed handedness for stings).
- **b** The central target of the different stings. Stings 1 and 2 have been previously shown to target the first thoracic ganglion (T1) and the brain and subesophageal ganglion, respectively. Stings 3–5 are made into the 2nd thoracic ganglion. The central target for sting 6 has not been determined.
(2) a pause during which the wasp backed away from the cockroach and often groomed for several minutes before returning. In the latter case, upon return the wasp usually stung the cockroach again, in a manner similar to that described above, but abbreviated. Specifically, wasps delivered a single sting below the basisternum and a single sting at the base of the front leg, before beginning the search and oviposition process. For sake of clarity, these 2 stings (which may be repeated multiple times) were designated sting 7 and sting 8 (Fig. 14). However, sting 7 was indistinguishable from stings 3–5, and sting 8 was indistinguishable from sting 6. Similarly, their function seemed analogous to these previous stings, in that during the wasp’s pause (after stings 3–5), the femur was seen to gradually become mobile once again in many cases. Stings 7 usually caused a repeat of the previously described extension of the femur, thus facilitating oviposition (online suppl. movie S7).

A serendipitous set of observations shed additional light on stings 7 and 8. Recall the experiments described above, in which the 2nd legs (including coxa) were either damaged or entirely removed (under CO₂ anesthesia). In such cases, rather than searching for the coxa and then abandoning the cockroach in its absence, the wasps often engaged in a repetitive series of behaviors, as if caught in a loop of sorts. The behavior consisted of stings 7 and 8 (to the basisternum and base of the front leg, respectively) followed by a search in the area that would normally be the location of the coxa. After failing to locate the target, the wasps backed away, and then returned to repeat stings 7 and 8. This was then followed, once again, by the search for the coxa. Online supplementary movie S8 shows an example for a wasp trying to oviposit on a cockroach that was missing the middle 2 legs (removed prior to the final molt). The wasp stung the cockroach over 60 times during the course of the recordings and continued thereafter. A similar behavior was observed when the coxa was damaged, and in one case after the sensory hairs were trimmed from the tip of a wasp’s abdomen. It seemed reasonable to suppose that under natural conditions, the
only reason the coxal plate would not be detected, would be when it was blocked by the femur. This follows because, unlike the more distal parts of the leg, the coxa is not autotomized (dropped) when a leg is grasped by a predator. In other words, it would be very unusual for a cockroach to lose this part of its body and survive.

To test the wasp’s response when the coxa was constantly blocked by the femur, the femurs of the 2nd legs of 2 cockroaches were glued in the retracted position. In both cases, wasps repeated the sting-search loop previously described. Online supplementary movie S9 shows part of this sequence for one example, during which the cockroach was stung over 120 times before it eventually became active and exited the oviposition chamber at which point filming was aborted.

**Discussion**

The original goal of this study was to simply investigate the hairs on the tip of the abdomen of the emerald jewel wasp to learn more about their potential role as sensors during the oviposition process [Veltman and Wilhelm, 1991; Gnatzy et al., 2018]. As often happens, the study expanded from there – in 2 different directions. The first expansion was an investigation of the wasp larvae and their choice of targets for a blood meal and subsequent entry into their cockroach host. This was required to understand the importance (or lack thereof) of proper egg placement by the female wasp – and by extension the importance of sensory feedback during oviposition. The second expansion was based on the surprising and entirely unexpected discovery of a new suite of stings by the wasp, made just prior to oviposition. It seemed warranted to include all of this research in a single manuscript, because the data are unified by an overarching theme – the need for proper egg placement by the wasp in order for the larvae to succeed.

Although there have been many studies of the jewel wasp’s remarkable reproductive strategy, little attention has been paid to the location at which larvae feed after hatching. Perhaps the most detailed discussion of this aspect of wasp development comes from Williams’ seminal 1942 paper [Williams, 1942]. Williams points out that the wasp’s egg is typically laid (on either one of the 2nd pair of legs) such that the upper portion is “very near to one of 2 delicate membranous areas that help hinge the leg to the thorax”. In Figure 1, I retained Williams’ designation of these 2 membranes as “A” and “B” referring to the lower and upper membrane, respectively. Williams further states that “A and B” show membranes through which the wasp grub bites its way into the interior of the cockroach. The membrane at “A” seems the more frequent of the 2.

In contrast to Williams’ suggestion that either membrane might be used, the results of the present study suggest that larvae are constrained to use the larger, lower membrane (“A” in Fig. 1) of the 2nd pair of legs, in order to obtain a blood meal (I refer to this membrane as the trochantinal membrane). The evidence for this conclusion comes from 100 documented cases of normally laid eggs, in addition to many documented abnormally laid eggs, some of which resulted in larvae attempting (but failing) to break through membrane “B” (between the meron and the epimeron).

When considering potential targets that were further afield, there was no evidence that larvae could use the other legs to obtain a blood meal (although the data were limited). In the case of the roach’s front legs, the space between the coxal plate and the trochantinal membrane appears to be far too great to be bridged by developing larvae (Fig. 9). In the case of the rear legs, it was not clear why the 3 eggs laid in this location failed, as the anatomy of the coxal plate and trochantinal membrane appeared similar to that of the 2nd pair of legs.

In any case, it seems clear that the newly hatched larva has very limited choices about where to obtain a blood meal from its host – *Periplaneta americana*. This may represent a barrier that limits the species of cockroach that could, potentially, serve as hosts for the emerald jewel wasp. Presumably, the larvae would have to find a similarly vulnerable membrane on any other species on which it hatched, and such might not exist. And, there is another potential barrier to a larva in its choice of feeding spots. When the later (much larger) instar larva enters its host, it typically destroys the entire trochantinal membrane and detaches the upper suture that connects the trochantin to the rest of the cuticle. This creates a relatively large hole that can later be readily seen on close examination of late-stage parasitized roaches. And yet even with this relatively large opening, the much larger larva appears to take much effort to slowly squeeze its soft body into the cockroach, and in some few cases it does not succeed. It is possible many other soft, membranous points on the cockroach would not offer a suitable entry point, even if a blood meal could be obtained (on a different note, the immune system of other, atypical hosts may represent a second challenge, e.g., Lackie [1975]).

The finding that larvae seem to be constrained to make use of a single small location (on either the right or left side) of the cockroach emphasizes the critical importance
of the female jewel wasp’s precision when it comes to oviposition. Clearly, the sensory hairs at the tip of the abdomen play an important role in this process, as has been previously suggested [Gnatzy et al., 2018; Veltman and Wilhelm, 1991]. The removal of these hairs resulted in a myriad of poorly placed eggs. Importantly, wasps that had these hairs trimmed off were still able to accurately target their stings. Thus, the sensory hairs at the tip of the abdomen seem most important for oviposition. This result not only clarified the function of these prominent hairs, but also provided a unique opportunity to track and observe larvae that hatched in abnormal locations.

The result of the latter observations reinforced the importance of the wasp’s choice of oviposition site. Larvae that hatched in unusual locations demonstrated little ability to find the target membrane. Hours of microscopic video revealed that first instar larvae are capable of slow forward movements, but often moved less than a millimeter before dying in the first 24 h if no blood meal was obtained. While attempting to find the target membrane, most larvae periodically bent the upper half of their body to either the left, right, or upward. These apparently exploratory movements brought the head and jaws into contact with parts of the cockroach exoskeleton in a fan-shaped region in front, and on either side of the larva. And yet many larvae spent long periods of time attempting to chew through tough, impenetrable cuticle in between such exploratory movements, before they died. This came as a surprise, because one could easily imagine the main challenge to survival being egg adherence until hatching, after which larvae might use any of a number of landmarks or chemical cues to move towards and identify the target membrane. Such was not the case.

The results discussed above, highlighting the importance of the oviposition site, provide a context for interpreting the function of the newly discovered stings. It is not only the case of a retracted femur on the 2nd leg that presents a potential obstacle to the wasp. Rather the femur might interfere at any point during oviposition, because the position of the femur at any given time is transitory. Although the wasp can (and often does) pull the cockroach forward by an antenna before initiating oviposition, that does not ensure the femur will end up extended (or that it will remain extended) for the duration of the search and egg-laying process, which takes between 1 and 2 min. Also, recall from Figure 3b that the typical sequence of abdominal movements used to locate the oviposition site requires access to the entire dorsal half of the coxa, not merely the smaller wedge of surface where the egg is eventually deposited. Moreover, as has been thoroughly documented [Liberstat and Gal, 2013], the zombified cockroach is still capable of the full range of movements if sufficiently stimulated. The wasp’s 1–2 min of continuous tactile probing on the roach’s vulnerable underside is a good candidate stimulus for eliciting leg movements, during which the femur may be retracted over the coxal plate.

The wasp’s solution to this dilemma is a further testament to their remarkable ability to manipulate the central nervous system of the cockroach. The newly discovered bout of stinging includes 3 successive stings into the 2nd thoracic ganglion. This can be confidently inferred from the short-latency (2–5 s) reaction of the leg musculature that often occurs after each of these stings, and the direct visualization of the stinger entering the ganglion when the ganglion was exposed (Fig. 12 and online suppl. movie S5).

In further testament to the wasp’s skill at manipulating its host, the wasps stung into the side of the ganglion ipsilaterally to oviposition. It should be noted however that it was fairly common to see a reaction from both of the middle legs after the stings (e.g., online suppl. movie S4). This might be the result of stings that were not restricted to a single side of the ganglion, or it might be due to diffusion of venom. The latter seemed likely on some occasions, when the contralateral leg responded with some delay after the ipsilateral leg.

Although I have emphasized the response of the femur, it seemed clear on many occasions that a wide range of leg musculature was strongly activated by the sting. This was evident from the extreme expansion of the joints at the base of the leg that coincided with hyperextension of the femur (online suppl. movie S7). And yet femur extension seems to be the key result for the wasp. I should point out that the femur’s response to the stings is quite variable. On some occasions the movement was subtle, consisting of only a slight extension of the ipsilateral femur, but almost inevitably also including an expansion of the space between the coxa and the basisternum. It may be that on some occasions the distal aspect of the roach’s leg was in direct contact with the side of the filming chamber, thus preventing the femur from extending. On other occasions the leg extended so dramatically (though usually smoothly) that the cockroach was nearly flipped on its back.

Venoms are generally a complex mixture with correspondingly complex effects on an animal’s physiology, and the jewel wasp seems to be a prime example of this truism [Moore et al., 2006, 2018; Banks and Adams, 2012; Arvidson et al., 2016, 2019; Kaiser et al., 2019]. Neverthe-
less, for the case of the wasp’s stings to the 2nd thoracic ganglion, it seems reasonable to hypothesize about a mechanism based on the well-known anatomy and neurophysiology of the roach locomotor system. The femur is extended by the coxal depressor muscles, which are in turn innervated by 2 motor neurons with cell bodies located in the ventral portion of the ganglion (the wasp stings into the ventral portion of the ganglion). Moreover, the comparatively smooth and slow hyperextension of the leg on many occasions suggests the primary effect might well be at the “slow” motor neurons in the ganglion (including “Ds,” the slow motor neuron innervating the coxal depressor muscle). Activation of this final common pathway by a cholinergic agonist, or perhaps even by acetylcholine, would presumably cause leg extension. In fact, acetylcholine is a prominent component of some wasp venoms [e.g., Piek, 1986]. However, as far as the author knows, acetylcholine has not been identified in the jewel wasp’s venom [Banks and Adams, 2012; Arvidson et al., 2016, 2019; Moore et al., 2018; Kaiser et al., 2019]. And yet, milked venom for previous analysis is presumably taken from wasps prior to sting 1 and sting 2. Perhaps the long delay between these early stings and stings 3–5 would allow for synthesis of other venom components? The tractability of the roach’s well-known and simple neural circuit that controls leg movements, and the simplicity of the cockroach response, might allow this piece of the wasp’s venom puzzle to be solved.

The function of sting 6 remains a mystery. Given that every other sting made by the jewel wasp targets the CNS, and given that sting 1 is directed into the first thoracic ganglion [Haspel and Libersat, 2003], it seems likely the same is true for sting 6 (which resembles sting 1). There was no obvious effect noted for sting 6, but perhaps the function of sting 6 is analogous to the function of sting 1, that is, preventing the cockroach from using its front legs to interfere with the wasp, in this case with oviposition (sting 1 prevents the front legs from interfering with sting 2 to the brain and subesophageal ganglion). Additional studies are needed to clarify the target and function of sting 6.

On many occasions, the cockroach was stung additional times after sting 6. This occurred when the wasp completed stings 3–6 but then paused in the oviposition process. Although the reason for a pause in the oviposition process was not obvious, the reason for an additional bout of stinging seemed clear; the extension of the femur resulting from stings 3 to 5 lasted only a few minutes. When the wasps paused, it was common for the femur to retract or be re-engaged in the normal sequence of leg movements that happen as the cockroach moves about. When the wasp returned to the cockroach to resume the process of oviposition, an additional sting to the ganglion (followed by sting 6 to the base of the front leg) usually caused at least some re-extension of the femur (online suppl. movie S7) facilitating oviposition.

I have referred to these latter stings as sting 7 and sting 8 for clarity, but they seem in every way identical to stings 3–5 and sting 6, respectively. Stings 7 and 8 took on more significance when they were noted to recur many dozens of times under certain unusual conditions. This was first discovered during experiments that were aimed at getting the wasp to oviposit on the 3rd pair of legs (to assess larval survival in this unusual location). It was presumed that removal of the 2nd pair of legs (coxa included) would result in oviposition on the hind legs, because they are anatomically similar to the middle legs. This was, in fact, borne out on several occasions. But before the wasp’s increasingly desperate-seeking search for an oviposition site expanded to encompass the hind legs, it was caught in a loop of behavior that included repeated bouts of sting 7 and sting 8, followed by a search for the coxal plate of the 2nd leg. Wasps might switch sides in the course of this search, at which point the looping behavior was re-engaged (both 2nd legs were missing). The duration of some of these behavioral loops was remarkable, as it seemed the wasp should be exhausted after hours of stinging. On 2 occasions the cockroach was sufficiently stimulated by the repeated piercing of its sensitive underside (over 120 stings) that the wasp’s zombifying “spell” was broken, and the cockroach ran from the chamber under its own power, at which point the wasp gave up its efforts. A similar, repetitive stinging behavior was observed when the coxal plate was badly damaged (cauterized) bilaterally, leaving a deep depression in the usually smooth plate.

Under natural conditions, the absence of the smooth coxal plate would almost always be caused by a blocking femur, because roaches do not normally lose the coxa (whereas loss of the leg at the trochanter joint is relatively common). Presumably, over the wasp’s evolutionary history the usual solution to an “absent” coxal plate would be a sting to the 2nd thoracic ganglion, thus moving the femur out of the way. In support of this possibility, the repeated bouts of stinging were observed when the femurs of 2 cockroaches were glued in the retracted position (covering the coxal plate) thus mimicking a natural condition that might be (briefly) encountered by the wasp.
Getting the Most Out of Your Zombie

Here I suggest a possible twist on the common mechanism for triggering a fixed action pattern [Lorenz, 1981]. Rather than being triggered by a sign stimulus (releaser) that has one or a few hallmark features, it may be that jewel wasps engage a fixed action pattern in the absence of a stimulus (the stimulus being the smooth coxal plate). This interpretation would account for the repetition of the stings and search that occurred under all of the observed conditions: that is, when the smooth coxal plate was damaged, when the second legs were missing entirely, and when the femur permanently blocked the coxal plate. If so, the result is a cautionary tale for interpreting animal behavior. Had the femurs been glued in place at the outset, the interpretation might be instead that a fixed action pattern was triggered by the stimulus of the blocking femur, rather than the absence of the coxal plate.

Finally this investigation emphasizes the unpredictable outcome of any given investigation. It is only by happenstance that the first part of this study – examining the wasp’s sensory hairs – led to microscopic examination of the events surrounding oviposition, which normally occur in darkness. It was this closer look at one subject (oviposition) that brought an unexpected subject (new stings) into clear focus.

References

Arvidson R, Kaiser M, Lee SS, Urenda JP, Dail C, Mohammed H, et al. Parasitoid jewel wasp mounts multipronged neurochemical attack to hijack a host brain. Mol Cell Proteomics. 2019 Jan;18(1):99–114.

Arvidson R, Kaiser M, Pan S, Liberst F, Adams ME. Bioinformatic and Functional Analysis of Venom from the Jewel Wasp Ampulex compressa. FASEB J. 2016;30(51):819.1.

Banks CN, Adams ME. Biogenic amines in the nervous system of the cockroach, Periplaneta americana following envenomation by the jewel wasp, Ampulex compressa. Toxicon. 2012 Feb;59(2):320–8.

Catania KC. How Not to Be Turned into a Zombie. Brain Behav Evol. 2018;92(1–2):32–46.

Comer CM, Dowd JP. Multisensory processing for movement: antennal and cercal mediation of escape turning in the cockroach. In Proceedings of the workshop on “Locomotion Control in Legged Invertebrates” on biological neural networks in invertebrate neuroethology and robotics. San Diego, Academic Press; 1993. pp 89–112.

Comer CM, Mara E, Murphy KA, Getman M, Mungy MC. Multisensory control of escape in the cockroach Penplaneta americana. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 1994;174(1):13–26.

Denburg JL. Identified motor neurons in the cockroach exhibit stereotypic peripheral branching and nerve terminal structure. J Comp Neurol. 1982 Jul;208(4):325–34.

Fouda K, Liberst F, Rathmayer W. The venom of the cockroach-hunting wasp Ampulex-Compressa changes motor thresholds–a novel tool for studying the neural control of arousal. Zool- Anal Complex Sy. 1994;98:23–34.

Fox EG, Bressan-Nascimento S, Eizenberg R. Notes on the biology and behavior of the jewel wasp, Ampulex compressa (Fabricius, 1781)(Hymenoptera: Ampulicidae), in the laboratory, including first record of gregarious reproduction. Entomol News. 2009;120(4):430–7.

Gal R, Rosenberg LA, Liberst F. Parasitoid wasp uses a venom cocktail injected into the brain to manipulate the behavior and metabolism of its cockroach prey. Arch Insect Biochem Physiol. 2005 Dec;60(4):198–208.

Gnatzy W, Volkandnt W, Dzwooneck A. Egg-laying behavior and morphological and chemical characterization of egg surface and egg attachment glue of the digger wasp Ampulex compressa (Hymenoptera, Ampulicidae). Arthropod Struct Dev. 2018 Jan;47(1):74–81.

Haspel G, Liberst F. Wasp venom blocks central cholinergic synapses to induce transient paralysis in cockroach prey. J Neurobiol. 2003 Mar;54(4):628–37.

Haspel G, Rosenberg LA, Liberst F. Direct injection of venom by a predatory wasp into cockroach brain. J Neurobiol. 2003 Sep;56(3):287–92.

Iles JF, Pearson KG. Coxal depressor muscles of the cockroach and the role of peripheral inhibition. J Exp Biol. 1971 Aug;55(1):151–64.

Kaiser M, Arvidson R, Zarivach R, Adams ME, Liberst F. Molecular cross-talk in a unique parasitoid manipulation strategy. Insect Biochem Mol Biol. 2019 Mar;106:64–78.

Liberst F, Gal R. Neuro-manipulation of hosts by parasitoid wasps. Recent Advances in the Biochemistry, Toxicity and Mode of Action of Parasitic Wasp Venoms. Research Signpost; 2007. pp. 93–114.

Liberst F, Gal R. What can parasitoid wasps teach us about decision-making in insects? J Exp Biol. 2013 Jan;216(1):47–55.

Liberst F, Gal R. Wasp voodoo rituals, venom-cocktails, and the zombification of cockroach hosts. Integ Comp Biol. 2014 Jul;54(2):129–42.

Lorenz K. The foundations of ethology. New York: Springer-Verlag; 1981.

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Statement of Ethics

“Animal experiments conform to internationally accepted standards.” The ethics statement is not applicable, as there are no review boards for work on insects.

Conflict of Interest Statement

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Moore EL, Arvidson R, Banks C, Urenda JP, Duong E, Mohammed H, et al. Ampulexins: A New Family of Peptides in Venom of the Emerald Jewel Wasp, Ampulex compressa. Biocchemistry. 2018 Mar;57(12):1907–16.

Moore EL, Haspel G, Libersat F, Adams ME. Parasitoid wasp sting: a cocktail of GABA, taurine, and β-alanine opens chloride channels for central synaptic block and transient paralysis of a cockroach host. J Neurobiol. 2006 Jul;66(8):811–20.

Pearson KG, Iles JF. Discharge patterns of coxal levator and depressor motoneurones of the cockroach, Periplaneta americana. J Exp Biol. 1970 Feb;52(1):139–65.

Piek T. Synergistic effects of agonists and antagonists in insect venoms—a natural way of insecticidal action. Pestic Sci. 1987;19(4):317–22.

Piek T, Visser JH, Veenendaal RL. Change in behaviour of the cockroach, Periplaneta americana, after being stung by the sphecid wasp Ampulex compressa. Entomol Exp Appl. 1986;35(2):195–203.

Richardson BA, Paul RK. Quantitative aspects of behaviour and reproduction in the Jewel wasp Ampulex compressa. Int Zoo Yearb. 1993;32(1):177–83.

Veltman J, Wilhelm W. Husbandry and display of the Jewel wasp: ampulex compressa and its potential value in destroying cockroaches. Int Zoo Yearb. 1991;30(1):118–26.

Weisel-Eichler A, Haspel G, Libersat F. Venom of a parasitoid wasp induces prolonged grooming in the cockroach. J Exp Biol. 1999 Apr;202(Pt 8):957–64.

Whittington PM. The specificity of innervation of regenerating motor neurons in the cockroach. J Comp Neurol. 1979 Aug;186(3):465–71.

Williams FX. Ampulex Compressa (Fabr.). A Cockroach-Hunting Wasp Introduced from New Caledonia Into Hawaii. Proc Haw Ent Soc. 1942;11:221–33.

Ye S, Leung V, Khan A, Baba Y, Comer CM. The antennal system and cockroach evasive behavior. I. Roles for visual and mechanosensory cues in the response. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2003 Feb;189(2):89–96.