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Life History of the Emerald Jewel Wasp *Ampulex compressa*

Ryan Arvidson[1,3], Victor Landa[2], Sarah Frankenberg[3], Michael E. Adams[1,2,3]

1 *Graduate Program in Biochemistry and Molecular Biology, University of California, Riverside, CA, 92521, USA* **2** *Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA* **3** *Department of Entomology, University of California, Riverside, CA 92521, USA*

Corresponding author: *Michael E. Adams* (michael.adams@ucr.edu)

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Abstract

The Emerald Jewel Wasp *Ampulex compressa* (Fabricius) is an endoparasitoid of the American cockroach *Periplaneta americana* (Linnaeus). Its host subjugation strategy is unusual in that envenomation is directed into the host central nervous system, eliciting a long-term behavior modification termed hypokinesia, turning stung cockroaches into a lethargic and compliant, but not paralyzed, living food supply for wasp offspring. *A. compressa* manipulates hypokinesic cockroaches into a burrow, where it oviposits a single egg onto a mesothoracic leg, hatching three days later.

Herein we describe the life history and developmental timing of *A. compressa*. Using head capsule measurements and observations of mandibular morphology, we found that the larvae develop through three instars, the first two ectoparasitoid, and the third exclusively endoparasitoid. The first two instars have mandibles sufficient for piercing and cutting the cuticle respectively, while the third instar has a larger and blunter mandibular structure. During ecdysis to the third instar, the larva enters the body cavity of the cockroach, consuming internal tissues selectively, including fat body and skeletal muscle, but sparing the gut and Malpighian tubules. The developmental timing to pupation is similar between males and females, but cocoon volume and mass, and pupation duration are sexually dimorphic. Further, we show that the difference in cocoon mass and volume can be used to predict sex before eclosion, which is valuable for studies in venom pharmacology, as only females produce venom.

Keywords

Instar, Dyar's rule, pupation, development, parasitoid, *Periplaneta americana*

Introduction

The Emerald Jewel Wasp *Ampulex compressa* (Hymenoptera, Ampulicidae) employs a unique strategy to subdue and exploit its host, the American cockroach *Periplaneta americana* (Blattodea, Blattidea). Upon encountering the cockroach, *A. compressa* hunts and aggressively attacks its prey (Keasar et al. 2006). The attack is characterized by two stings: first a swift sting into the thorax resulting in transient paralysis of the prothoracic legs, followed by a sting into the head cavity, targeting both the subesophageal ganglion and brain (Haspel et al. 2003). The head sting is remarkably precise, as the wasp is able to sense the location of cephalic ganglia through mechanosensory inputs from the stinger (Gal et al. 2014). Envenomation of head ganglia induces long-term (7–10 days) behavioral changes characterized by increased escape threshold, decreased escape distance, and decreased spontaneous walking. This modification in behavior, known as hypokinesia, facilitates the parasitization process by rendering the cockroach submissive to manipulation by the wasp (Fouad et al. 1996). After the cockroach is subdued, the wasp clips the antennae at precise locations with buzzsaw actions of its mandibles, then commences to drink hemolymph, using the antennal stumps as straws (Piek et al. 1984) (video 1:45). The wasp leads the stung cockroach into its burrow by grasping the truncated antennae (Veltman and Wilhelm 1991) and proceeds to lay a single egg on a mesothoracic leg of the host, then entombs it by sealing the burrow entrance with miscellaneous debris (Williams 1942). This provides the young wasp larva with a fresh food supply during its development. More recently it has been shown that *A. compressa* larvae secrete antimicrobial compounds into the cockroach as it is being consumed to sanitize and perhaps preserve the host (Herzner et al. 2013; Weiss et al. 2014).

A. compressa larvae hatch approximately three days after oviposition (Fox et al. 2009). The first instar larva pierces the soft cockroach cuticle near the base of the leg to feed on hemolymph. The animal remains in this position through the second instar, continually obtaining nourishment from host hemolymph (Fox et al. 2006). At the end of the second instar, the larva enters the body cavity of the still-living cockroach and begins to consume internal organs in preparation for pupation. Once ready to pupate, the larva spins a cocoon with silk that forms two layers: a hard shell that encases the pupa and a thick, woven silk outer covering that surrounds the shell (Williams 1942). Pupal development time lasts several weeks, after which the adult emerges from the desiccated husk of the cockroach to complete the life cycle.

Herein we describe that *A. compressa* develops through three instars and characterize the unique mandibular structures specific to each instar as well as the developmental time course for both male and female wasps. We report that the third instar larva consumes organs of the host selectively, presumably to preserve its life until development is completed. Lastly, we show that measurements of cocoon volume or mass can be predicative of sex, since females are larger than males.

Materials and methods

Animal husbandry

Ampulex compressa adults were reared in 2 ft^3 plexiglass cages and provided with honey and water ad libitum. One female and two to three males were maintained in each breeding cage. Cockroaches were reared in 55-gallon plastic containers with an 18 volt electric barrier along the perimeter at the top of the container with dry dog food and water *ad libitum*. All insects were maintained at 28 °C and ~50% humidity, on a 16:8 light: dark cycle. Adult female cockroaches were provided to female *A. compressa* for parasitization five times a week but no more than one in 24 hours. Female cockroaches were chosen for parasitization because they contain a significantly larger store of fat body, which provides more energy for the developing larva. Wasp development was completed in French vials in a humidified incubator. Eclosed wasps were placed into gender-specific holding cages.

Larval and pupal development duration

Differences in development times between male and female *A. compressa* were determined relative to the time a cockroach host was introduced into *A. compressa* cages, the time between parasitization and cocoon spinning, and the time between cocoon spinning and eclosion. Sex of the wasp was determined upon emergence. Seven breeding cages were maintained, each containing a single female wasp and one to three males. Cockroaches were introduced once a day, five days a week for approximately one year. When a female wasp would die or lose fecundity, she was replaced with a 10-day posteclosion, virgin female.

Cocoon volume and adult mass

Since pupae of male and female *A. compressa* are visually similar, we distinguished gender prior to eclosion by measuring cocoon volume and mass. To accomplish this, cocoons from thirty parasitized cockroaches were gently removed from the cockroach husk and silk was removed to allow measurements of mass, length and width. Out of the thirty selected, eight were female, 21 were male, and one failed to eclose. Since the cocoon is a prolate spheroid, similar to a parabola revolving about its axis, the following equation was derived to obtain volume as a function of length and width, where l is the long axis of the cocoon, L is length of the cocoon at the longest point and W is the corresponding width. These data were used to generate a binary logistic regression analysis useful for predicting the probability of a female eclosing from a cocoon based on its volume or mass.

Eq. 1 $$Volume \sim \pi \int_0^L [(-W/2)/(L/2)^2(l - L/2)^2 + W/2]^2 \, dl$$

Head capsule measurements

Head capsule measurements were taken from 24 larvae varying in age over a 10-day period. The age of the larva was referenced to time of oviposition. Larval body length and width were measured using a calibrated ruler. Head capsule width was taken as distance between the eyes and length from mandibles to head apex. The product of length and width yielded head capsule area. Head capsule area was used instead of length or width to emphasize differences between instars.

Electron microscopy

For each *A. compressa* larva measured for head capsule size, mandibles were dissected and imaged under a Hitachi TM-1000 tabletop scanning electron microscope at the Microscopy Core at the Institute of Integrative Genome Biology at the University of California, Riverside.

Data analysis and figure generation

Data for Figures 1–3 were analyzed on Prism Graphpad version 7.02. Photoplates in Figures 4–6 were generated with Adobe Photoshop version CS5. Probability of female emergence was determined using logistic regression from cocoon volume and mass data.

Results

Over the course of the study, of 1896 cockroaches introduced to female *A. compressa*, 1656 were parasitized, and 1033 eggs progressed to pupation, of which 962 emerged as adult wasps. Of those, 135 (14%) were female and 818 (80%) were male. We observed no sexual dimorphism in duration of larval development. In contrast, pupal development time, defined as the interval between pupation and adult eclosion, shows significant sexual dimorphism (Fig. 1A). The mass of newly eclosed adult wasps also differs significantly between males and females (Fig. 1B). Given that adult mass and cocoon volume are sexually dimorphic, the probability that a female will emerge from a cocoon can be estimated using cocoon volume (Fig. 2A) or mass (Fig. 2B).

We observed that *A. compressa* develops through three larval instars distinguished by quantum changes in head capsule size over the developmental period of eight days (Fig. 3). Average head capsule length and width by instar are listed in Table 1. We

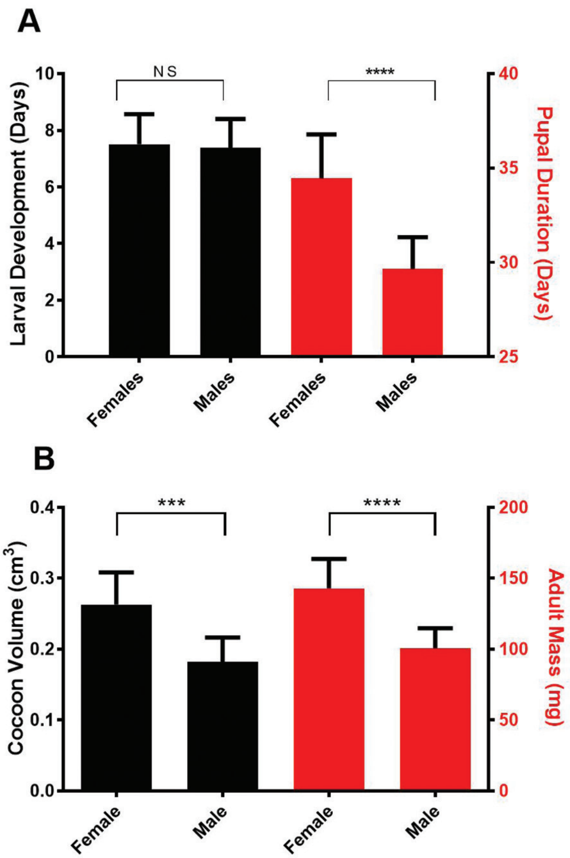

Figure 1. *A. compressa* development time and adult size are sexually dimorphic. **A** Larval development as defined by the duration between egg laying and pupation shows no difference between males and females (black, $p = 0.26$). Pupal duration - time between cocoon spinning and eclosion - is sexually dimorphic (red, $p < 0.0001$). Statistical difference determined by Welch's T test, NS = not significant, **** = $p < 0.0001$. (n = 200, males; n = 135, females) **B** Cocoon volume (black) of females is significantly larger than that of the males. (female n = 8, male n=21). Adult mass (red) is significantly larger for females (Female n = 8, male n=17). (*** = $p < 0.001$, **** = $p < 0.0001$, by Welch's T test). Error bars indicate standard deviation.

observed three distinct mandibular morphologies during larval development (Fig. 4). Mandibles of the first instar are ~100 µm in length. They are slender and pointed, with 2-3 teeth at the end (Fig. 4A). Mandibles of the second instar are ~250 µm long and have a serrated edge (Fig. 4B). Third instar mandibles are larger still at 325 µm and are relatively blunt (Fig. 4C). A comparison between all mandibular morphologies is shown in Fig. 4D.

The first instar hatches within 3 days of oviposition, the second instar appears during the 4th day of development, the third and final instar appears on day 6 with head capsule about double the size of the first instar. First and second instar larvae were observed in all cases to develop outside the cockroach, whereas the third instar is spent

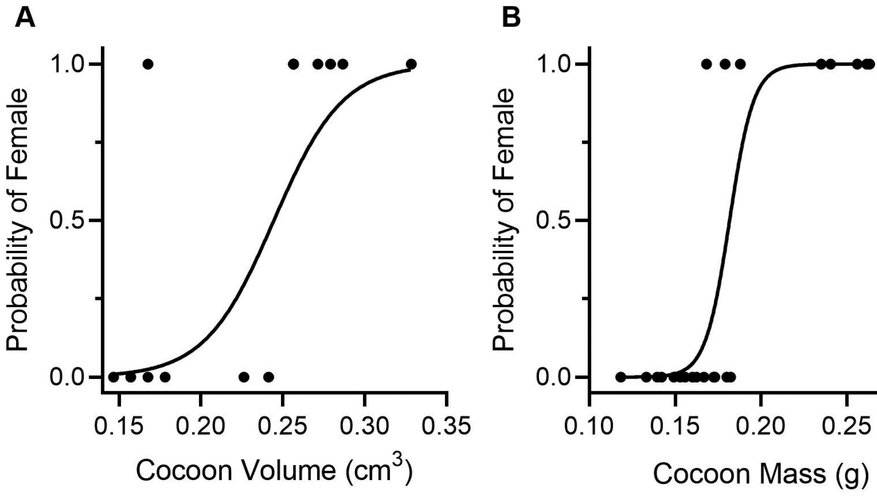

Figure 2. Cocoon mass and volume can be predictors of sex. The probability that a cocoon will emerge as a female is given as **A** a function of pupal volume (p < 0.0001, Chi Square = 16.5), and **B** a function of cocoon mass (p < 0.0001, Chi Square = 19.6)

Table 1. Size of larval body and head capsule by instar. n = 6 for instar 1, n = 5 for instar 2, and n = 13 for instar 3.

	Larva		Head Capsule	
Instar	Length (mm)	Width (mm)	Length (μm)	Width (μm)
1	3.53 ± 0.77	1.07 ± 0.18	514 ± 36	561 ± 128
2	10.0 ± 2.88	4.00 ± 0.99	885 ± 88	780 ± 72
3	28.4 ± 7.27	8.79 ± 2.06	1181 ± 104	885 ± 64

exclusively inside of the host. Ecdysis from second to third instar appears to occur upon entry into the cockroach body cavity. This is corroborated by appearance of larval cuticle on the outside of the cockroach near the entry wound (video 4:25). We were not able to observe shedding of cuticle resulting from ecdysis to the second instar, however it has been reported that layers of cuticle appear between larva and host, indicating ecdysis occurs while larvae develop on the outside of the cockroach (Williams 1942).

Once inside the host, the third instar larva consumes all fat body and muscle within its reach, even removing muscle from the coxa and femur of the prothoracic legs. After separating itself from the gut with a layer of silk, the wasp spins a cocoon and pupates inside the cockroach, where it develops into an adult after several weeks and ecloses, ready to mate and hunt, completing the life cycle (Fig. 5). Priority tissues for consumption during the third instar clearly are muscle tissue and fat body, which were first observed to be missing following entry of the larva into the host. Next consumed were the tracheal system, ovaries and accessory glands. The final "meal" was the central nervous system, though a significant portion of the ventral

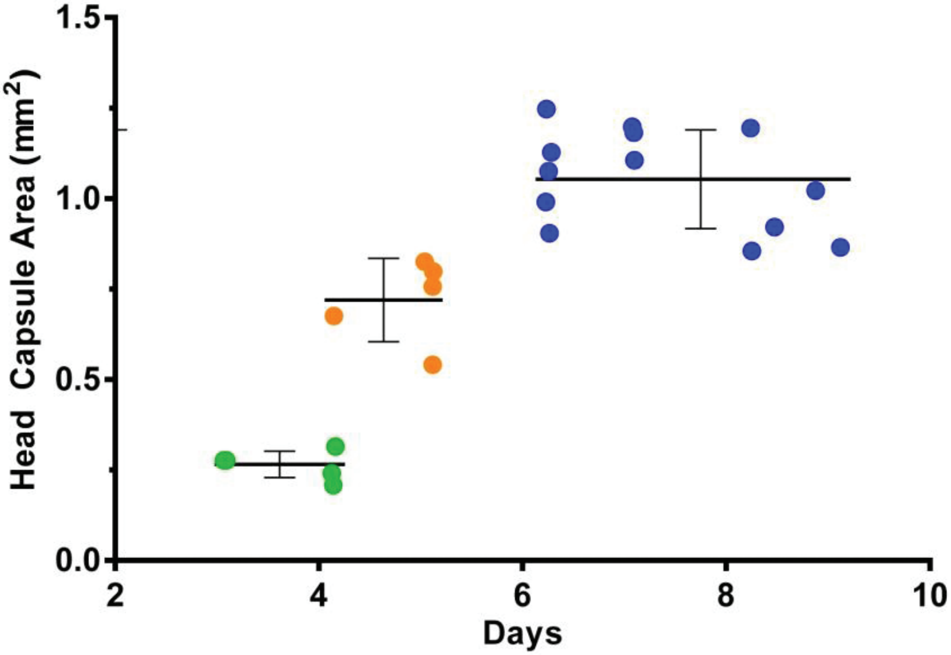

Figure 3. *Ampulex compressa* larvae develop through three instars. The number of instars of *A. compressa* were determined by taking representative larva from different time intervals and plotting the product of the length and width of the head capsule (area) against age of the larva. The data plots as three distinct groups, indicative of three instars (green - first instar, orange - second instar, blue - third instar. Horizontal bars represent average area of the head capsule in each instar and vertical error bars are standard deviation. Each mean head capsule size is significantly different from the others, as indicated by the Kruskal-Wallis test ($p < 0.0001$).

nerve cord remained following wasp pupation. The gut also remains intact following pupation (Fig. 6). Eventually the host desiccates without rotting and the wasp pupa develops in its surrogate womb.

Discussion

Ampulex compressa is a remarkable wasp renowned for its unique host subjugation strategy, in essence turning the cockroach into a "zombie" that becomes a complacent, living source of nourishment for the single offspring (Gal and Libersat 2010). Like all parasitoids, *A. compressa* has evolved an instinctive recognition of its host. We observed that this instinctive familiarity goes beyond just adult behavior, as the larva displays selectivity in its consumption of host tissues. By avoiding the gastrointestinal tract and CNS and focusing instead on fat body, muscle and ovaries, host lifespan may be pro-

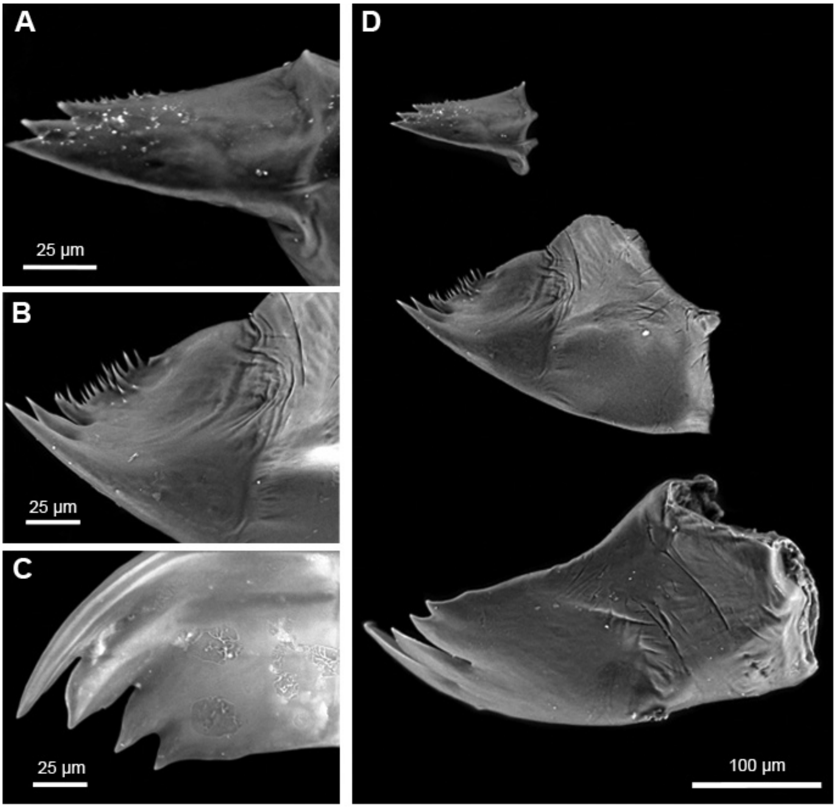

Figure 4. Comparative morphology of mandibles of the three larval instars of *A. compressa*. Mandible morphology is unique to each instar and appears to suit the needs of each stage. **A** First instar mandibles are suitable for piercing the cockroach cuticle and facilitating a steady flow of hemolymph without serious injury to the cockroach **B** The second instar mandible is larger and contains a serrated edge suitable for cutting into the cockroach to facilitate entry into the host **C** Third and last instar mandibles appear after the larva has entered the body cavity of the cockroach to consume fat body and muscle. These mandibles appear to be suited for crushing and macerating the internal tissues **D** All three mandible types together to scale for comparison.

longed until larval development is completed. In fact, *A. compressa* larvae take measures to avoid contact with the host gut by laying down a bed of silk from esophagus to rectum. The cockroach remains alive throughout the first two larval instars and many hours into the third as its organs are consumed selectively.

The wasp larva develops through three instars, the first two outside of the cockroach and the third exclusively inside. This is indicated by distinctive jumps in head capsule size. Dyer's rule asserts that heavily sclerotized body segments such as head capsule and mandibles grow in predictable quantum jumps between instars (Dyar 1890). Dyar's Rule has been applied to many Hymenoptera, where the number of parasitoid wasp instars can vary from two to five (Giannotti 1997; Hansell 1982; Odebiyi and Bokonon-Ganta 1986; Solis et al. 2010; Whitfield et al. 1987). Ad-

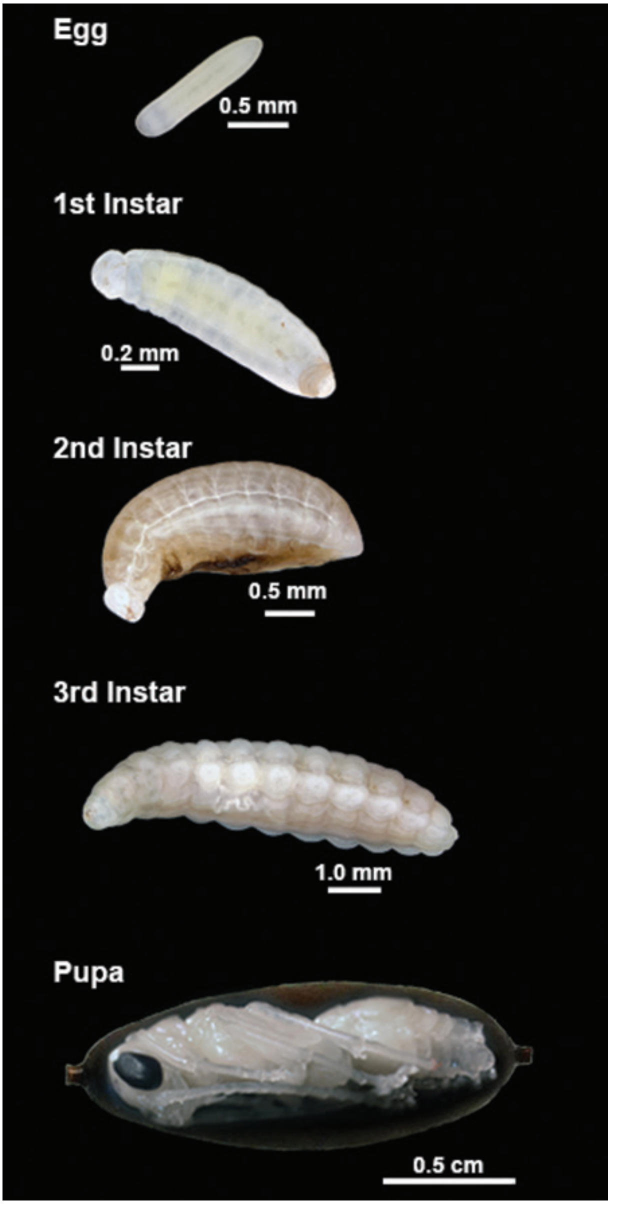

Figure 5. Images of the *Ampulex compressa* life cycle. Life cycle is depicted from egg to pupa.

ditionally, each larval instar is characterized by distinct mandibular morphologies, which appear well-suited to the foraging demands of each instar. The first instar pierces the intersegmental membrane in order to draw hemolymph from the host. The vampiric behavior of the first instar is supported by a circular labium that may be ideal for sucking and ingesting hemolymph (video 3:49). Second instar mandibles are larger, more complex, and serrated, similar to a saw. This may facilitate cutting

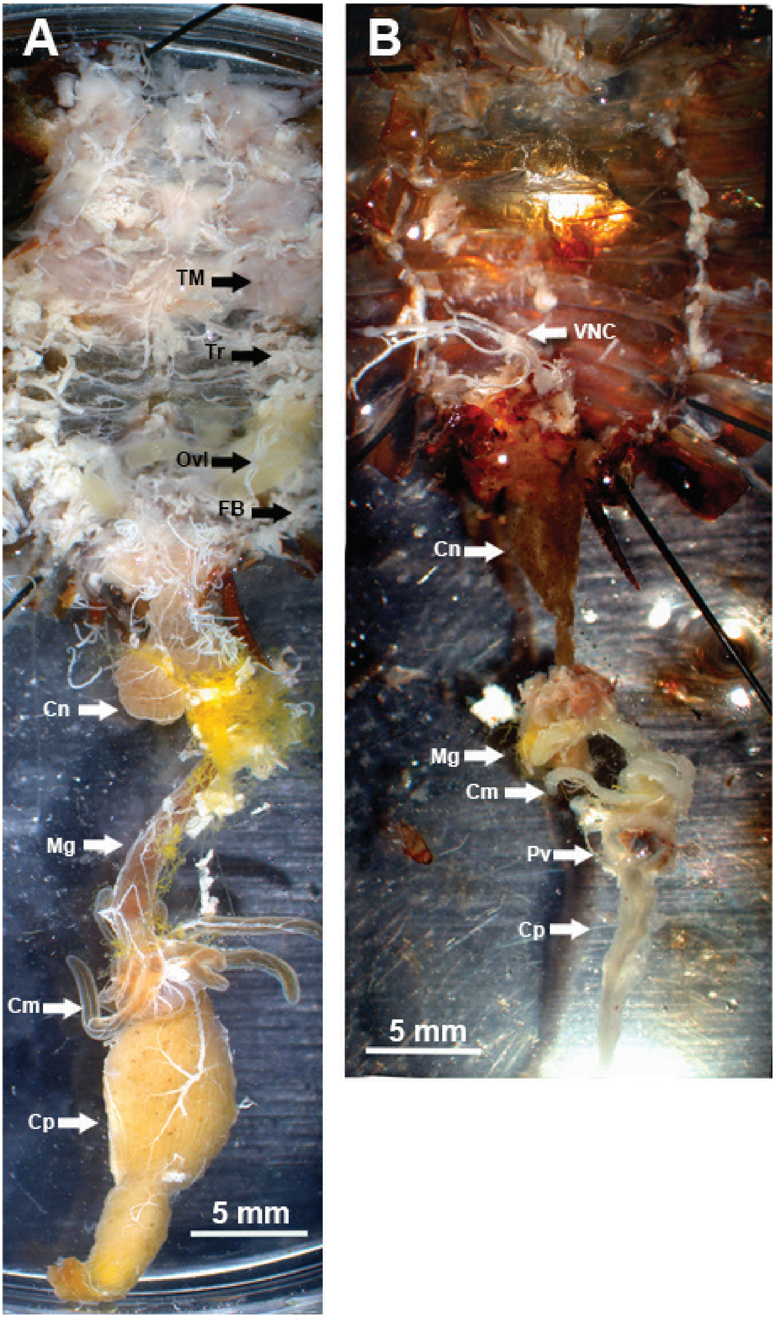

Figure 6. Viscera of *P. americana* before (**A**) and after (**B**) parasitization by *A. compressa*. **A** The entire digestive system has been isolated to more thoroughly illustrate all parts of the gut as well as tissues previously obstructed such as trachea, ovaries, thoracic muscle and fat body **B** Isolated gut from after completion of *A. compressa* larval development. Abbreviations: TM = thoracic muscle, Tr = trachea, Ovl = ovarioles of ovary, FB = fat body, Cn = colon, Mg = midgut, Cm = caeca, Cp = crop, VNC = ventral nerve cord, Pv = proventriculus.

Figure 7. Estimation of pupal size by modeling as a parabola. Shape of the *Ampulex compressa* pupa is a prolate spheroid. Thus, volume of the cocoon can be estimated by modeling it as parabola, rotated about the longitudinal axis, using only length and width measurements.

a hole into the cuticle of the cockroach for entry into the hemocoel. The third and final instar mandibles are larger and have a blunt, crushing surface suitable for maceration and consumption of internal organs.

Development and morphology of *A. compressa* immature stages has been described previously (Fox et al. 2009; Fox et al. 2006; Haspel et al. 2005; Veltman and Wilhelm 1991). Our findings in the present study indicate that developmental timing is more rapid than previously reported, though expected, in that our rearing conditions were 2-3 °C warmer (Fox et al. 2009). Sexual dimorphism also was observed with respect to developmental timing, though this was not found to be statistically significant (Fox et al. 2009). Sexual dimorphism was also suggested as a means to pre-select males from females, though not formally addressed (Veltman and Wilhelm 1991). First and second larval instars are well described as ectoparasitoid (Fox et al. 2006; Haspel et al. 2005). However, we find that, in our colony, the third and last instar, is found exclusively inside the host, which is contradictory to previous reports, despite agreement on body and mandible morphology (Fox et al. 2009; Fox et al. 2006).

Given that female pupae are larger than males, measuring the mass or determining the volume of the cocoon may allow prediction of sex weeks before adult eclosion. *Ampulex compressa* are haplodiploid; females may or may not fertilize their eggs, such that fertilized eggs generate females and non-fertilized eggs generate males. This may result in different numbers of male and female offspring. Only female *A. compressa* envenomate cockroaches and pharmacological research of the envenomation mechanism relies on consistent generation of females. It is therefore useful to predict which pupa will yield female adults and measurement of cocoon mass and volume constitutes a practical way to predict the likelihood that the pupa will develop into a female (Fig. 7).

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References

Dyar HG (1890) The number of molts of lepidopterous larvae. Psyche 5: 420–422. https://doi.org/10.1155/1890/23871

Fouad K, Rathmayer W, Libersat F (1996) Neuromodulation of the escape behavior of the cockroach *Periplaneta americana* by the venom of the parasitic wasp *Ampulex compressa*. Journal of Comparative Physiology A 178: 91–100. https://doi.org/10.1007/BF00189593

Fox EGP, Bressan-Nascimento S, Eizemberg R (2009) Notes on the biology and behaviour of the Jewel wasp, *Ampulex compressa* (Fabricius, 1781) (Hymenoptera; Ampulicidae), in the laboratory, including first record of gregarious reproduction. Entomological News 120: 430–437. https://doi.org/10.3157/021.120.0412

Fox EGP, Buys SC, Mallet RS, Bressan-Nascimento S (2006) On the morphology of the juvenile stages of *Ampulex compressa* (Fabricius, 1781) (Hymenoptera, Ampulicidae). Zootaxa 1279: 43–51. https://doi.org/10.11646/zootaxa.1279.1.2

Gal R, Kaiser M, Haspel G, Libersat F (2014) Sensory arsenal on the stinger of the parasitoid Jewel wasp and its possible role in identifying cockroach brains. PLoS One 9: e89683. https://doi.org/10.1371/journal.pone.0089683

Gal R, Libersat F (2010) On predatory wasps and zombie cockroaches: Investigations of "free will" and spontaneous behavior in insects. Communicative & Integrative Biology 3: 458–461. https://doi.org/10.4161/cib.3.5.12472

Giannotti E (1997) Biology of the wasp *Polistes (Epicnemius) cinerascens* (Saussure) (Hymenoptera: Vespidae). Anais da Sociedade Entomológica do Brasil 26: 61–67. https://doi.org/10.1590/S0301-80591997000100008

Hansell MH (1982) Brood development in the subsocial wasp *Parischnogaster mellyi* (Saussure) (Stenogastrinae, Hymenoptera). Insectes Sociaux 29: 3–14. https://doi.org/10.1007/BF02224523

Haspel G, Gefen E, Ar A, Glusman JG, Libersat F (2005) Parasitoid wasp affects metabolism of cockroach host to favor food preservation for its offspring. Journal of Comparative Physiology A 191: 529–534. https://doi.org/10.1007/s00359-005-0620-1

Haspel G, Rosenberg LA, Libersat F (2003) Direct injection of venom by a predatory wasp into cockroach brain. Journal of Neurobiology 56: 287–292. https://doi.org/10.1002/neu.10238

Herzner G, Schlecht A, Dollhofer V, Parzefall C, Harrar K, Kreuzer A, Pilsl L, Ruther J (2013) Larvae of the parasitoid wasp *Ampulex compressa* sanitize their host, the American cockroach, with a blend of antimicrobials. Proceedings of the National Academy of Sciences 110: 1369–1374. https://doi.org/10.1073/pnas.1213384110

Keasar T, Sheffer N, Glusman G, Libersat F (2006) Host-handling behavior: an innate component of foraging behavior in the parasitoid wasp *Ampulex compressa*. Ethology 112: 699–706. https://doi.org/10.1111/j.1439-0310.2006.01214.x

Odebiyi JA, Bokonon-Ganta AH (1986) Biology of *Epidinocarsis [=Apoanagyrus] lopezi* [Hymenoptera: Encyrtidae] an exotic parasite of cassava mealybug, *Phenacoccus manihoti* [Homoptera: Pseudococcidae] in Nigeria. Entomophaga 31: 251–260. https://doi.org/10.1007/BF02373334

Piek T, Visser JH, Veenendaal RL (1984) Change in behaviour of the cockroach, *Periplaneta americana*, after being stung by the sphecid wasp *Ampulex compressa*. Entomologia Experimentalis et Applicata 35: 195–203. https://doi.org/10.1111/j.1570-7458.1984.tb03379.x

Solis DR, Fox EGP, Rossi ML, Moretti TDC, Bueno OC (2010) Description of the immatures of workers of the ant *Camponotus vittatus* (Hymenoptera: Formicidae). Florida Entomologist 93: 265–276. https://doi.org/10.1653/024.093.0218

Veltman J, Wilhelm W (1991) Husbandry and display of the Jewel wasp: *Ampulex compressa* and its potential value in destroying cockroaches. International Zoo Yearbook 30: 118–126. https://doi.org/10.1111/j.1748-1090.1991.tb03473.x

Weiss K, Parzefall C, Herzner G (2014) Multifaceted defense against antagonistic microbes in developing offspring of the parasitoid wasp *Ampulex compressa* (Hymenoptera, Ampulicidae). PLoS One 9: e98784. https://doi.org/10.1371/journal.pone.0098784

Whitfield GH, Richards KW, Kveder TM (1987) Number of instars of larvae of the alfalfa leafcutter bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae). The Canadian Entomologist 119: 859–865. https://doi.org/10.4039/Ent119859-10

Williams FX (1942) Ampulex Compressa (Fabr.), A cockroach-hunting wasp introduced from New Caledonia into Hawaii. Proceedings of the Hawaiian Entomological Society 11: 221–233.

Supplementary material 1

Video

Authors: Ryan Arvidson, Victor Landa, Sarah Frankenberg, Michael E. Adams

Data type: multimedia

Link: https://doi.org/10.3897/jhr.63.21762.suppl1