ELSEVIER

Journal of Insect Physiology 49 (2003) 967–974

Journal of Insect Physiology

www.elsevier.com/locate/jinsphys

Changes in juvenile hormone biosynthetic rate and whole body content in maturing virgin queens of *Solenopsis invicta*

Colin S. Brent *, Edward L. Vargo

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613, USA

Received 14 April 2003; received in revised form 7 July 2003; accepted 7 July 2003

Abstract

Studies were conducted on the physiological and hormonal changes following the release of alates from developmentally suppressive pheromones produced by mature queens of the fire ant *Solenopsis invicta* Buren. Winged virgin queens were removed from the pheromonal signal and placed in colony fragments. The time for dealation, degree of ovarian development, and biosynthesis rate and whole body content of juvenile hormone (JH) were measured. The production rate and content of JH were highly correlated. Dealation and the initiation of oviposition corresponded to peak production of JH. JH production rose sharply following separation from the natal nest, peaking after 3 days. After 8 days of isolation, JH production gradually subsided to levels similar to that found in pre-release queens, but began to increase again after 12 days. Mature queens had highly elevated levels of JH relative to recently dealate females, probably reflecting the increased reproductive capability of these older females. The results support the hypothesis that the pheromone released by functional queens inhibits reproduction in virgin alates by suppressing corpora allata activity and the production of JH.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Reproduction; Formicidae; Queen; Juvenile hormone; Primer pheromone

1. Introduction

An integral attribute of eusociality is that reproduction is limited to one or a few individuals within a colony (Wilson, 1971). These reproductively active individuals are able to directly inhibit the reproductive behavior and development of their nestmates. One of the principle means by which nestmate development is suppressed is through the release of pheromones that can act as behavioral releasers and physiological primers (Wilson and Bossert, 1963). These chemical stimuli likely act on the neuroendocrine system of the insects to induce inhibition, either by providing a recognition cue that the nestmates can use to self-regulate development or by directly inducing the inhibitory effect. Despite ample evidence of the direct link between the release of these pheromones and the inhibition of nestmate reproduction, little is known about the specific physiological mechanisms through which pheromones influence development, even in well-studied systems such as the honeybee (reviewed by Robinson and Vargo, 1997).

Among the ants, one of the most highly studied pheromone systems is that of the red imported fire ant, *Solenopsis invicta* Buren. Fire ant queens can indirectly influence nestmate reproduction by producing releaser pheromones that incite workers to eliminate sexual brood (Vargo and Fletcher, 1986, 1987; Klobuchar and Deslippe, 2002) or any supernumerary queens that might arise within the nest (Fletcher and Blum, 1983a,b; Vander Meer and Alonso, 2002). Queens also produce a pheromone that can directly influence the development of conspecifics, and thus appears to be a primer pheromone (reviewed by Vargo, 1998). For instance, functional queens are able to mutually inhibit each other's ability to produce eggs (Vargo, 1992). Queens are also able to prevent virgin alates, the winged sexuals that disperse from their natal colony to initiate new colonies, from dealating and becoming reproductively active (Fletcher and Blum, 1981).

* Corresponding author. Tel.: +1-919-515-3784; fax: +1-919-515-7746.

E-mail address: csbrent@nscu.edu (C.S. Brent).

0022-1910/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0022-1910(03)00166-5

An alate that has been separated from the inhibitory stimulus of a functional queen will quickly shed its wings, enhance its ovarian development and initiate oogenesis (Fletcher and Blum, 1981, 1983a,b). She will also begin to produce her own inhibitory pheromones (Glancey et al., 1981; Willer and Fletcher, 1986; Vargo, 1999). The queen pheromone may affect nestmate development by acting on the corpora allata thereby altering the rate of juvenile hormone (JH) production and/or release (Barker, 1978, 1979; Vargo and Laurel, 1994). Juvenile hormone has been widely implicated as a primary regulator of reproductive behavior and development in the eusocial Hymenoptera (reviewed by Robinson and Vargo, 1997). Treating fire ant alates with natural (Burns et al., 2002) or synthetic JH (Kearney et al., 1977), or the JH analog methoprene (Vargo and Laurel, 1994), can induce alates to dealate and initiate oogenesis even in the presence of a functional queen. Similarly, alates that are allatectomized (Barker, 1978, 1979) or treated topically with precocene (Burns et al., 2002), a disruptor of CA biosynthetic activity, will retain their wings when living in queenless colonies, but will quickly dealate with an application of JH. Although these findings strongly support the role of the CA and JH in mediating the inhibitory effect of fire ant queen pheromones, little is known about the endocrine events following disinhibition and their timing with regard to dealation and oogenesis. It is also not known how these pheromones reduce circulating JH, since they may increase the activity of JH esterases or decrease the activity of the CA, as occurs in roaches (Tobe et al., 1985) and honeybees (Huang et al., 1991).

Here, we report the biosynthetic activity of the CA, the whole body content of JH and ovarian activity of inhibited alates and functional queens during the first 25 days of development after alates are isolated from queen pheromone. We present evidence that dealation and oogenesis are triggered by a rapid surge in JH production and provide a more complete model of the mode of action of the queen pheromone.

2. Materials and methods

2.1. Source and maintenance of ants

Ants used in these experiments originated from Alachua and Marion Counties, FL, Wake County, NC, and Richland County, SC. Colonies were collected by excavation of mounds and separated from soil by flooding (Jouvenaz et al., 1977). Only ants of the polygyne (multiple queen) form were used in this experiment. Colonies were housed in plastic trays (40 × 52 × 8 cm) supplied with three to four nests (14 cm diameter covered Petri dishes half-filled with damp dental plaster) and were maintained in an environmental chamber at LD, 14:10 h and 26 °C. The ants were fed crickets (*Acheta domesticus*) and given 20% sugar water and tap water ad libitum. Maturing queens were produced for this experiment by placing two winged females in smaller plastic trays (40 × 26 × 8 cm) along with 25–40 workers. They were supplied with a nest, crickets, sugar water and tap water.

2.2. Juvenile hormone biosynthesis rate

The in vitro biosynthetic rates of juvenile hormone production in individual females were measured using a rapid partition radiochemical assay (RCA) (Pratt and Tobe, 1974; Tobe and Pratt, 1974; modified by Feyereisen and Tobe, 1981). The paired glands of the corpora allata and corpora cardiaca complex, hereafter referred to as the CA, were dissected under sterile conditions and cleaned of any attached tissue. Once excised, the CA was preincubated for 30 min in a Petri-dish at 26 °C in 100 μl of modified TC199 medium (Specialty Media, Phillipsburg, NJ), with 50 mM Hepes buffer, pH 7.4, without methionine or bicarbonate, and containing 2% Ficoll 400 (Sigma Chemical Co.). After the pre-incubation, the CA was transferred to a 6 × 50 mm borosilicate culture tube containing 100 μl fresh medium supplemented with 5 μCi L-[methyl-^{3}H]-methionine (specific activity of 70–85 Ci/mmol; NEN Life Science Products, Inc.). All glands were floated in the surface of the medium to ensure an adequate supply of oxygen (Holbrook et al., 1997). Culture tubes were maintained at 26 °C and were rotated at 90 rpm at a 15° pitch on an orbital shaker. Following incubation, radiolabeled JH was extracted from the medium and CA together with 250 μl ice-cold iso-octane. A 100 μl aliquot from each sample was evaporated under N_2 and then mixed with 3 ml Scintiverse BD (Fisher) scintillation fluid. Radiolabeled methionine incorporation was measured using a scintillation counter (Beckman LS-5801). JH-III was verified as the principle product synthesized by *S. invicta* CA by comparison to the products of *Blattella germanica* females as separated by thin-layer chromatography. Pooled samples were analyzed on Whatman linear-K high performance silica gel plates (200 μ) using benzene, ethyl ether and acetic acid (84:15:1) as developing solvents. The proportion of JH-III from the total radioactivity was calculated as the ratio of the radioactivity in the JH zone in thin-layer chromatography and the radioactivity in an equivalent aliquot from the same extract after subtraction of blanks of ^{3}H-methionine. Other, more polar compounds were also detected.

An appropriate incubation time for the RCA was established by measuring the time course of JH production over a 6-h period. For each hour, 10–18 CA were sampled as described above. Each CA was taken from a virgin queen that had been reared away from a functional queen for 4 days and was used in only one sample. Based

on the results of this linearity test, a standard incubation time of 5 h was used for the remainder of the experiment.

The effect that release from the inhibitory queen pheromone has on JH production was determined by rearing *S. invicta* virgin queens in colony fragments then destructively sampling them for each of the first 15 days and after 25 days (n = 13–34) following isolation. Additional samples were obtained from 29 virgin queens prior to isolation, 26 ovipositing queens known to have been dealates for at least 60 days, and 25 workers.

2.3. Juvenile hormone content

The whole body content of JH was measured according to Bergot et al. (1981) as modified by Shu et al. (1997). Virgin queens reared in colony fragments were sampled after 0, 3, 6, 9, 12, 15 and 25 days of isolation from the pheromonal signal of functional queens. Older ovipositing queens and workers were also sampled. There were 5–8 pooled samples for each day. For each sample, 10 females, 100 µl 2% NaCl, 100 µl acetonitrile and 200 pg JH-III ethyl ester (internal standard; supplied by Ramaswamy) were homogenized three times in 1 ml 50% acetonitrile. Samples were centrifuged at 6000 rpm for 8 min, and the supernatant was collected and filtered through a C-18 Sep-Pac cartridge (Waters). The JH was collected from the cartridge with 8 ml 75% methanol and the solution dried under nitrogen. After being resuspended in 1 ml 50% acetonitrile, the samples were extracted three times with 2.5 ml hexane. The extracts were combined and concentrated in a SpeedVac concentrator (SVC 100H; Savant Instruments Inc.). The extract was resuspended in hexane and eluted through an Al_2O_3 (activated with 6% water) column with hexane, 10% ethyl ether–hexane and 30% ethyl ether–hexane. The 30% ethyl ether–hexane fraction was dried in the SpeedVac concentrator. The residue was resuspended with 75-µl methyl d alcohol, then 75 µl of 5% trifluoroacetic acid in methyl d alcohol was added. The sample was derivatized in an oven at 60 °C for 20 min. Derivitization was stopped with the addition of 500 µl hexane and the sample was dried in the SpeedVac concentrator. The residue was dissolved in 600 µl hexane and eluted through an Al_2O_3 column with hexane, 30% ethyl ether–hexane to remove any underivatized compounds, and ethyl acetate–hexane (1:1) to obtain d_3-methoxyhydrins. The ethyl acetate–hexane fraction was dried in the SpeedVac concentrator, the residue resuspended with 200 µl hexane and again dried in SpeedVac. The residue was dissolved in 100 µl hexane and dried down to less than 3 µl under nitrogen. JH d_3-methoxyhydrins were analyzed on a HP 6890 Series gas chromatography (Hewlett-Packard) equipped with a 30 m $\times$ 0.25 mm (ID) Carbowax Econo-Cap GC column (Alltech) coupled to a HP 5973 mass selective detector with helium as the carrier gas. The JH-III d_3-methoxyhydrins derivative was monitored for fragments at m/z 76 and 225 to ensure JH specificity. Derivatives for JH-I and JH-II were monitored for fragments at m/z 90 and 239 and m/z 90 and 225, respectively. Total abundance for specific JHs was quantified against that for internal standards of JH-I, JH-II, JH-III and JH-III-ethyl ester (supplied by Ramaswamy).

2.4. Timing of dealation and ovarian activity

Winged queens in colony fragments were observed for wing loss every 12 h following separation from the natal colony. These were not the same individuals used in the previous experiments, but they came from the same natal colonies and were housed under identical conditions. The reproductives were considered dealates once three of their four wings were absent, and the time to dealation was recorded. Ovarian activity was assessed by dissecting virgin queens in 70% ethanol under a dissecting microscope and counting the total number of chorionated eggs (see Vargo and Laurel, 1994) in the ovarioles and common oviduct of each female. On each of days, 3, 6, 9, 12, 15 and 25 following separation, at least 15 females were collected for dissection. Oocyte production was also assessed for virgin females taken directly from their natal colony (day 0), and for fully functional queens heading mature colonies (marked as Q). Total oocyte production was assessed in queens that had been isolated for 15 days by counting the total number of eggs oviposited.

2.5. Statistical analysis

Statistical tests used are reported with the results.

3. Results

3.1. Juvenile hormone biosynthesis rate

Thin-layer chromatography was used to compare the biosynthetic products of corpora allata dissected from both *Blattella germanica* and *S. invicta* females. The results indicate that fire ants produce primarily JH-III during in vitro incubation, confirming the findings of Burns et al. (2002). Of the radiolabeled constituents measured in the sample, JH-III accounted for 36.2% of the total activity sampled, and the remaining portion was primarily background radiation. Results of GC-MS analysis also confirmed that JH-III was the only homologue of the hormone found in the hemolymph. For the remainder of this paper JH-III will be referred to simply as JH.

The in vitro biosynthesis rate of JH by corpora allata

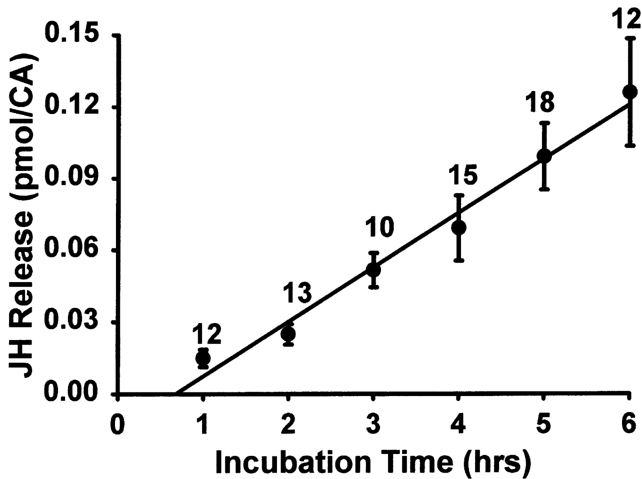

Fig. 1. Time course of juvenile hormone biosynthesis in maturing fire ant queens. Each point represents the mean (±S.E.) cumulative hormone production of 10–16 virgin queens sampled independently 4 days after separation from queen pheromone. The rate of production is very linear (r = 0.630, F = 51.439, $p < 0.001$).

was found to be linear for at least a 6-h period (Fig. 1; linear correlation, R = 0.630, $p < 0.001$, n = 80). Because of this long-term consistency in production rate, each CA was incubated for 5 h in all the subsequent measures of biosynthetic rate. This sample period maximized the concentration of JH in the samples while ensuring an accurate assessment of the biosynthetic rates.

The average rate of JH biosynthesis in day 0 alates was 0.076 ± 0.009 pmol/h (Fig. 2), which was significantly greater than in workers (0.024 ± 0.005 pmol/h; Mann–Whitney, $T_{25,29}$ = 381.0, $p < 0.001$), but significantly less than in mature queens ("Q"; 0.140 ± 0.012 pmol/h; Mann–Whitney, $T_{29,36}$ = 660.0, $p < 0.001$). During the 25 days following release from the inhibitory stimulus produced by a functional female, the amount

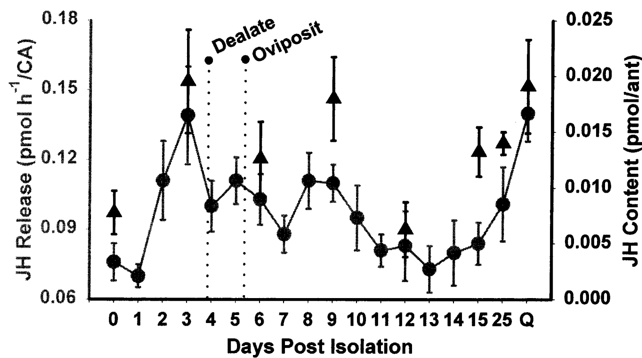

Fig. 2. The means (±S.E.) of the in vitro rate of biosynthesis (●) and whole body content (▲) of JH-III in mature queens (Q) and in alates during the first 25 days of isolation from inhibitory pheromones. Each circle represents 14–34 females and each triangle represents 5–8 pooled assays. The timing of dealation (3.8 ± 0.2 days) and the start of ovipositing (5.3 ± 0.3 days) are indicated.

of JH produced by virgin queens varied considerably (Fig. 2; Kruskal–Wallace ANOVA, H = 32.037, d.f. = 16, p = 0.01). After 3 days of isolation the rate had almost doubled to 0.139 pmol/h (Mann–Whitney, $T_{22,29}$ = 673.0, $p < 0.01$). There was a subsequent decline in JH production so that after 13 days there was no significant difference between maturing queens and day 0 alates (Mann–Whitney, $T_{15,29}$ = 333.0, p = 0.921). Juvenile hormone production began to rise steadily again as females continued to mature, so that the highest observed rates were in the oldest queens.

3.2. Juvenile hormone content

The profiles of the biosynthetic rate and the whole body content of JH appear to follow similar patterns in reproductives isolated from the inhibitory influence of queen primer pheromones (Fig. 2). Based on the means for 8 days on which both measures were determined, the biosynthetic rate and content are significantly correlated, (linear correlation, r = 0.884, F = 21.513, p = 0.004). The total content of JH per ant was initially low (0.008 ± 0.002 pmol), rose sharply to peak after 3 days (0.020 ± 0.005 pmol), dropped to low levels again after 12 days and rose once more in older females. Mature queens (Q) had more than twice (0.019 ± 0.004 pmol) the JH of day 0 alates sampled directly from their natal nest. Workers were also sampled, but the quantity of JH was too low to the distinguished from background noise.

3.3. Timing of dealation and ovary activity

Subsequent to separation from their natal nest, alates shed their wings within an average of 3.8 ± 0.2 days (n = 75). Females began ovipositing within 5.3 ± 0.3 days (n = 57), and by day 15 had laid an average of 233.9 ± 13.1 eggs (n = 34). The rate at which new eggs were being produced increased steadily during the first 15 days of maturation (Fig. 3; r = 0.765, F = 233.307, $p < 0.001$). During this time, the number of vitellogenic oocytes found in the ovaries increased from an average of 2.1 ± 0.3 (n = 38) to 41.1 ± 3.1 (n = 34). After this the rate of oocyte production varied little through day 25. However, there must be a subsequent rise in ovarian activity since an average of 65.2 ± 4.0 vitellogenic oocytes is observed in older queens drawn from mature colonies, although this number varied greatly between females. The variability was likely due to normal age differences between queens randomly selected from natural populations, or it may be a result of the queens producing eggs in batches rather than at a steady rate.

4. Discussion

The results of this experiment demonstrate distinct changes in juvenile hormone biosynthesis and whole

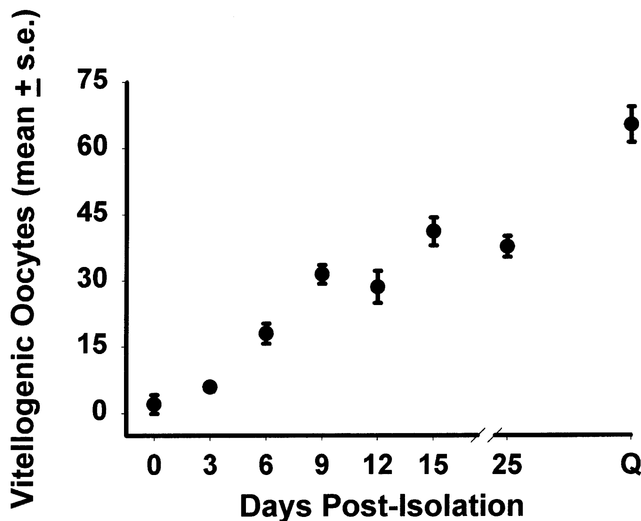

Fig. 3. Number of vitellogenic oocytes found in the ovaries of maturing queens following isolation. Extent of vitellogenic activity is highly correlated with age ($r = 0.630$, $F = 51.439$, $p < 0.001$).

body content in maturing *S. invicta* queens after separation from their natal nest. Although the quantities involved are small, especially when compared to the high titers of other Hymenoptera such as honey bee foragers (Huang et al., 1991; Robinson et al., 1991) and bumblebees (Röseler and Röseler, 1978; Bloch et al., 1996, 2000), we observed significant differences between the days sampled. Burns et al. (2002) similarly found that the JH titer of maturing *S. invicta* queens were relatively low, although their values were roughly twice what we found. However, the difference can be readily explained by the minute quantities involved, variations in experimental procedure, and potential differences between the populations sampled.

The significant changes we observed provide additional support for the hypothesis that the pheromone released by *S. invicta* queens inhibits dealation and reproduction in alates by suppressing the production of juvenile hormone in the corpora allata, thus presumably acting as a primer pheromone (Barker, 1978, 1979; Vargo and Laurel, 1994). Previous research had indicated that exogenous application of JH and its analogs could release fire ant alates from these inhibitory effects (Kearney et al., 1977; Vargo and Laurel, 1994; Burns et al., 2002), however, there was little information about the hormonal events which this external application was mimicking. This study shows that isolation from the queen pheromone causes increases in corpora allata activity and JH content that are well coordinated with dealation and ovarian activity, highlighting the regulatory role of JH.

It is possible that the queen pheromone may regulate alate development by altering the activity of JH-esterases, thereby reducing levels of circulating JH (Hammock, 1985). However, the significant correlation between biosynthesis rate and JH content (Fig. 3) suggests that it is CA activity alone that determines the level of circulating JH, which also seems to be the case in honey bees (Rachinsky and Hartfelder, 1990; Huang et al., 1991) and roaches (Tobe et al., 1985). The queen pheromone may control reproductive development by manipulating CA activity, as occurs in *Bombus terrestris* workers exposed to their queen's mandibular pheromone (Röseler and Röseler, 1978; Röseler et al., 1981; Bloch et al., 1996; but see Bloch et al., 2000). Although the role of esterases cannot be wholly excluded without direct measurements, their activity appeared relatively constant; the amount JH circulating in the body was roughly one-tenth of what was being produced in the CA over the entire sample period, despite major fluctuations in the biosynthesis rate. Similar differences in rate and titer have been observed in both bumble bees (Röseler and Röseler, 1978; Bloch et al., 1996, 2000) and honey bees (Huang et al., 1991; Robinson et al., 1991) even though substantially larger quantities of JH are involved in both of these species.

The response of virgin queens to isolation from the queen pheromone is not immediate. The inhibitory effect on the CA appears to persist for at least 24 h. Once the inhibition has faded, the rate of JH biosynthesis rises sharply, peaking just prior to dealation. On that day, the concentration of JH might reach a threshold necessary to elicit wing-shedding behaviors, either by priming or directly activating the nervous system. Exogenous application of JH may trigger dealation by similarly exceeding this threshold level (Burns et al., 2002). However, dealation can also take place within 30 min of leaving the natal nest if an alate participates in a nuptial flight (Vinson and Greenberg, 1985; Burns et al., 2002). The substantial delay observed in 'orphaned' colonies (Fletcher and Blum, 1981; Vinson and Greenberg, 1985) and under the artificial conditions of this experiment may indicate the flight and/or mating provide important trigger stimuli for initiating dealation by strongly stimulating corpora allata activity. Similar trigger stimuli have been identified in the rapid up-regulation of CA activity in other insects (Engelmann, 1970; Feyereisen, 1985; Gadot et al., 1989), and in most cases they are not obligatory for CA activation to occur. Alternatively, the nuptial flight may trigger a separate mechanism to initiate dealation that is independent of JH titer (Burns et al., 2002). Regardless of the particular mechanism triggered by the nuptial flight, the dealation of unflown/unmated queens appears to be highly coordinated with JH production.

Following the initial increase in CA activity, JH remains elevated for several days, gradually drops to a level similar to that in inhibited alates, and then rises again in older females (Fig. 2). If JH serves as a gonadotropic hormone in *S. invicta*, as it does for most other insects (Koeppe et al., 1985; but see Robinson et al.,

1991, 1992), then the sustained elevation in JH titer may be necessary to initiate and maintain oogenesis, while fluctuations in CA activity may moderate the rate of egg production. The role of JH in reproduction can be varied, promoting accessory gland activity, vitellogenesis by the fat body, DNA replication within the follicular epithelium surrounding the terminal oocytes, ovarian protein synthesis, and the deposition of vitellin within developing eggs (Engelmann, 1970, 1983; Koeppe et al., 1985). In *S. invicta*, an elevated JH titer may also promote wing muscle histolysis (Vargo and Laurel, 1994; but see Jones et al., 1978), making additional amino acids available for vitellogenesis (Toom et al., 1976a; Barker, 1979; Lewis et al., 2001).

Each physiological response to circulating JH may occur independently, being triggered at different concentrations, and each response may be graded to match the concentration of JH. Such a tiered response system would enable queens to maintain a greater level of control over reproduction. One example of such differential responses to JH is an apparent separation of vitellogenesis and vitellogenin uptake by the ovaries. Vitellogenin is produced in virgin alates still under inhibitory control, but it is not incorporated into the oocytes and therefore accumulates in the hemolymph (Vargo and Laurel, 1994; Lewis et al., 2001). Vargo and Laurel (1994) proposed that vitellogenesis and vitellogenin uptake were promoted at two different threshold concentrations of JH. Consistent with this hypothesis was our finding that although JH biosynthesis is suppressed prior to isolation, inhibited virgin queens still produced significantly more of the hormone than workers (Fig. 2). The steady production and storage of vitellogenin in virgin alate queens may be a secondary effect of incomplete CA inhibition. Once the alates are released from the queen pheromone, the resulting elevation in JH production may further promote vitellogenesis and initiate vitellogenin uptake. We also observed that the number of vitellogenic oocytes rose significantly even before JH production had peaked, so an intermediate concentration of JH may be sufficient to promote uptake.

The decrease in JH content observed between days 8 and 13 (Fig. 2) might reflect a normal interruption in the cycle of egg production in monogyne queens. Previous studies have shown that egg production declines steeply for a short time after the initial clutch is produced as the queen delays producing a second clutch of eggs until the first generation of offspring molts into workers (Markin et al., 1972; Lewis et al., 2001). Although we observed only a small decrease in vitellogenic oocytes during this period (Fig. 3), egg production may well have ceased after the eighth day, and the maturation of the oocytes may have been suspended for several days. Because an independently founding queen does not forage, she must rely wholly on the nutrient resources stored in her fat body until her first brood matures, and therefore is limited in the number of brood she can support. The resource drain on a queen at this stage is manifested in a significant reduction in her body mass (Markin et al., 1972) and total protein content (Toom et al., 1976b). Cessation of oogenesis could be triggered by a feedback mechanism that lowers JH production when the fat body or other endogenous resources have become depleted to a threshold level. This mechanism would prevent the queen from over-investing in egg production, ensuring she maintains sufficient energetic reserves for the incipient colony to survive.

The resumption of egg production appears to coincide with the emergence of the first brood of workers. These helpers assume the energetic burdens of maintaining the colony, tending brood, and foraging (Oster and Wilson, 1978; Porter and Tschinkel, 1986). They also provide the queen with amino acids produced by fourth instar larvae that may be necessary for egg production (Tschinkel, 1988). This allows the queen to replenish her endogenous resources and begin producing her next brood. However, the queens in this experiment were provided with a small number of workers from the start, which might have reduced the effects of resource depletion on egg production. Unlike solitary monogyne queens that produce 20–100 eggs during the first two weeks following dealation (Fincher and Lund, 1967; Kahn et al., 1967; Markin et al., 1972), the females in this experiment produced, on average, over 230 eggs. The presence of workers during this stage of development may stimulate a queen to invest more of her resources in egg production, given that she would not have to bear the cost of rearing the first brood. Such a high rate of egg production would quickly deplete a queen's endogenous reserves. The rapidity of the depletion would be exacerbated in these colony fragments by the absence of fourth instar larvae, which have been shown to be crucial in replenishing proteins for oogenesis (Tschinkel, 1988). The resource demands of the queens would likely exceed the workers provisioning ability, again triggering reproductive inhibition. Similar "boom or bust" cycles of egg production appear to occur regularly in newly founded colonies of *S. invicta* (Markin et al., 1972; O'Neal and Markin, 1975), possibly until there are sufficient workers and larvae available to ensure a queen is adequately supplied at all times (Tschinkel, 1988).

In summary, the results of this experiment show that exposure to queen pheromone inhibits JH production in *S. invicta*, that the rate of JH biosynthesis is the primary determinate of JH titer, and that both the onset of dealation and oogenesis are strongly correlated with an increase in the concentration of circulating JH. These data support the hypothesis that the queen pheromone inhibits the reproductive development in nestmates by suppressing CA activity, however, it remains to be determined how this putative primer pheromone acts on

the CA and the specific means by which JH might promote dealation and oogenesis in *S. invicta*. It is also not clear how CA activity is affected by the endogenous state of the queen, although this interaction must play an important role in determining her ability to reproduce.

Acknowledgements

We thank Dr. S. Ramaswamy and Dr. D. Crook, Department of Entomology, Kansas State University, for supplying internal standard, and Dr. C. Gemeno, North Carolina State University, for assistance with GC analysis, Dr. C. Schal and Dr. Y. Fan, Department of Entomology, North Carolina State University, for assistance with radiochemical analysis, Dr. W. Boss, Department of Botany, North Carolina State University, for use of the TLC plate scanner, and Dr. M. Roe, Department of Entomology, North Carolina State Univeristy, for use of the GC-MSD. This research was supported by the Keck Center for Behavioral Biology and by a grant from the Texas Imported Fire Ant Research and Management Plan.

References

Barker, J.F., 1978. Neuroendocrine regulation of oocyte maturation in the imported fire ant *Solenopsis invicta*. General and Comparative Endocrinology 35, 234–237.

Barker, J.F., 1979. Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*. Experientia 35, 552–553.

Bergot, B.J., Ratcliff, M., Schooley, D.A., 1981. Method for quantitative determination of the four known juvenile hormones in insect tissue using gas-chromatography–mass spectroscopy. Journal of Chromatography 204, 231–244.

Bloch, G., Borst, D.W., Huang, Z.-Y., Robinson, G., Cnaani, J., Hefetz, A., 2000. Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. Journal of Insect Physiology 46, 47–57.

Bloch, G., Borst, D.W., Huang, Z.-Y., Robinson, G.E.J., Hefetz, A., 1996. Effects of social conditions on juvenile hormone mediated reproductive development in *Bombus terrestris* workers. Physiological Entomology 21, 257–267.

Burns, S.N., Teal, P.E.A., Vander Meer, R.K., Nation, J.L., Vogt, J.T., 2002. Identification and action of juvenile hormone III from sexually mature alate females of the red imported fire ant, *Solenopsis invicta*. Journal of Insect Physiology 48, 357–365.

Engelmann, F., 1970. The Physiology of Insect Reproduction. Pergamon Press, New York.

Engelmann, F., 1983. Vitellogenesis controlled by juvenile hormone. In: Downer, R.G.H., Laufer, H. (Eds.), Endocrinology of Insects. Invertebrate Endocrinology, vol. 1. Alan R. Liss, New York, pp. 260–270.

Feyereisen, R., 1985. Regulation of juvenile hormone titer: synthesis. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 1. Pergamon Press, Oxford, pp. 391–429.

Feyereisen, R., Tobe, S.S., 1981. A rapid partition assay for routine analysis of juvenile hormone release by corpora allata of adult female *Diplopetera punctata*. Analytical Biochemistry 111, 372–375.

Fincher, G.T., Lund, H.O., 1967. Notes on the biology of the imported fire ant, *Solenopsis saevissima richteri* Forel (Hymenoptera: Formicidae) in Georgia. Journal of the Georgia Entomological Society 2, 91–94.

Fletcher, D.J.C., Blum, M.S., 1981. Pheromonal control of dealation and oogenesis in virgin queen fire ants. Science 212, 73–75.

Fletcher, D.J.C., Blum, M.S., 1983a. Regulation of queen number by workers in colonies of social insects. Science 219, 312–314.

Fletcher, D.J.C., Blum, M.S., 1983b. The inhibitory pheromone of queen fire ants: effects of disinhibition on dealation and oviposition by virgin queens. Journal of Comparative Physiology 153, 467–475.

Gadot, M., Burns, E., Schal, C., 1989. Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: effects of grouping and mating. Archives of Insect Biochemistry and Physiology 11, 189–200.

Glancey, B.M., Glover, A., Lofgren, C.S., 1981. Pheromone production by virgin queens of *Solenopsis invicta* Buren. Sociobiology 6, 119–127.

Hammock, B.B., 1985. Regulation of juvenile hormone titer: degradation. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 7. Pergamon Press, Oxford, pp. 431–472.

Holbrook, G., Chiang, A.-S., Schal, C., 1997. Improved conditions for culture of biosynthetically active cockroach corpora allata. In vitro Cellular and Developmental Biology—Animal 33, 452–458.

Huang, Z.-Y., Robinson, G.E., Tobe, S.S., Yagi, K.J., Strambi, C., Strambi, A., Stay, B., 1991. Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. Journal of Insect Physiology 37, 733–741.

Jones, R.G., Davies, W.L., Hung, A.C., Vinson, S.B., 1978. Insemination induced histolysis of the flight musculature in fire ants (*Solenopsis* spp.): an ultrasonic study (1). American Journal of Anatomy 151, 603–610.

Jouvenaz, D.P., Allen, G.E., Banks, W.A., Wojcik, D.P., 1977. A survey for the pathogens of fire ants, *Solenopsis* spp. in the southeastern United States. Florida Entomologist 60, 275–279.

Kahn, A.R., Green, H.B., Brazzel, J.R., 1967. Laboratory rearing of the imported fire ant. Journal of Economic Entomology 60, 915–917.

Kearney, G.P., Toom, P.M., Bloomquist, G.J., 1977. Induction of dealation in virgin female *Solenopsis invicta* with juvenile hormones. Annals of the Entomological Society of America 70, 699–701.

Klobuchar, E.A., Deslippe, R.J., 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). Naturwissenschaften 89, 302–304.

Koeppe, J.K., Fuchs, M., Chen, T.T., Hunt, L.M., Kovalick, G.E., Briers, T., 1985. The role of juvenile hormone in reproduction. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 7. Pergamon Press, Oxford, pp. 165–203.

Lewis, D.K., Campbell, J.Q., Sowa, S.M., Chen, M.-E., Vinson, S.B., Keeley, L.L., 2001. Characterization of vitellogenin in the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Apocrita: Formicidae). Journal of Insect Physiology 47, 543–551.

Markin, G.P., Collins, H.L., Dillier, J.H., 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Annals of the Entomological Society of America 65, 1053–1058.

O'Neal, J., Markin, G.P., 1975. Brood development of the various castes of imported fire ant, *Solenopsis invicta* Buren. Journal of the Kansas Entomological Society 48, 152–159.

Oster, G.F., Wilson, E.O., 1978. Caste and Ecology in the Social Insects. Princeton University Press, Princeton.

Porter, S.D., Tschinkel, W.R., 1986. Adaptive value of nanitic workers in newly founded red imported fire ant colonies (Hymenoptera:

Formicidae). Annals of the American Entomological Association 79, 723–726.
Pratt, G.E., Tobe, S.S., 1974. Juvenile hormones biosynthesized by corpora allata of adult female locust in vitro. Life Sciences 14, 575–586.
Rachinsky, A., Hartfelder, K., 1990. Corpora allata activity, a prime regulating element for caste-specific juvenile hormone titer in honey bee larvae (*Apis mellifera carnica*). Journal of Insect Physiology 36, 189–194.
Robinson, G.E., Strambi, C., Strambi, A., Feldlaufer, M.F., 1991. Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult workers and queen hone bees (*Apis mellifera*). Journal of Insect Physiology 37, 929–935.
Robinson, G.E., Strambi, C., Strambi, A., Huang, Z., 1992. Reproduction in worker honey bees is associated with low juvenile hormone titers and rates of biosynthesis. General and Comparative Endocrinology 87, 471–480.
Robinson, G.E., Vargo, E.L., 1997. Juvenile hormone in adult eusocial Hymenoptera; gonadotropin and behavioral pacemaker. Archives of Insect Biochemistry and Physiology 35, 559–583.
Röseler, P.-F., Röseler, I., 1978. Studies on the regulation of the juvenile hormone titer in bumblebee workers, *Bombus terrestris*. Journal of Insect Physiology 24, 707–713.
Röseler, P., Röseler, I., van Honk, C.G.J., 1981. Evidence for inhibition of corpora allata activity in workers of *Bombus terrestris* by a pheromone from the queen's mandibular glands. Experientia 37, 348–351.
Shu, S., Park, Y.I., Ramaswamy, S.B., Srinivasan, A., 1997. Hemolymph juvenile hormone titer in pupal and adult stages of southwestern cornborer, *Diatraea grandiosella* (Pyralidae), and its relationship with ovarian development. Journal of Insect Physiology 43, 719–726.
Tobe, S.S., Pratt, G.E., 1974. The influence of substrate concentrations on the rate of insect juvenile hormone biosynthesis by corpora-allata of the desert locust in vitro. Biochemistry Journal 144, 107–113.
Tobe, S.S., Ruegg, R.P., Stay, B., Baker, F.C., Miller, C.A., Schooley, D.A., 1985. Juvenile hormone titer and regulation in the cockroach *Diploptera punctata*. Experientia 41, 1028–1034.
Toom, P.M., Johnson, C.P., Cupp, E.W., 1976a. Utilization of body reserves during preoviposition activity by *Solenopsis invicta*. Annals of the Entomological Society of America 69, 145–148.
Toom, P.M., Cupp, E.W., Johnson, C.P., Griffin, I., 1976b. Utilization of body reserves for minimum brood development by queens of the imported fire ant, *Solenopsis invicta*. Journal of insect Physiology 22, 217–220.
Tschinkel, W.R., 1988. Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. Physiological Entomology 13, 327–350.
Vander Meer, R.K., Alonso, L.E., 2002. Queen primer pheromone affects conspecific fire ant (*Solenopsis invicta*) aggression. Behavior, Ecology and Sociobiology 51, 122–130.
Vargo, E.L., 1992. Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*. Behavioral, Ecology and Sociobiology 31, 205–210.
Vargo, E.L., 1998. Primer pheromones in ants. In: Vander Meer, R.K., Breed, M.D., Winston, M.L., Espelie, K.E. (Eds.), Pheromone Communication in Social Insects: Ants, Wasps, Bees and Termites. Westview Press, Boulder, CO, pp. 293–313.
Vargo, E.L., 1999. Reproductive development and ontogeny of queen pheromone production in the fire and *Solenopsis invicta*. Physiological Entomology 24, 370–376.
Vargo, E.L., Fletcher, D.J.C., 1986. Evidence of pheromonal queen number control over the production of female sexuals in the fire ant *Solenopsis invicta*. Journal of Comparative Physiology A 159, 741–749.
Vargo, E.L., Fletcher, D.J.C., 1987. Effect of queen number on the production of sexuals in natural populations of the first ant *Solenopsis invicta*. Physiological Entomology 12, 109–116.
Vargo, E.L., Laurel, M., 1994. Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. Journal of Insect Physiology 40, 601–610.
Vinson, B., Greenberg, L., 1985. The biology, physiology and ecology of imported fire ants. In: Vinson, S.B. (Ed.), Economic Impact and Control of Social Insects. Praeger, New York, pp. 193–226.
Willer, D.E., Fletcher, D.J.C., 1986. Difference in inhibitory capability among queens of the fire ant *Solenopsis invicta*. Physiological Entomology 11, 475–482.
Wilson, E.O., 1971. The Insect Societies. Belknap Press, Cambridge, MA.
Wilson, E.O., Bossert, W.H., 1963. Chemical communication among animals. Recent Progress in Hormone Research 19, 673–716.