E-Article

The Behavior and Reproductive Physiology of a Solitary Progressive Provisioning Vespid Wasp: Evidence for a Solitary-Cycle Origin of Reproductive Castes

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abstract: The emergence of queens and workers from solitary antecedents mark a major evolutionary transition in the history of life. The solitary progressive provisioning wasp *Synagris cornuta*, a member of the subfamily Eumeninae (basal to eusocial vespid wasps), alternates between behavioral states characterized as queenlike and worker-like. Akin to a queen in eusocial wasps, a *S. cornuta* female initiates construction of a cell into which she oviposits and then, similar to a worker, cares for the brood as it develops. The ovarian groundplan (OGP) hypothesis for caste origins predicts that these behavioral states are associated with cyclical changes in ovarian status, where females performing queenlike tasks have eggs and those performing worker-like tasks possess only small oocytes. Our findings show strong support for the OGP hypothesis: the ovaries of *S. cornuta* females undergo differential oogenesis depending on the behavioral phase: the largest oocyte in the ovaries of females building a cell progresses faster compared to that of females attending brood. Yet contrary to the OGP hypothesis, neither juvenile hormone nor ecdysteroids is associated with the reproductive cycle. Finally, the cuticular hydrocarbon profile showed no link with ovarian status, suggesting that fertility signals evolved subsequent to the emergence of group living.

Keywords: ecdysteroids, juvenile hormone, ovarian groundplan hypothesis, reproductive groundplan hypothesis, Vespidae.

Introduction

The origin of novel traits is largely achieved through the reordering of old, conserved modules within the context of organismal development (West-Eberhard 1996, 2003). Such change can occur at the macroevolutionary scale, involving reconfiguration in genomic regulatory networks, as well as within species, associated with phenotypic plasticity. A good illustration of such evolutionary recruitment is how caste phenotypes (queens and workers) likely emerged from a decoupling of an ancestral reproductive cycle operating in their solitary ancestors. In social vespid wasps, for example, the egg-laying, reproductively dominant queen initiates cell construction and oviposits, whereas the facultatively sterile worker has inactive ovaries and performs tasks relating to brood care. As highlighted by the ovarian groundplan (OGP) hypothesis (West-Eberhard 1987, 1996), these basic caste-specific behavioral repertoires are also expressed in solitary vespids but with the major distinction that they are performed by a single female in chronological sequence: she builds a cell, lays an egg, and with her ovaries diminished after oviposition, protects and provisions the brood. The ovarian cycle is assumed to be accentuated in solitary progressive provisioning species, that is, in those wasps that feed their growing larvae continuously as they develop. They oviposit much less frequently and have fewer oocytes compared to solitary mass provisioning wasps that provide a complete supply of food to their young before or shortly after laying an egg (Iwata 1964; reviewed in O'Neill 2001). Furthermore, the continued physical contact of the progressively feeding female wasp with her immature offspring is thought to have been a stepping-stone toward the evolution of social behavior (Wilson 1971).

The OGP hypothesis predicts that progressive provisioning vespids have smaller oocytes when engaged in brood-care activities, a widespread association in eusocial species. The OGP hypothesis thus argues that what was and still is expressed cyclically in a single phenotype in nonsocial wasps became dissociated into two mutually dependent, noncyclical phenotypes, where one caste lacks (and compensates for) the distinct suite of characters expressed in the other (see West-Eberhard 1996). In its most simple form, the OGP

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hypothesis predicts that the ovaries of solitary progressive provisioning wasps alternate between egg-possessing and non-egg-possessing states. Yet plasticity in the cycle should also be expected, as it would be disadvantageous for a female to lose her brood and endure a long delay before recommencing the reproductive cycle. In this case, the possession of at least one nearly or fully formed egg throughout the reproductive cycle is envisaged, with the ovaries nonetheless showing phase-specific oogenesis. Obviously, this is not a complete alternative to the OGP hypothesis but rather an extension. An alternative hypothesis to the OGP would predict that the ovaries of a solitary progressive provisioning wasp show no relationship with behavior and/or contain multiple eggs throughout the reproductive cycle (i.e., as in mass provisioning species).

Support for the OGP framework, as well as for the abovementioned extended version, comes from the observation that ovary-correlated changes in behavior are evident within workers as they age. This widespread behavioral sequence, called temporal polyethism, characterizes many wasps, bees, and ants and may owe its origin to the ovarian and behavioral maturation of ancestral solitary females (West-Eberhard 1996). In eusocial paper wasps, for example, young workers with activated ovaries perform queenlike activities, such as building, and only later, in concomitance with ovarian regression, transit to energy-demanding tasks outside the nest, namely, foraging (West-Eberhard 1981; O'Donnell 2001). This sequence of tasks, underlaid and likely affected by a change in ovary size, mirrors the behavioral maturation of a solitary wasp in her first reproductive cycle (West-Eberhard 1996).

Key to assessing the OGP hypothesis is the identification of factors that regulate the maturation (both ovarian and behavioral) and the reproductive cycle of solitary hymenopterans that progressively provision. The OGP hypothesis already predicted that juvenile hormone (JH), a widespread gonadotropin in solitary insects and primitively eusocial wasps, is centrally involved. Nonetheless, in its original version, the OGP hypothesis was essentially based on behavioral data; with the advance in understanding of hormonal control of the female reproductive cycle of insects, it came to include molecular factors and gained an important reformulation as the reproductive groundplan (RGP) hypothesis (Amdam et al. 2004). Other factors currently under discussion in the context of a molecular tool kit concept version of the RGP hypothesis are the insulin/target of rapamycin signaling pathways, JH, ecdysteroids, and the yolk precursor protein vitellogenin (for references, see Corona et al. 2015). Nevertheless, the basic prediction of West-Eberhard (1996) that the ovaries of solitary progressive provisioners cycle between reproductive and nonreproductive phases has never been tested.

In nonsocial insects that show a pronounced ovarian cycle, such as cockroaches, tsetse flies, and others, there is evidence of a marked rise and fall of JH during ovarian maturation, which is essential for normal development (Tobe and Stay 1977; Stay and Tobe 1978; Baumann et al. 2013). In turn, ovarian growth is important for regulating JH biosynthesis (Stay and Tobe 1981). Might a similar mechanism have evolved in solitary progressive provisioning vespids, especially those species that take weeks to complete their reproductive cycle? Given that JH is important or is at least associated with cell building in a number of eusocial wasps and bees (Röseler et al. 1985; Shpigler et al. 2014), the surge of JH is expected to occur when a solitary progressive provisioning female is building her cell. It is also possible that JH drives transitions in nesting behavior in solitary wasps, just as it does in workers of independently derived eusocial Hymenoptera. Two major hypotheses have been proposed in this respect. The novel-function hypothesis states that JH became neofunctionalized as a regulator of age polyethism in wasps, bees, and ants (i.e., JH had no role in brood-care activities in the solitary ancestor, and the worker-specific functions of JH are independently derived). The split-function hypothesis, on the other hand, argues in favor of dual functions for JH in the solitary ancestor, one gonadotropic and one involved in brood care, and that these may have evolved prior to and were important for the recurrent evolution of a temporal polyethism in the Hymenoptera (Giray et al. 2005). An implicit prediction of the split-function hypothesis is that JH levels (or its receptor) will fluctuate according to the behavioral phase in a solitary progressive provisioning wasp. Other regulatory factors in the hemolymph that may also be involved in reproductive behavior are ecdysteroids, ovarian-produced hormones that are associated with reproductive behaviors in some social bees and wasps (Röseler et al. 1985; Geva et al. 2005; Hartfelder and Emlen 2012). We would therefore expect the ecdysteroids in the hemolymph, if present, to correlate with the size of the ovaries.

Besides the co-option of these endocrine regulators of fertility, another important aspect to the evolution of social organization in insects is the communication of the reproductive status of the dominant female toward the subdominant helpers through behavioral (aggression) or chemical means or a combination of both in inducing sterility. Saturated cuticular hydrocarbons (CHCs) are the most common fertility cue in solitary through derived social insects and show large degrees of evolutionary conservatism (van Oystaeyen et al. 2014). In most eusocial vespids that have been studied, and in most eusocial Hymenoptera, relative ovarian development is linked to an individual's CHC profile (Turillazzi et al. 2004; Kelstrup et al. 2014*b*). These so-called fertility cues may have evolved as a byproduct of the ovarian cycle in presocial hymenopterans and be intrinsically linked to hormone cycles. We would therefore expect a tight link between ovarian development,

hormone cycles, and the CHC profile, which is ostensibly in line with the OGP framework. Alternatively, these cues are derived from sex pheromones of solitary insects, and only after group living evolved were these compounds secondarily co-opted to signal dominance or induce sterility in subordinates (for a full discussion, see Oi et al. 2016). In this case, there would be no predicted linkage between CHCs and the ovarian cycle in solitary progressive provisioning wasps but rather differences between nonmated and mated females.

So while there is clear interest for hypotheses on the evolution of social behavior in insects, the rarity of solitary progressively feeding vespids in nature goes a long way toward explaining why physiological work on them is essentially lacking. Within the Eumeninae, the subfamily basal to all or most eusocial vespids (Hines et al. 2007; Pickett and Carpenter 2010; Hermes et al. 2014), only a few species are known to progressively provision their brood (West-Eberhard 1978; O'Neill 2001), all of which are tropical, including the four species of the casteless, group-living eumenines (West-Eberhard 2005). Still rarer are species that are full progressive provisioners (Cowan 1991), which, like eusocial vespids, provision their larvae until they stop eating and are fully grown. Full progressive provisioning is known to occur in only one eumenine, the Afrotropical eumenine *Synagris cornuta*, whose females masticate hunted prey to a pulp before passing it to the mouth of the larva (as illustrated in fig. 2.7 of Cowan 1991), a behavior otherwise restricted to eusocial wasps (Roubaud 1910; Longair 2004). For this reason, *S. cornuta* has long been cited as an important species for the study of hypothetical caste origins (Roubaud 1910; Bequaert 1918; Wheeler 1923; Wilson 1971; Cowan 1991; West-Eberhard 1996; Longair 2004; Hunt 2007). Yet, since 1918, only one article with original data on female behavior of *S. cornuta* (Longair 2004) has been published, and the primary focus of this work was on the behavior and tusk morphology of males (fig. A1*A*, A1*B*; figs. A1–A6 are available online). Here, in what is the most direct and complete assessment of the OGP hypothesis to date, we set out to determine whether oocyte growth is dependent on the behavioral phase of *S. cornuta*. We also tested whether changes in JH levels and/or the ecdysteroid titer associate with the ovarian cycle, reproductive status, and behavioral phenotype. Finally, we searched for mating-, phase-, ovary-, and hormone-correlated CHC patterns to assess hypotheses regarding how queen pheromones might have originated in the Hymenoptera.

Material and Methods

Field Observations

Fieldwork on *Synagris cornuta* (Hymenoptera: Vespidae: Eumeninae) was conducted within a 3-km radius of the International Stingless Bee Centre (ISBC; http://www.stinglessbeecenter.com) in Abrafo, Ghana, from June 9 to August 8, 2013. Nests were most commonly found attached to the undersides of man-made structures (fig. A1*A*, A1*C*) close to or inside forest areas, the sloping undersides of large cacti (fig. A1*D*), and especially, the undersides of sturdy African oil palm fronds (fig. A1*E*). All nests were studied in situ, and those females studied for a week or more were marked with Sharpie (Oak Brook, IL) paint pens. Virtually all the active nests were monitored daily or on alternating days, where at any given moment in time, the activity of the female, if present, was recorded. Behaviors (or positions) of a female included (1) ensconced in the brood cell, with head facing outward (as in figs. 1*B*, fig. A1*C*, A1*C*), (2) on the surface of the nest (e.g., walking, resting, inspecting the cell), (3) flying to or from the nest, (4) cell building (fig. 1*B*), (5) cell sealing (female closing the cell with mud; fig. A1*F*), or (6) absent. The recordings of these events were separated by at least 30-min intervals, amounting to a total of 4,445 observations from 85 nests. For all cells that could be visually inspected (~85% of cells), the presence or absence of an egg was also checked.

Of the 85 nests, 12 nests were observed consecutively for 2–5 days, 33 for 6–10 days, 24 for 11–19 days, and 16 for 20–49 days (mean = 12.5 days, median = 9 days of observation). Females were observed to be active (i.e., away from the nest) as early as 06:30 (~30 min after sunrise during study period) and as late as 18:00 (~20 min before sunset) but were more often present (63%–82%) at the nest before 07:00 and after 17:30. For each time point between 07:30 and 17:00, females were present for ~50% of event recordings, showing no obvious circadian trend in time spent away from the nest (fig. A2). Therefore, only event recordings made within this period (n = 4,189) were used for calculating the overall percentage of time females (of a given phase) spent in, on, or away from the nest.

The event recordings were supplemented with sustained observations of focal females that nested in close proximity to others (up to four active nests within a 2 m^3 space, but with each nest separated by >0.2 m), affording the ability to observe several females and their respective nests simultaneously.

Based on the event recordings of all females and the sustained observations of focal females, the behavioral cycle of free-living *S. cornuta* females could be split into four distinct sequential, repeating phases: cell building → egg guarding → larval provisioning → cell sealing. We collected females in the first three phases to investigate differences in physiological parameters that might correspond to caste-like phenotypes in eusocial wasps. Moreover, the cell-sealing phase was brief (see "Results") and infrequently observed. All free-living wasps were captured in the afternoon (12:00–17:00) with a net or by placing a glass vial over the female while she was inside the cell or on the nest.

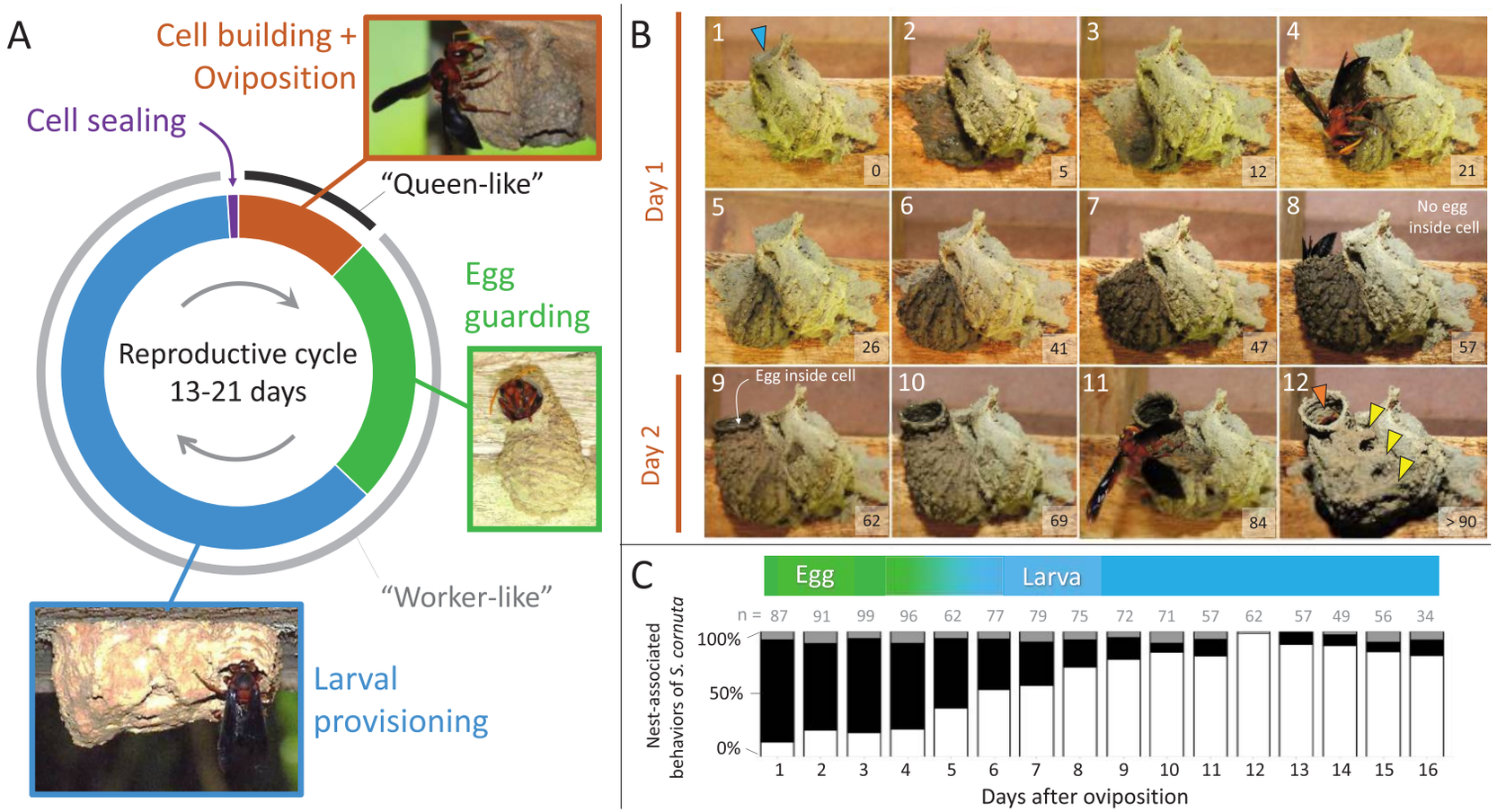

Figure 1: Natural history observations of *Synagris cornuta*. *A*, Summary of the reproductive cycle of *S. cornuta*. According to the ovarian groundplan framework, the cell-building and egg-laying phases are considered queenlike, whereas the brood-care phase is considered worker-like (West-Eberhard 1987). *B*, The building of a cell by *S. cornuta*. The nest was positioned directly overhead on a rafter beam just inside the roof of a workshop. Numbers in the bottom right corner indicate the completed number of trips by this female. Frame 1: A single cell recently sealed with mud (blue arrowhead indicates wet, recently molded material). The new cell will be adjoined to a recently constructed one. Frames 2–8: Construction of the foundation and a basic cell over the first day, prior to oviposition. Frame 9: The initial construction of the turret. An egg had been laid by this stage. Frames 10–12: Completion of the cell followed by wall thickening and fortification (yellow arrowheads indicate reinforcement ribs added apparently to strengthen the attachment to the old cell). With the cell completed and an egg inside, the cell builder now rests ensconced inside her cell (top of head indicated by orange arrowhead), entering the egg-guarding phase. *C*, The presence and position of 24 females observed from the onset of cell initiation, based on snapshot observations ($n = 1{,}124$), calibrated to the number of days following oviposition. Sample size of snapshot observations are indicated. White sections of bars indicate the female was off nest (i.e., absent), black sections indicate the female was inside the cell (i.e., ensconced), and gray sections indicate the female was on the nest (full body exposed), inspecting a cell headfirst or in transition between states (e.g., leaving or returning to the nest). All eggs hatched within 4–5 days of being laid, coinciding with an increase in the frequency of the female being absent from the nest.

Unattended nests with closed cells (e.g., from females taken for physiological study) were removed and brought to the ISBC and placed in a cubic wooden frame (50 × 40 × 40 cm) with mesh sides. Each day, the box was regularly checked for emerged adults. Newly emerged females ($n = 6$), which were never observed to emerge after 10:00, were bled (see below) within 2.5 h of daybreak.

Juvenile Hormone Quantification

For hemolymph sampling, all wasps ($n = 65$) were immediately buried in ice where they were cold immobilized and, within 2 h, bled according to established methods for wasps (Kelstrup et al. 2014*a*). Only four of six newly emerged females were successfully bled, with 2–12 μL of hemolymph removed from between the abdominal sternites with a flame-tapered capillary tube. When more than 3 μL of hemolymph could be withdrawn, the hemolymph was split into two portions: one was transferred into 500 μL of acetonitrile for JH radioimmunoassay (RIA) and the other into 500 μL of methanol for ecdysteroid RIA. Both sample types were stored in 2-mL Teflon-capped glass vials. For JH measurement, which was done for each female bled, at least 2 μL of hemolymph was used (mean = 4.0 μL); for ecdysteroid measurements, at least 1 μL (mean = 2.4 μL) was used.

For the JH RIA, we used (10-3H[N])-JH III (specific activity 19.4 Ci/nmol; Perkin Elmer Life Sciences, Waltham, MA), JH-III (Fluka, Munich), and a JH-specific antiserum (Goodman et al. 1990). For the ecdysteroid RIA, we used (23, 24-3H[N])ecdysone (Perkin Elmer, specific activity 102 Ci/mmol), an antiserum prepared against a hemisuccinate derivative of ecdysone (Bollenbacher et al. 1983; Feldlaufer and Hartfelder 1997), and 20-hydroxyecdysone (20E; Sigma, St. Louis).

Cuticular Hydrocarbon Analysis

After bleeding, the entire individual was then placed in a vial containing 2 mL of 97% hexane for 2 min to extract the CHCs. The CHC extracts were concentrated to ~100 μL using a stream of pure nitrogen, and 1 μL of this extract was injected into an Agilent 6850 gas chromatograph (GC). The GC system was fitted with a splitless inlet, flame-ionization detection, and a DB-5 capillary column (30 m × 0.32 mm × 0.25-μm film thickness; Agilent Technologies, Santa Clara, CA). The injection port and the detector were set at 290°C and 320°C. Helium was used as the carrier gas at a flow rate of 30.4 mL/min, with nitrogen acting as the makeup gas. The temperature was programmed as follows: 1 min at 150°C, increased (15°C/min) to 250°C, and then more slowly increased (3°C/min) to 310°C, where the temperature was held for 30 min. Gas chromatograms were generated using GC ChemStation software (Rev. A.09.03, Agilent Technologies, 1990–2002).

Representative samples were then analyzed by GC-coupled mass spectrometry (GC/MS) using an Agilent GC 6890N with a 5975 MSD fitted with a ZB-5MS Guardian (30 m, 0.25 mm ID, 0.25-μm film thickness) ZB 7HG-G010-11 column. An authentic C_7– C_{40} straight-chain hydrocarbon series (Supelco Analytical, Bellefonte, PA) was used as a standard to identify n-alkanes. Nonlinear alkanes were identified by analyzing the fragmentation patterns of the EI-MS and the presence of diagnostic ions (see Blomquist et al. 1987). The position of double bounds in alkenes and alkadienes was not determined.

Assessment of Ovary Status

After the brief hexane extraction for CHC analysis, the ovaries were dissected from the abdomen, placed in cold Ephrussi-Beadle Ringer's solution (7.5 g NaCl and 0.35 g KCl/L distilled water), and photographed alongside a scale with a Canon PowerShot camera (Canon, Tokyo). Oocyte area (not including the adjoining trophocyte chamber) was measured using ImageJ 1.45 (NIH, Bethesda, MD; Abràmoff et al. 2004). This work was done without a microscope, so we were unable to verify whether the spermatheca contained sperm or not.

Statistic Analysis

To test for differences in oocyte growth and hormone titers between the four female categories, a Kruskal-Wallis H test was used, followed by the Steel-Dwass all pairs test, a nonparametric equivalent to the post hoc Tukey's honest significant difference test, using JMP 11.1 (Cary, NC). A general linearized mixed model (GLMM) was performed on SPSS 23 (IBM, Armonk, NY) to determine whether the date of collection influenced JH titers. To evaluate whether oogenesis progresses at a constant rate, which involved the area measurements of nine oocytes (three oocytes from each of three behavioral phases), only 8 of the 36 comparisons were appropriate for a statistical test. In this case, we used Bonferroni corrections to adjust the *P* level of significance to .0063 (see "Results"). CHCs were quantified by determining their peak areas in the chromatograms. We excluded small peaks (<0.1%) only if they were unidentifiable as hydrocarbons and were not associated with a group (i.e., only hydrocarbons were consistently detected). In cases where a peak was undetectable, we assigned a proportional area percentage of 0.001%. The compound percentage for each sample was then adjusted to 100%, and all percentages were subjected to log ratio transformation (Aitchison 1982). To discriminate the CHC profiles of female categories, we performed principal coordinate analyses (PCoA) using a PerMANOVA test in R

software (ver. 3.1.1; vegan package, ver. 2.0–10), including 10,000 permutations.

Results

Behavior

Synagris cornuta females required 13–21 days to raise their brood from oviposition to the cell-sealing larval stage ($n = 12$, mean = 16.7, median = 16.5), a period intermediate between that of populations from Cote d'Ivoire (11–14 days; Longair 2004) and the Republic of Congo (~4 weeks; Roubaud 1910). The duration of brood rearing increased throughout the study period (Pearson's $r = 0.88$, $P = .0007$), a correlation that may in part be explained by the ~2.5°C drop in ambient temperature from early June to early August ($n = 61$ days, Pearson's $r = -0.88$, $P < .0001$; average daily temperature in Abrafo: 25.4°C [range = 22°–32°C]).

Based on event recordings and sustained focal observations, the behavioral cycle of free-living *S. cornuta* females could be split into four distinct sequential phases: cell building, egg guarding, larval provisioning, and cell sealing (fig. 1*A*).

Cell Building. Brood cell construction was characterized by frequent mud-collecting trips and required 1.5–2 days to complete (fig. 1*B*), a period intermediate between that reported by Longair (2004) and Roubaud (1910; ~1 and 2–3 days, respectively). We twice observed the construction of a cell from its initiation: one cell built adjacent to a recently sealed cell (fig. 1*B*) and another cell built in isolation (i.e., a new nest). Each required at least 100 trips with mud, occurring at a rate of up to 12 trips/h. Oviposition occurred before the completion of cell construction, just prior to the addition of the turret or neck of the cell (fig. 1*B*). To our knowledge, this is the first time that this behavior, which is common in eusocial species, has been observed in a solitary eumenine wasp (Cowan 1991; O'Neill 2001). As reported by others (Roubaud 1910; Longair 2004), the egg is suspended by a silk thread deep inside the cell (fig. A1*G*).

Egg Guarding. A female tending a cell containing an egg spent the majority of the day ensconced within the cell or on the nest (81.6% of the total 347 event recordings [see fig. 1*C*] and 88.3% of the time based on 21 h of focal observations of three egg-possessing females), corroborating the largely anecdotal observations of Roubaud (1910) and Longair (2004). As noted by Roubaud (1910), females were never observed to return with caterpillars during this phase, and only one of 18 egg-containing cells inspected after adult removal contained caterpillar parts, and this cell contained an egg that was near hatching (e.g., the segments of the larva were visible through the chorion). The larva hatched 4–5 days after oviposition, whereupon the larva would move to the bottom of the cell.

Larval Provisioning and Cell Sealing. Concomitant with the hatching of the larva, females began spending more and more time away from the nest, so that from day 8 after oviposition (or ~4 days posthatch), females were present for only 16.6% of the total 477 event recordings (see fig. 1*C*). Focal females observed with brood over 8 days old ($n = 8$ nests) were present 24.0% of the time (for a visual portrayal of activity of one cluster of three larval provisioning females, see fig. A3). Caterpillars were brought to the nest, either whole or masticated into paste, and deposited into the cell (cf. Roubaud 1910 and Longair 2004, where whole prey were never observed). Many of the caterpillars delivered to the nest were crushed (body appearing flattened with the cuticle still intact), but it was not clear if all prey were dead on arrival (i.e., some may have been only paralyzed). Nonetheless, as evident from the larva-containing cells that were removed ($n = 26$), no stash of excess intact caterpillars was present. Therefore, the day-by-day increase in female activity off the nest is likely explained by the hunting behavior of females provisioning the growing larva with more and more food. The larval-provisioning phase concluded when the turret was deconstructed with foraged water, and the resulting mud reused to seal the cell (fig. A1*F*), a process that could be completed within 2 h.

Cell Reuse and Offspring Mortality Unite. Most nests (76.4%) contained several cells (up to eight), and as reported by Longair (2004), only the newest cell was used for brood rearing. Of the 37 cells that were sealed, 70.3% of the females remained on the nest to construct a new cell, which always adjoined the recently sealed one (as in fig. 1*B*). The only cells to be reused for brood rearing were those that were never sealed (i.e., the original brood had perished and was replaced by an egg); cells that were vacated by emerging adults were never reused for brood rearing. In the one instance a vacated cell was occupied, it was by a broodless female with fully regressed ovaries residing in a very worn (i.e., probably old) inactive nest. Cell reuse for brood rearing by a female suspected not to be the original builder was observed only once, and neither cell usurpation nor prey stealing was observed.

A stark change in the daily behavior of a mother that did not switch cells, from being mostly absent (foraging) to being mostly ensconced (guarding) in the cell, was a reliable sign of larva mortality, confirmed by the appearance of an egg inside the cell no sooner than 3 days later. Most brood survived until the cell sealing stage, while 32.7% ($n = 55$) of brood was lost. Five instance of brood loss (two old larvae, two younger larvae, and one egg) were due to the com-

plete ($n = 4$) or incomplete ($n = 1$) destruction of the nest. In 12 cases, the brood ($n = 7$ older larvae, four younger larvae, and one egg) was lost but the cell was undamaged. Ants were found to occupy three of the larva-possessing cells (e.g., fig. A1*H*), and brood killing was observed once, where the mother was seen to bite, sting, and remove a very large larva, dropping it from the nest as she flew away (fig. A1*I*). The causes of egg and young larva mortality were not observed.

Focal Observations of Close-Nesting Females. Focal observations were mostly restricted to nests that were located within 0.2–2.5 m of one another. In 62.5 h of observation of three separate nest clusters consisting of 2–4 active nests (equating to 202 h of individual nest observation) and additional casual daily observations over the 2-month period of study, no social interaction or nest interlopers were observed. In fact, the only time two females were observed on the same nest was when a newly eclosed female emerged from her cell, while her putative mother was ensconced inside a brood-possessing cell. Two males, jostling for position, immediately seized the virgin female at emergence and fell to the ground. The mother remained inside her cell the entire time.

Ovarian Growth according to Phase and Mating Status

As with all social vespids, *S. cornuta* has three ovarioles per ovary. Most sets of ovaries had three or four discernible oocytes in different stages of development (fig. 2*B*). The ovaries of all free-living females collected had yellow bodies that are likely to represent relics of previously laid eggs (Tyndale-Biscoe 1984) or resorbed eggs (Bell and Bohm 1975). Therefore, we refer to these females, which encompassed all behavioral phases described above, as reproductive females. Newly emerged females, which emerged from field-collected nests placed in a cage, lacked yellow bodies and had relatively small oocytes. Each female ($n = 6$) possessed one or two vitellogenic oocytes (all of which were <30% of the area of a mature oocyte) with intact adjoining trophocyte chambers, indicating that newly emerged females require at least a few days of maturation before their first oviposition.

When comparing the summed areas of the three largest oocytes of females, there was a significant difference according to behavioral phase (egg guarding, larva provisioning, and cell building; Kruskal-Wallis H test: $n = 57$, $H(2) = 31.52$, $P < .0001$). Cell builders had a 46% larger summed oocyte area than larval provisioners (Steel-Dwass all pairs test: $Z = 4.11$, $P < .0001$), and larval provisioners in turn had 35% larger oocytes than egg guards ($Z = 3.64$, $P = .0008$). To determine whether oogenesis is constant throughout the reproductive cycle or discontinuous and dependent on the behavioral phase, we compared the area of an oocyte as it would progress from being the smallest to the largest oocyte in the reproductive cycle. This includes eight comparisons, that is, from the third-largest oocyte of the egg guard (smallest oocyte in reproductive cycle) to the largest oocyte, the incipient egg, of the cell builder (see

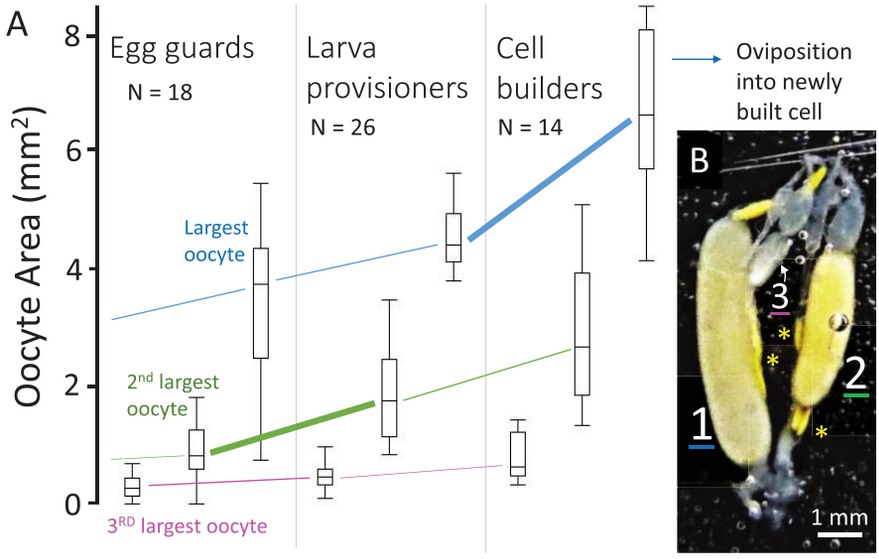

Figure 2: Oocyte growth through the behavioral phases of *Synagris cornuta*. *A*, The three largest oocytes from each phase were measured, with sample size shown. The boxplots show the median and the inner two quartiles; the whiskers indicate the 1.5 interquartile range. Lines indicate the growth trajectory of a given oocyte, and thickened lines indicate a significant difference between oocyte areas. *B*, A photograph of a well-developed ovary of a cell builder, with the three largest oocytes indicated. Yellow asterisks at the base of the ovarioles indicate yellow bodies, likely to be relics of past ovipositions (Tyndale-Biscoe 1984).

connecting lines in fig. 2*A*). Only two significant differences in oocyte area were found between the behavioral phases: the second-largest oocyte between egg guards and larval provisioners ($P = .0037$) and the largest oocyte between larval provisioners and cell builders ($P = .0014$; fig. 2*A*). Thus, while the largest oocyte of a cell builder exhibits accelerated growth, the smaller oocytes would not. Conversely, the second-largest oocyte appears to grow most rapidly in the larval-provisioning phase. Data for oocyte area (mm^2) are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2v0hj (Kelstrup et al. 2018).

JH and Ecdysteroid Titers according to Phase and Age

The hemolymph JH titer did not differ between egg guards, larval provisioners, cell builders, and newly emerged females (Kruskal-Wallis H test: $n = 62$, $H(3) = 2.55$, $P = .47$; fig. 3*A*). Additionally, there was no correlation with summed area of the three largest oocytes ($n = 62$ females; Spearman's $\rho = -0.12$, $P = .36$) or with the largest oocyte ($n = 62$; Spearman's $\rho = -0.11$, $P = .4$). To take into account potential day-to-day variation in JH titers among reproductive females ($n = 57$), we also performed a general linearized mixed model (GLMM) analysis using phase as a fixed effect and date (of collection) as a random effect (attempts were made to collect females from each phase on any given day). Again, there was no difference ($F_{2, 54} = 1.69$, $P = .19$; date had no effect).

The ecdysteroid titer, on the other hand, did differ between female categories (Kruskal-Wallis H test: $n = 59$, $H(3) = 15.63$, $P = .001$; fig. 3*B*), with newly emerged females (despite the small sample size) having significantly higher titers than each of the three reproductive female subtypes ($Z = 2.92 - 3.0$; $P < .02$). Notably, the ecdysteroid titers were very low and invariable in reproductive females, irrespective of phase (fig. 3*B*), and so a GLMM was not performed. Finally, there was no relationship between ecdysteroid titer and summed oocyte area ($n = 57$ females; Spearman's $\rho = -0.19$, $P = .16$). Data for both ecdysteroid and JH titers are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2v0hj (Kelstrup et al. 2018).

Cuticular Hydrocarbon Profiles of Females

A total of 42 hydrocarbon peaks were detected (see fig. A4). The average CHC profile consisted of alkenes (60.57% of total composition), linear alkanes (33.84%), alkadienes (5.27%), and monomethyl-alkanes (0.32%). The CHC profile of newly emerged females did not significantly differ from that of reproductive females (PerMANOVA test: pseudo-$F = 3.32$, $P = .076$; fig. 4), probably due to the low sample size for newly emerged females ($n = 6$). A cluster dendrogram analysis based on Euclidean distances and 10,000 bootstrap replicates, however, confirmed that newly emerged females grouped separately from reproductive

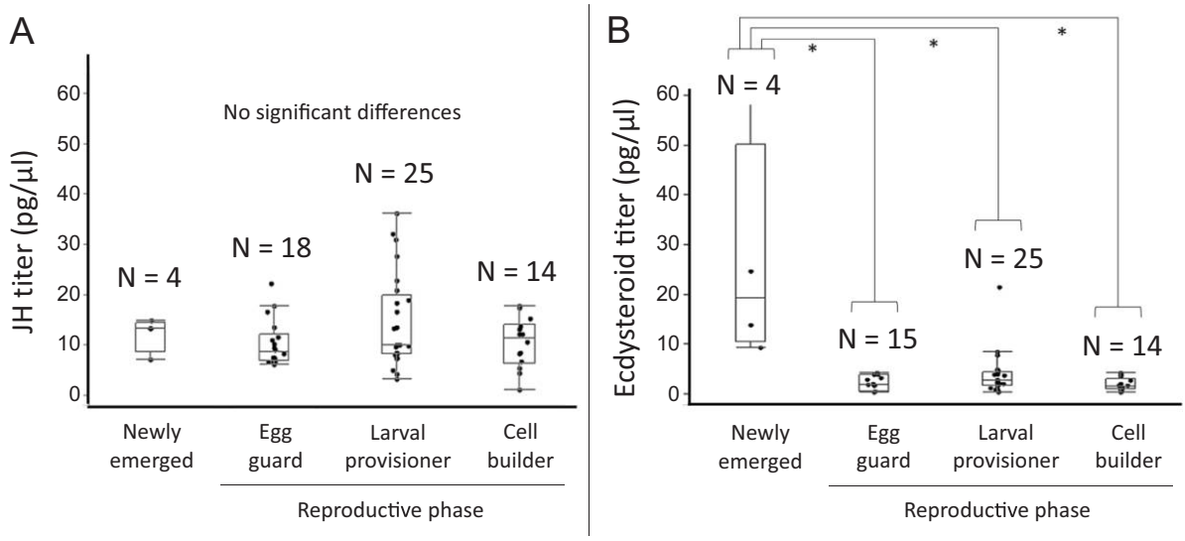

Figure 3: Juvenile hormone (JH) and ecdysteroid titers according to age and phase. *A*, JH titers did not differ according to behavioral phase and showed no suggestive difference between newly emerged females and mature, reproductive ones. *B*, Ecdysteroid titers were highest in the newly emerged females (asterisks indicate $P < .02$) and very low in reproductive wasps. Sample sizes are indicated. The boxplots show the median and the inner two quartiles; the whiskers indicate the 1.5 interquartile range.

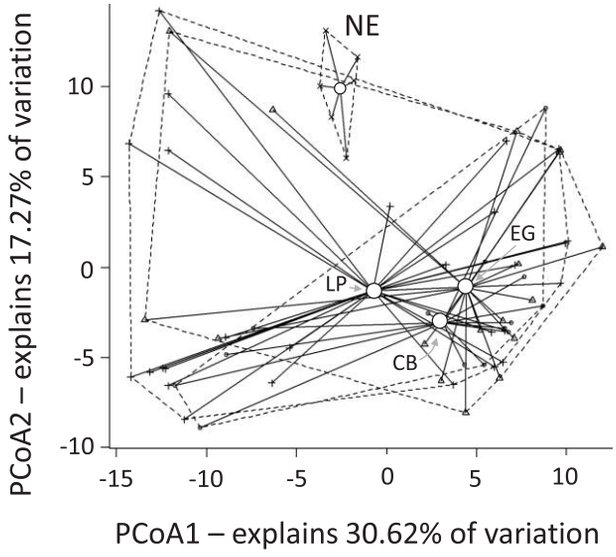

Figure 4: Principal coordinate analysis (PCoA) of cuticular hydrocarbon profiles of *Synagris cornuta*. Axes are represented by principal coordinates. The centroid is represented by a white circle for each group, the dashed lines encompass all individuals of the group, and the lines connect the sample to the group centroid. The centroid of newly emerged (NE) females is separated from the cluster of mature reproductive females split, according to phase (EG = egg guards, LP = larval provisioners, CB = cell builders), although the difference between NE females and mature ones was not significant (see text and fig. A4).

females (fig. A5). The proportional representation of 17 hydrocarbons significantly differed between the two types of females, with all six methyl-branched alkanes being higher in newly emerged females (fig. A6). Whether the apparent chemical distinction in young females relates to age or mating status was not determined. Finally, only one of the 42 showed hydrocarbon proportions significantly correlated with oocyte area (ZZ-C37: Spearman's $\rho = 0.49$; $P < .0001$), and no hydrocarbon proportion significantly correlated with JH titer (Bonferroni correction: $P < .0012$). Data underlying figure 4 are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2v0hj (Kelstrup et al. 2018).

Discussion

The Ovarian Cycle of S. Cornuta*: Support for the Ovarian Groundplan Hypothesis*

Our data on the natural history and reproductive physiology of the solitary progressive provisioning eumenine *Synagris cornuta* supports the primary prediction of the OGP hypothesis: the caste-like behaviors expressed in a temporal sequence in solitary-nesting progressive provisioning wasp are associated with differences in ovarian status. When building new cells, the wasps possess nearly or fully mature oocytes (like queens in eusocial colonies), and in the egg guarding and larval-provisioning phase, they possess smaller oocytes (like workers in eusocial colonies). More importantly, we show that the predicted growth rate of an oocyte in *S. cornuta* correlates with the behavioral phase of the female and the position (or relative size) of the respective oocyte in the ovary.

Taking the (cyclical) separation of tasks to the extreme, the queens of eusocial species in colonies containing workers rarely hunt, despite the presence of brood and, therefore, persist in the aggressive cell acquisition phase but may also guard recently laid eggs (West-Eberhard 1981). Consequently, queens are continuously exposed to the sensory stimulus, namely, the presence of empty cells, that are thought to activate ovarian growth and oviposition behaviors (Evans 1966). On the rare occasion that a vespid queen does perform a foraging task, it is as exceptional as it is illustrative: she collects pulp (or water) used for building but never prey (West-Eberhard 1969; Kelstrup et al. 2014*b*), thus supporting the OGP hypothesis that the cell-initiating phase in solitary wasps is likely homologous to the queen phenotype in eusocial vespids (West-Eberhard 1987, 1996).

Potential Determinants of the Ovarian Cycle of S. Cornuta

As with most eumenines, the majority of *Synagris* species are probably mass provisioners and may complete their reproductive cycle within a few days (Roubaud 1910, 1916; H. Kelstrup, personal observation of *Synagris analis* in St. Lucia, South Africa). As suggested by Roubaud (1910), the exogenous cue that selectively retards and thus regulates ovarian development of progressive provisioning *Synagris* females may be the presence of a larva. During the protracted larval-provisioning phase of *S. cornuta*, the longest oocyte shows limited growth; only after the cell is sealed and interactions between mother and larva cease does the oocyte appear to mature. Yet the second-largest oocyte follows its own rules, appearing to grow most rapidly in the larval-provisioning phase and slowing down in the cell-building phase.

Elucidating the environmental and proximate mechanisms that regulate such differential oogenesis in solitary wasps will be key to a full assessment of the OGP hypothesis. A proximate mechanism underlying such regulation could be the ecdysteroid production by the follicle epithelium cells of the polytrophic meroistic ovarioles. In previous studies on ecdysteroid levels in ovaries and hemolymph of social wasps, we had shown that intraovarian ecdysteroid levels can be very high while these are not released into hemolymph (Kelstrup et al. 2014*a*, 2014*b*, 2015). How such intraovarian ecdysteroid signaling may regulate the dynamics of critical oogenesis steps is, however, still a dark spot in insect reproduction, but expression studies of ecdy-

sone receptors and response genes recently reviewed for *Drosophila melanogaster* (Bellés and Piulachs 2015) indicate that these steroid hormones are fine-tuning several critical oogenesis steps, including their adjustment to the nutritional status of the females.

The Endocrinology of S. Cornuta*: Possible Functions for Juvenile Hormone and Ecdysteroids in the Hemolymph*

Given the involvement of JH and ecdysteroids in reproduction in insects, in general (Wyatt and Davey 1996), and a solitary vespid (Tibbetts et al. 2013) and social vespids, in particular (Jandt et al. 2013; Kelstrup et al. 2015), we expected that these hormones would be highest in cell builders. Yet, neither of these hormones showed an obvious phase-related change in titer. Ecdysteroid titers were so low in the hemolymph of reproductive adults that we doubt these ovarian-produced molecules are of physiological relevance outside the ovary itself, for instance, as a factor that would stimulate fat body vitellogenin production, as seen in mosquitoes (Raikhel et al. 2005). The results also strongly suggest that JH does not coordinate the behavioral and ovarian cycles in *S. cornuta*. Rather, it may simply function as a general gonadotropin, since ovarian growth occurs in all phases, albeit at different rates. This means that oogenesis, or fat body vitellogenin production, is not necessarily shut down cyclically for phases, as it does in insects that produce one ootheca or oocyte at a time (Tobe and Stay 1977; Baumann et al. 2013). These data also indicate that JH is not associated with transitions in brood-care activities (e.g., from egg guarding to larval provisioning) and so do not support the split-function hypothesis for JH in *S. cornuta*.

Nonetheless, there are three caveats to consider. First, the sample size for hormone titers of newly emerged females was not large enough to allow for an evaluation of the hypothesis that JH and/or ecdysteroids regulate the ovarian and/or behavioral maturation of young adults. Second, newly emerged and reproductive females were collected at different times of the day, and this could have affected their hormone levels. Third, the behavior of newly emerged females was restricted due to their emergence in a cage; for example, they could not disperse. These caveats aside, we suspect that the high ecdysteroid titer of newly emerged females relates either to the completion of adult development, emergence behavior, or the regulation of hydrocarbons that advertise sexual receptivity (see below). JH titers of newly emerged females, on the other hand, were not strikingly different from those of reproductive females. What remains to be determined, and will be difficult to evaluate in the field, is how these hormones change as a female mates, matures, and becomes reproductive, as has been done with some success in the facultatively eusocial bee, *Megalopta genalis* (Smith et al. 2013).

The Cuticular Hydrocarbon Profile of S. Cornuta*: No Link with Ovarian Status*

Newly emerged (virgin) females had a distinct CHC profile compared to reproductive ones, with the former showing a higher proportion of methyl-branched alkanes. These saturated hydrocarbons may, therefore, serve as signals of virginity and/or age but probably not ovary size, since these chemicals did not correlate with oocyte area in reproductive females. Nevertheless, the elevated and highly variable ecdysteroid titer of young females (fig. 3*B*) suggests a mechanism by which the CHC profile of virgin females may be transiently regulated. In young virgin female house flies, for example, the ovarian-produced ecdysteroids regulate sex pheromone production but are not required for the continued production of the sex pheromone (Dillwith et al. 1983; Blomquist 2003). Yet the origin of a fertility-linked pheromone signal, so pervasive in social Hymenoptera (Liebig 2010; Oi et al. 2015), is thought to have been linked to ovarian status and not specifically to mating status or age, and this hypothesis receives support from studies of solitary insects, where ovarian growth itself has an effect on the CHC chemical profile of a female. However, our results for *S. cornuta* revealed that the CHC profile of reproductive females does not change according to the reproductive cycle, in contrast to nonsocial insects that mate repeatedly and have a hormonally driven reproductive cycle, for example, cockroaches (Liang and Schal 1993), or form breeding pairs, for example, burying beetles (Scott et al. 2008). Indeed, there would be no obvious adaptive value for a truly solitary insect that mates once in early adulthood, as do many eumenines (Budrienë and Budrys 2007), to produce fertility signals when cycling through the reproductive phases, since there is no biologically relevant information to convey. This is highly relevant to the discussion of queen pheromone origins in the Hymenoptera, since the majority of eusocial species also mate just once, usually early in adulthood (Thornhill and Alcock 1983; Strassmann 2001). Therefore, the recruitment of cuticular or glandular odors to reflect fertility may have emerged subsequent to the evolution of social living in wasps, when communication between regularly interacting females becomes important (Anderson et al. 2002; Oi et al. 2015). Clearly, the links between sexual receptivity, ovarian status, and chemical profiles in solitary and casteless nest-sharing hymenopterans merit further investigation, and hypotheses on these links should not be based so strongly on evidence from nonsocial, nonhymenopterans that mate repeatedly and have pronounced reproductive cycles.

Conclusions

Vespid wasps afford an excellent opportunity to study how queen and worker phenotypes emerged from solitary ancestors in a biologically relevant setting: their natural hab-

itat. There is a stunning diversity of lifestyles in extant solitary and social vespids, many of which serve as signposts for conceivable intermediate stages of social evolution (West-Eberhard 1978; Cowan 1991; Gadagkar 1991; Hunt 2007). Although we make no claim that *Synagris* is on its way toward evolving castes, it may possess traits that were likely present in a solitary ancestor of social vespids. Some species of the genus *Synagris*, including *S. cornuta*, can form massive, dense aggregations of hundreds of nests, some of which are conjoined (Roubaud 1910; Bequaert 1918). In our population, however, social interactions among females were never observed, even among those nesting near one another.

The queen- and worker-like behaviors of *S. cornuta* not only correlated with ovarian status but also the size of particular oocytes. What remains to be determined are the exogenous and endogenous factors that control the ovarian cycle and synchronize it with the behavioral phases. Possibly, the presence of a larva is the environmental cue that upregulates provisioning behaviors and slows egg growth, and brood-swap experiments in *S. cornuta* or other solitary eumenines will be needed to confirm this. Although OGP-based molecular tool kit hypotheses regarding JH and ecdysteroid functions in a solitary progressive provisioning eumenine were not supported, the basic behavioral prediction of the OGP framework holds: an ovarian cycle of growth underlies the caste-like cyclical phases of maternal behaviors observed in a solitary vespid wasp. Furthermore, our results cast doubt on the hypothesis that fertility-linked CHCs were implicitly already present in the hypothetical presocial ancestor.

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Literature Cited

Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics International 11:36–42.

Aitchison, J. 1982. The statistical analysis of compositional data. Journal of the Royal Statistical Society B 44:139–177.

Amdam, G. V., K. Norberg, M. K. Fondrk, and R. E. Page. 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proceedings of the National Academy of Sciences of the USA 101:11350–11355.

Anderson, P., M. Hilker, and T. Meiners. 2002. Oviposition pheromones in herbivorous and carnivorous insects. Pages 235–263 *in* M. Hilker and T. Meiners, eds. Chemoecology of insect eggs and egg deposition. Blackwell, Berlin.

Baumann, A. A., J. B. Benoit, V. Michalkova, P. O. Mireji, G. M. Attardo, J. K. Moulton, T. G. Wilson, and S. Aksoy. 2013. Juvenile hormone and insulin suppress lipolysis between periods of lactation during tsetse fly pregnancy. Molecular and Cellular Endocrinology 372:30–41.

Bell, W. J., and M. K. Bohm. 1975. Oosorption in insects. Biological Reviews 50:373–396.

Belles, X., and M. D. Piulachs. 2015. Ecdysone signaling and ovarian development in insects: from stem cells to ovarian follicle formation. Biochimica et Biophysica Acta 1849:181–186.

Bequaert, J. 1918. A revision of the Vespidae of the Belgian Congo based on the collection of the American Museum Congo Expedition: with a list of Ethiopian diplopterous wasps. Bulletin of the American Museum of Natural History 39:1–384.

Blomquist, G. J. 2003. Biosynthesis and ecdysteroid regulation of housefly sex pheromone production. Pages 231–252 *in* Insect pheromone biochemistry and molecular biology. Academic Press, Cambridge, MA.

Blomquist, G., D. Nelson, and M. de Renobales. 1987. Chemistry, biochemistry and physiology of insect cuticular lipids. Archives of Insect Biochemistry and Physiology 6:227–265.

Bollenbacher, W. E., M. A. O'Brien, E. J. Katahira, and L. I. Gilbert. 1983. A kinetic analysis of the action of the insect prothoracicotropic hormone. Molecular and Cellular Endocrinology 32:27–46.

Budrienë, A., and E. Budrys. 2007. Comparison of mating of ten eumeninae wasp species with a brief review of sexual selection theories: a framework for future research. Acta Zoologica Lituanica 17:87–104.

Corona, M., R. Libbrecht, and D. E. Wheeler. 2015. Molecular mechanisms of phenotypic plasticity in social insects. Current Opinion in Insect Science 13:55–60.

Cowan, D. 1991. The solitary and presocial Vespidae. Pages 33–73 *in* K. Ross and R. Matthews, eds. The social biology of wasps. Comstock, Ithaca, NY.

Dillwith, J. W., T. S. Adams, and G. J. Blomquist. 1983. Correlation of housefly sex pheromone production with ovarian development. Journal of Insect Physiology 29:377–386.

Evans, H. 1966. The comparative ethology and evolution of the sand wasps. Harvard University Press, Cambridge, MA.

Feldlaufer, M. F., and K. Hartfelder. 1997. Relationship of the neutral sterols and ecdysteroids of the parasitic mite, *Varroa jacobsoni* to those of the honey bee, *Apis mellifera*. Journal of Insect Physiology 43:541–545.

Gadagkar, R. 1991. *Belonogaster*, *Mishcocyttarus*, *Parapolybia*, and independent-founding *Ropalidia*. Pages 149–187 *in* K. Ross and S. Matthews, eds. The social biology of wasps. Comstock, Ithaca, NY.

Geva, S., K. Hartfelder, and G. Bloch. 2005. Reproductive division of labor, dominance, and ecdysteroid levels in hemolymph and ovary of the bumble bee *Bombus terrestris*. Journal of Insect Physiology 51:811–823.

Giray, T., M. Giovanetti, and M. J. West-Eberhard. 2005. Juvenile hormone, reproduction, and worker behavior in the Neotropical social wasp *Polistes canadensis*. Proceedings of the National Academy of Sciences of the USA 102:3330–3335. http://dx.doi.org/10.1073/pnas.0409560102.

Goodman, W. G., D. C. Coy, F. C. Baker, L. Xu, and Y. C. Toong. 1990. Development and application of a radioimmunoassay for the juvenile hormones. Insect Biochemistry 20:357–364.

Hartfelder, K., and D. J. Emlen. 2012. Endocrine control of insect polyphenism. Pages 464–522 *in* L. I. Gilbert, ed. Insect endocrinology. Academic Press, San Diego, CA.

Hermes, M. G., G. A. R. Melo, and J. M. Carpenter. 2014. The higher-level phylogenetic relationships of the Eumeninae (Insecta,

Hymenoptera, Vespidae), with emphasis on *Eumenes*. Cladistics 30:453–484.

Hines, H. M., J. Hunt, T. K. O'Connor, J. J. Gillespie, and S. A. Cameron. 2007. Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proceedings of the National Academy of Sciences of the USA 104:3295–3299.

Hunt, J. 2007. The evolution of social wasps. Oxford University Press, Oxford.

Iwata, K. 1964. Egg giganticism in subsocial Hymenoptera, with ethological discussion on tropical bamboo carpenter bees. Pages 399–435 *in* T. Kira and T. Umesao, eds. Nature and life in Southeast Asia. Vol. 3. Fauna and Flora Research Society, Kyoto.

Jandt, J., E. Tibbetts, and A. Toth. 2013. *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insectes Sociaux 61:11–27.

Kelstrup, H. C., K. Hartfelder, F. S. Nascimento, and L. M. Riddiford. 2014*a*. Reproductive status, endocrine physiology and chemical signaling in the Neotropical, swarm-founding eusocial wasp *Polybia micans*. Journal of Experimental Biology 217:2399–2410.

———. 2014*b*. The role of juvenile hormone in dominance behavior, reproduction and cuticular pheromone signaling in the caste-flexible epiponine wasp, *Synoeca surinama*. Frontiers in Zoology 11:e78.

Kelstrup, H. C., K. Hartfelder, and T. C. Wossler. 2015. *Polistes smithii* vs. *Polistes dominula*: the contrasting endocrinology and epicuticular signaling of sympatric paper wasps in the field. Behavioral Ecology and Sociobiology 69:2043–2058.

———. 2018. Data from: The behavior and reproductive physiology of a solitary progressive provisioning vespid wasp: evidence for a solitary-cycle origin of reproductive castes. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.2v0hj.

Liang, D., and C. Schal. 1993. Ultrastructure and maturation of a sex pheromone gland in the female German cockroach, *Blattella germanica*. Tissue and Cell 25:763–776.

Liebig, J. 2010. Hydrocarbon profiles indicate fertility and dominance status in ant, bee, and wasp colonies. Pages 254–281 *in* G. Blomquist and A.-G. Bagnères, eds. Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge University Press, Cambridge.

Longair, R. W. 2004. Tusked males, male dimorphism and nesting behavior in a subsocial Afrotropical wasp, *Synagris cornuta*, and weapons and dimorphism in the genus (Hymenoptera: Vespidae: Eumeninae). Journal of the Kansas Entomological Society 77:528–557.

O'Donnell, S. 2001. Worker age, ovary development, and temporal polyethism in the swarm-founding wasp *Polybia occidentalis* (Hymenoptera: Vespidae). Journal of Insect Behavior 14:201–213.

O'Neill, K. 2001. Solitary wasps: behavior and natural history. Comstock, Ithaca, NY.

Oi, C. A., J. S. van Zweden, R. C. Oliveira, A. Van Oystaeyen, F. S. Nascimento, and T. Wenseleers. 2015. The origin and evolution of social insect queen pheromones: novel hypotheses and outstanding problems. BioEssays 37:808–821.

Pickett, K., and J. Carpenter. 2010. Simultaneous analysis and the origin of eusociality in the Vespidae (Insecta: Hymenoptera). Arthropod Systematics and Phylogeny 68:3–33.

Raikhel, A., M. Brown, and X. Belles. 2005. Endocrine control of reproductive processes. Pages 433–491 *in* L. I. Gilbert, K. Iatrou, and S. Gill, eds. Comprehensive molecular insect science. Vol. 3. Elsevier, Oxford.

Röseler, P., I. Röseler, and A. Strambi. 1985. Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. Behavioral Ecology and Sociobiology 18:9–13.

Roubaud, E. 1910. The natural history of the solitary wasps of the genus *Synagris*. Pages 507–525 *in* the Smithsonian Report for 1910. Smithsonian Institution, Washington, DC.

———. 1916. Recherches biologiques sur les guepes solitaires et sociales d'Afrique. Annales des Sciences Naturelles, 10^{e} Serie: Zoologie 1:1–160.

Scott, M. P., K. Madjid, and C. M. Orians. 2008. Breeding alters cuticular hydrocarbons and mediates partner recognition by burying beetles. Animal Behaviour 76:507–513.

Shpigler, H., E. Amsalem, Z. Y. Huang, M. Cohen, A. J. Siegel, A. Hefetz, and G. Bloch. 2014. Gonadotropic and physiological functions of juvenile hormone in bumblebee (*Bombus terrestris*) workers. PLoS ONE 9:e100650.

Smith, A. R., K. M. Kapheim, B. Pérez-Ortega, C. S. Brent, and W. T. Wcislo. 2013. Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Hormones and Behavior 63:1–4.

Stay, B., and S. Tobe. 1978. Control of juvenile hormone biosynthesis during reproductive-cycle of a viviparous cockroach. 2. Effects of unilateral allatectomy, implantation of supernumerary corpora allata, and ovariectomy. General and Comparative Endocrinology 34:276–286.

———. 1981. Control of the corpora allata during a reproductive cycle in a viviparous cockroach. American Zoologist 21:663–674.

Strassmann, J. 2001. The rarity of multiple mating by females in the social Hymenoptera. Insectes Sociaux 48:1–13.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Tibbetts, E., A. Mettler, and K. Donajkowski. 2013. Nutrition-dependent fertility response to juvenile hormone in non-social *Euodynerus foraminatus* wasps and the evolutionary origin of sociality. Journal of Insect Physiology 59:339–344.

Tobe, S., and B. Stay. 1977. Corpus allatum activity in vitro during reproductive cycle of viviparous cockroach, *Diploptera punctata*. General and Comparative Endocrinology 31:138–147.

Turillazzi, S., M. F. Sledge, L. Dapporto, M. Landi, D. Fanelli, L. Fondelli, P. Zanetti, and F. R. Dani. 2004. Epicuticular lipids and fertility in primitively social wasps (Hymenoptera Stenogastrinae). Physiological Entomology 29:464–471.

Tyndale-Biscoe, M. 1984. Age-grading methods in adult insects—a review. Bulletin of Entomological Research 74:341–377.

van Oystaeyen, A., R. C. Oliveira, L. Holman, J. S. van Zweden, C. Romero, C. A. Oi, P. d'Ettorre, et al. 2014. Conserved class of queen pheromones stops social insect workers from reproducing. Science 343:287–290.

West-Eberhard, M. 1969. The social biology of polistine wasps. Miscellaneous Publications of the Museum of Zoology, University of Michigan, Ann Arbor.

———. 1978. Polygyny and the evolution of social behavior in wasps. Journal of the Kansas Entomological Society 51:832–856.

———. 1981. Intragroup selection and the evolution of insect societies. Pages 3–17 *in* R. D. Alexander and D. W. Tinkle, eds. Natural selection and social behavior: recent research and new theory. Chiron, New York.

———. 1987. Flexible strategy and social evolution. Pages 35–51 *in* Y. Ito, L. Brown, and L. Kikkawa, eds. Animal societies: theories and facts. Japan Scientific Societies, Tokyo.

———. 1996. Wasp societies as microcosms for the study of development and evolution. Pages 290–317 *in* S. Turillazzi and M. West-Eberhard, eds. Natural history and evolution of paper wasps. Oxford University Press, Oxford.
———. 2003. Developmental plasticity and evolution. Oxford University Press, New York.
———. 2005. Behavior of the primitively social wasp *Montezumia cortesioides* Willink (Vespidae Eumeninae) and the origins of vespid sociality. Ethology, Ecology and Evolution 17:201–215.
Wheeler, W. 1923. Social life among the insects. Harcourt, Brace, New York.
Wilson, E. O. 1971. The insect societies. Harvard University Press, Cambridge, MA.
Wyatt, G. R., and K. G. Davey. 1996. Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. Advances in Insect Physiology 26:1–155.

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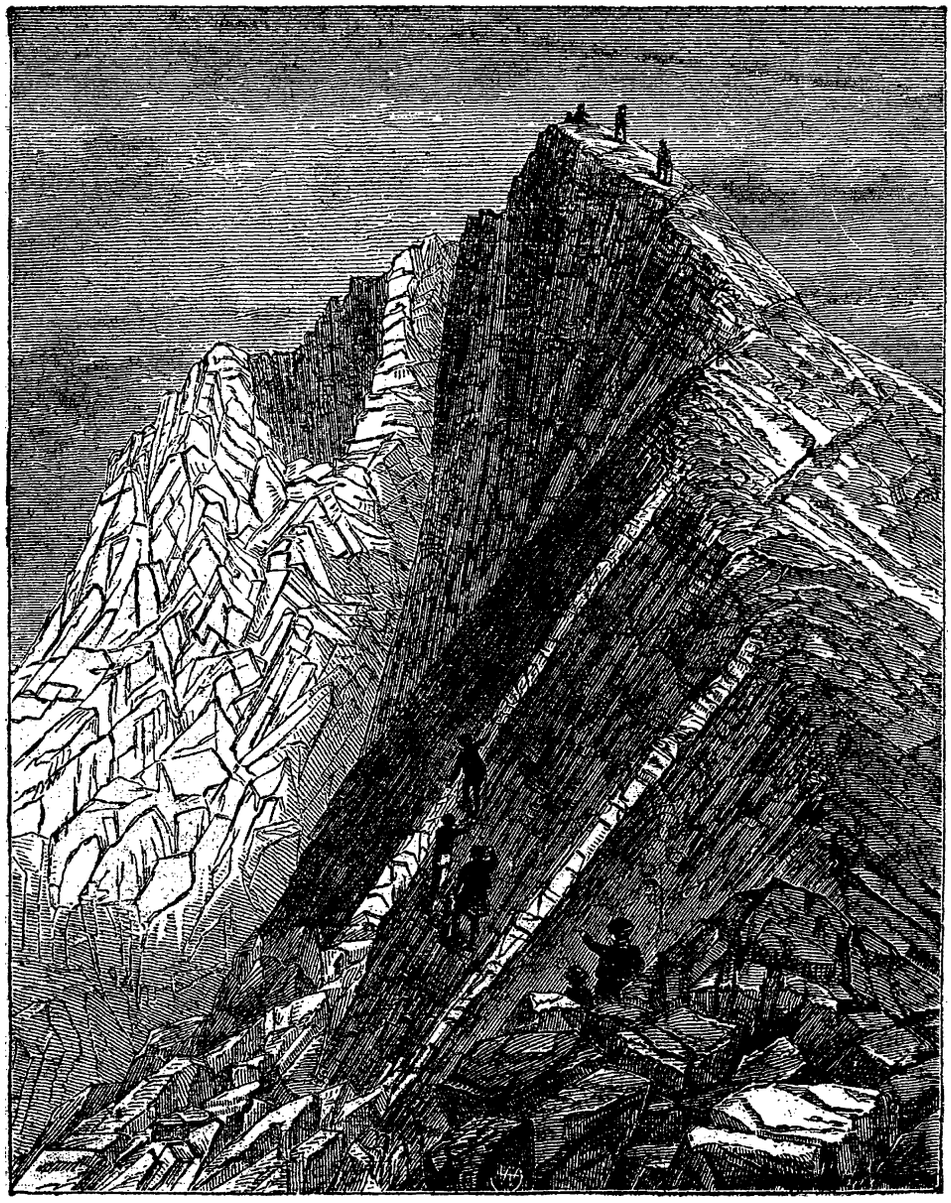

"The elevated plateau and mountains of Colorado have a unique interest to the naturalist. Most interesting questions in the distribution of life, both horizontal and vertical; the relation of the physical aspects of Colorado as compared with the plateau of Asia and the mountains arising from them, will find a partial solution in the data given in this report." Figured: Italian Mountain, Colorado. From the review of Hayden's *Geology of Colorado* (*The American Naturalist*, 1875, 9:173–178).