J Comp Physiol A (2006) 192: 315–320
DOI 10.1007/s00359-005-0065-6

ORIGINAL PAPER

Colin Brent · Christian Peeters · Vincent Dietmann
Robin Crewe · Ed Vargo

Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*

Received: 19 September 2005 / Revised: 4 October 2005 / Accepted: 8 October 2005 / Published online: 8 November 2005

Abstract In colonies of the queenless ant *Streblognathus peetersi*, dominance interactions produce a reproductive hierarchy in which one individual, the alpha, is capable of producing offspring while her subordinates remain infertile. Based on differences between behaviour and cuticular hydrocarbon profiles, the subordinates can be further divided into high and low ranking workers. Although it had been shown previously that alphas treated with a juvenile hormone analog lose their reproductive status, little was known of the endocrinological basis of dominance in this species. To elucidate the underlying endocrinology of these three ranks, we measured the individual in vitro rate of juvenile hormone (JH) production of excised corpora allata, and the ecdysteroid titer of pooled hemolymph samples. Production of JH was highest in low-ranking workers, intermediate in high rankers, and almost undetectable in alphas. Ecdysteroid titers were low for low rankers, but were more than twice as high for both high rankers and alphas. The results support the hypothesis that JH suppresses ovarian function in these queenless ants, and suggest that ecdysteroids may be responsible for stimulating vitellogenin production. The possible role of these hormones as behavioural modulators is also discussed.

Keywords Reproduction · Dominance hierarchy · Juvenile hormone · Ecdysteroids · Ant

C. Brent (✉)
School of Life Sciences, Arizona State University,
Box 4501, Tempe, AZ 85287, USA
E-mail: colin.brent@asu.edu
Tel.: +1-480-9657292
Fax: +1-480-9656899

C. Peeters
Laboratoire d'Écologie, CNRS UMR 7625,
Université Pierre et Marie Curie,
75252 Paris Cedex 05, France
E-mail: cpeeters@snv.jussieu.fr
Tel.: +33-1-44272668
Fax: +33-1-44273516

V. Dietmann
Department of Zoology and Entomology,
University of Pretoria,
0002 Pretoria, South Africa
E-mail: vdietemann@zoology.up.ac.za
Tel.: +27-12-4202548
Fax: +27-12-3625242

R. Crewe
Department of Zoology and Entomology,
University of Pretoria,
0002 Pretoria, South Africa
E-mail: robin.crewe@up.ac.za
Tel.: +27-12-4204094
Fax: +27-12-4203696

E. Vargo
Department of Entomology,
North Carolina State University, Box 7613,
Raleigh, NC 27695, USA
E-mail: ed_vargo@ncsu.edu
Tel.: +1-919-5132743
Fax: +1-919-5157746

Introduction

Streblognathus peetersi is one of just a few species of ant in which the queen caste has been lost and all the morphologically similar workers can potentially mate and reproduce (Peeters 1993; Robertson 2002). Their social organization is analogous to that of some primitively eusocial wasps (reviewed in West-Eberhard 1996), in which only one or a few high-ranking individuals mate to produce offspring, while the other females remain infertile and are responsible for brood care and nest maintenance. In queenless ants agonistic interactions among nestmates are used to establish a dominance hierarchy that regulates reproductive capability (Monnin and Peeters 1999; Cuvillier-Hot et al. 2004a, b). Three different social categories are established within this hierarchy; alpha, high rank, and low rank. During establishment of the hierarchy, higher-ranking workers will frequently engage in agonistic displays towards

nestmates, while lower rankers are seldom aggressive. The alpha is distinguished by its unique gaster curling behaviour. Once the hierarchy is established, its stability is maintained by the production of rank-specific hydrocarbon profiles (Cuvillier-Hot et al. 2004a, b).

It is highly likely that individual ranks correspond to differences in endocrine activity. Juvenile hormone (JH) and ecdysteroids, two of the major developmental regulators for most insects (Goodman and Granger 2005; Raikhel et al. 2005; Swever et al. 2005), have profound effects on individual behaviour, reproductive capability and pheromone production in other eusocial hymenoptera (reviewed in Fahrbach 1997; Robinson and Vargo 1997; Bloch et al. 2002; Hartfelder and Emlen 2005), and likely underlie the observed status differences among *S. peetersi* workers. Cuvillier-Hot et al. (2004b) have already shown that alpha females receiving topical applications of pyriproxyfen, a JH analogue, have decreased fertility and develop cuticular hydrocarbon profiles similar to that of low rankers; after several days they lose their reproductive status to a high ranking worker. These findings highlight the importance of JH, but accurately interpreting their implications is difficult, given the interspecific variation in the function of JH (Goodman and Granger 2005; Hartfelder and Emlen 2005) and the current dearth of hormonal information for this and other species of ants. In order to better understand the role of JH and ecdysteroids in maintaining status distinctions in *S. peetersi*, we undertook an examination of the hormonal profiles of differently ranked workers.

Materials and methods

Animals and housing conditions

Colonies of *S. peetersi* were collected on two separate occasions near Magoebaskloof, Limpopo province, South Africa. In 2003, eight colonies (32–204 workers/colony) were collected and used in determining JH production. Additional workers that had begun to display gaster curling behaviour (Cuvillier-Hot et al. 2004a) by 48 h after the removal of the original alpha female were also sampled from five of these eight colonies and were treated as replacement alphas. Eleven additional colonies (27–283 workers/colony) were collected in 2005 for ecdysteroid titration. Of these 11, the five largest colonies were split into equal halves to generate additional alpha-ranked workers. Colonies were reared in plaster nests, at 25°C, under a 12:12 light:dark cycle and were provided meal worms (*Tenebrio molitor*), 20% sugar water and tap water ad libitum. Each worker was individually marked with enamel paint applied to the thorax.

Behavioural assessment of rank

Behavioural observations were initiated 1 day after the colonies were housed under laboratory conditions. Individual behaviours in each colony were observed for 10 min periods twice a day for 6 days prior to final rank assignment and sampling, except for several alpha females sampled after 48 h as noted above. All instances of agonistic displays and the identities of the interacting individuals were recorded. High-ranking individuals were identified by their frequent aggressive behaviours, which included gaster rising, antennal boxing, and biting (Cuvillier-Hot et al. 2004a). A high ranker had to show a minimum of 15 aggressive acts during the combined observation periods. Among these high rankers, alpha workers were delineated by their stereotyped displays of gaster curling. Individuals selected as alphas had to be the only workers in their colony showing gaster curling, and such displays had to have been observed for at least three observation periods. Low-ranking workers were selected from among those individuals that spent the majority of their time at the nest periphery and never displayed aggression.

Juvenile hormone production

The in vitro biosynthetic rates of JH production in individual workers of low, high and alpha rank were measured using a rapid partition radiochemical assay (RCA; Pratt and Tobe 1974; Tobe and Pratt 1974; modified by Feyereisen and Tobe 1981; Brent and Vargo 2003). The paired glands of the corpora allata (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, USA), with 50 mM Hepes buffer, pH 7.4, without methionine or bicarbonate, and containing 2% Ficoll 400 (Sigma Chemical Co., MO, USA). After the preincubation, 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 BD on an orbital shaker. Following incubation, radiolabeled JH was extracted from the medium and CA together with 250 µl ice-cold isooctane. A 100 µl aliquot from each sample was evaporated under N_2, and then mixed with 3 ml Scintiverse (Fisher) scintillation fluid. Radiolabelled methionine incorporation was measured using a scintillation counter (Beckman LS-5801).

An appropriate incubation time for the RCA was established by measuring the time course of JH production over a 6-h period. The CA from five low ranking workers were sampled as described above. At each hour, the CA were transferred to fresh medium and the quantity of JH released during the previous hour was assessed as described above.

Titration of circulating ecdysteroids

Ecdysteroid titer was determined using a modified version (Zera and Bottsford 2001; Brent and Vargo 2003) of the radioimmunoassay developed by Warren et al. (1984). For each sample, two workers of the same rank were homogenized twice in 500 µl chilled 90% methanol. After centrifugation at 3500 rpm for 5 min, the supernatant was removed and pooled. The extracts were lyophilized, resuspended in 1 ml methanol, and stored at −80°C until analyzed. Duplicate 10 µl aliquots of each sample were incubated overnight with 100 µl of [^{3}H]-ecdysone (specific activity of 1.9–4.1 TBq mmol^{-1}; Perkin-Elmer Life Sciences Inc.) in Borate Buffer, and 100 µl of a polyclonal ecdysteroid antiserum (H-22 antibody, gift from L. Gilbert) at 4°C on an orbital shaker. The antiserum has been shown to be cross-reactive for ecdysone, ecdysterone, 20-hydroxyecdysone and makisterone A (Warren and Gilbert 1986). A standard competition curve was generated using 20-hydroxyecdysone (Sigma) in quantities from 15.6 to 2,000 pg. After 18 h, 20 µl of cleaned Protein-A solution (Pansorbin, CalBiochem) was added to each tube to precipitate the complex during another hour of incubation at room temperature. Samples were centrifuged at 5000 *g* and the remaining pellet was washed twice with 100 µl borate buffer. Radioactivity was determined by scintillation spectrometry and ecdysteroid concentrations were estimated by non-linear regression.

Statistics

Due to non-normally distributed data for JH production, analyses of variance based on ranks were used to determine whether significant differences existed between females of different rank. Dunn's method was used to correct for experiment-wise errors arising from multiple comparisons, with an overall alpha set at 0.05. The more normally distributed ecdysteroid data were analyzed by standard ANOVA, with application of the Holm-Šidák method to limit experiment-wise errors.

Results

Juvenile hormone production

The in vitro biosynthesis rate of JH by the CA was found to be linear for at least a 6-h period (linear correlation, $R = 0.731$, $P < 0.001$, $n = 30$). Because of this long-term consistency in CA activity, all subsequent incubations were conducted for 5 h to ensure accurate measures of production rate.

Low ranking workers had the fastest rate of JH production (Fig. 1; 12.431 ± 2.858 pmol/h, $n = 8$), producing almost four times more per hour than high ranking workers (3.331 ± 0.972 pmol/h, $n = 10$; ANOVA on ranks, $Q = 1.687$, $P > 0.05$), and nearly 240 times more than alpha females (0.059 ± 0.015 pmol/h, $n = 8$; ANOVA on ranks, $Q = 4.504$, $P < 0.05$). The difference in production rates between high rankers and alphas was also significant (ANOVA on ranks, $Q = 3.124$, $P < 0.05$).

The behavioural and endocrinological shift from a subordinate high ranker to a replacement alpha appears to be relatively rapid. Within 24 h of removal of the alpha, there were workers in most colonies that behaved aggressively and displayed gaster curling. After just 48 h, the putative replacement alphas had JH production rates equivalent to that of older alphas (0.189 ± 0.077 pmol/h, $n = 5$; ANOVA on ranks, $Q = 0.805$, $P > 0.05$).

Ecdysteroid whole body content

As observed with JH production, ecdysteroid content also varied considerably between ranks (Fig. 1). Low-ranking workers had the lowest ecdysteroid content (32.88 ± 8.85 ng), which was less than half the content found in high rankers (81.68 ± 5.82 ng; ANOVA, $t = 3.464$, $P = 0.003$) and one third that in alpha females (93.43 ± 11.36 ng; ANOVA, $t = 4.621$, $P < 0.001$). Although alphas had higher ecdysteroid titers than high rankers, the difference was not significant (ANOVA on ranks, $t = 0.860$, $P = 0.401$).

Discussion

Differentiation of status within a reproductive dominance hierarchy is often the result of behavioural and pheromonal interactions between nestmates, but the subsequent development of rank-associated traits results from specific alterations in endocrine activity that can influence an individual's behaviour and reproductive capability (reviewed in Robinson and Vargo 1997; Bloch et al. 2002; Hartfelder and Emlen 2005). These changes can be permanent, but they are more frequently dynamic, capable of responding to changing colony conditions. JH and ecdysteroids appear to be integrally involved in regulating these rank-specific differences in the social insects studied to date, but the functions of each hormone can vary considerably between species (reviewed in Hartfelder and Emlen 2005).

The role of JH in most adult insects is as a gonadotrope, stimulating the production and uptake of vitellogenin, and the onset of oviposition (Goodman and Granger 2005; Raikhel et al. 2005; Swever et al. 2005). JH has a similar function in many eusocial Hymenoptera (reviewed in Robinson and Vargo 1997), but there are exceptions such as reproductively active honeybee queens, in which JH has no apparent role and little is produced (reviewed in Hartfelder and Engels 1998). S. *peetersi* appears to be another such exception. Application of a JH analogue to fecund alpha females causes a decline in circulating vitellogenins, indicating

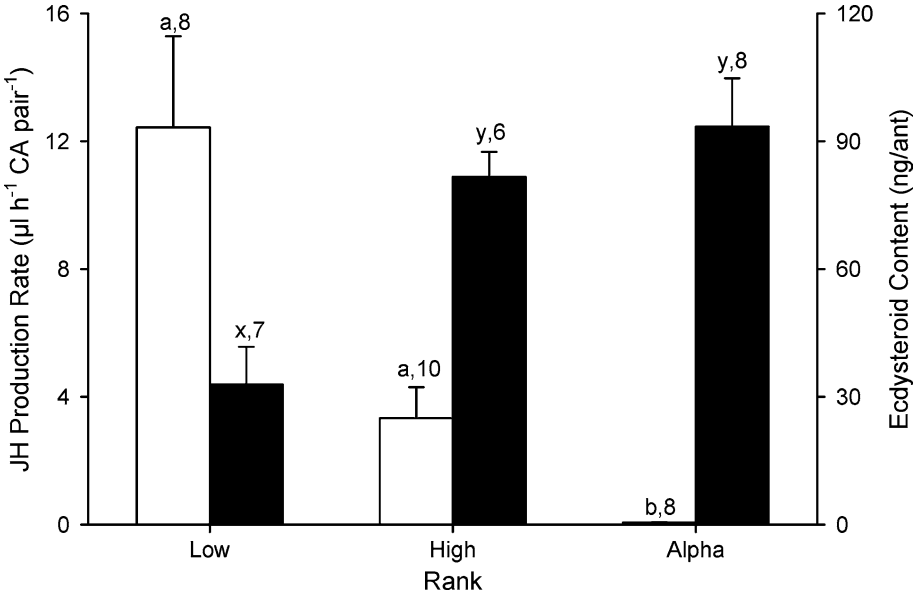

Fig. 1 The mean (±SE) in vitro rate of juvenile hormone (*JH*) release (*open bars*) and ecdysteroid titer (*filled bars*) for females with low, high or alpha ranking. Sample sizes are indicated above *each bar*. Significant ($P < 0.05$) pairwise differences between groups are indicated with *different letters*

that JH may actually suppress reproductive capability (Cuvillier-Hot et al. 2004b). Our results support this assessment; we found that JH production was negatively correlated with rank, so that corpora allata activity in alpha females was nearly undetectable, but grew significantly higher as rank decreased (Fig. 1). This is quite different from the reproductive endocrinology of queens of the fire ant *Solenopsis invicta*, in which an increase in circulating JH is necessary to initiate ovarian development and activity (Brent and Vargo 2003), and vitellogenin uptake (Vargo and Laurel 1994). In contrast, the pattern of JH production in *S. peetersi* is quite similar to that found in queenless *Diacamma* ants, in which JH is found in the subordinate ranks, but not in the reproductive workers (Sommer et al. 1993). The loss of the queen phenotype in these species would have necessitated the development of an alternative means for controlling reproductive activity. However, this modified endocrine system would have needed to develop within the constraints created by the worker phenotype. One potential explanation for our current findings is that the worker endocrine system retains some characteristics found in immature stages, for which high concentrations of circulating JH can inhibit the development of adult features, such as functional ovaries (Goodman and Granger 2005). Alternative neuroendocrine systems would then have to be relied upon to modulate ovarian activity.

With the loss of JH as a driver of egg production in *S. peetersi*, and possibly in other species of queenless Ponerinae, the role of ecdysteroids in oogenesis may have become much broader. Ecdysteroids can influence egg production in many insect species (Raikhel et al. 2005; Swever et al. 2005), and the modification of an existing hormonal system would have been easier than its de novo creation. We found that ecdysteroid titers were low in low-rankers but significantly higher in high rankers and alpha females (Fig. 1). This corresponds to the previous finding that ovarian development and vitellogenin titer was positively correlated with rank (Cuvillier-Hot et al. 2004a). The follicle cells surrounding the terminal oocytes of the ovaries are often the primary source of ecdysteroids in adult insects (reviewed in Lafont et al. 2005; Swevers et al. 2005), and the degree of ovarian development may dictate ecdysteroid production capacity. Assuming that our measure of the whole body content of the hormone accurately reflects the concentration of the ecdysteroids circulating in the hemolymph, alphas and other high rankers may be producing a threshold quantity of the hormone sufficient to induce vitellogenin production. Given that the majority of alpha workers in this experiment were sampled within just 6 days of attaining their reproductive rank, ecdysteroid production may continue to increase as the alphas mature. Fully mature alphas, with well-developed ovaries, might produce significantly more ecdysteroids than subordinate high rankers, driving greater vitellogenin production. Additional tests are needed to make this developmental distinction; hence the gonadotropic role of ecdysteroids remains undefined.

In addition to their putative roles as regulators of ovarian activity, both JH and ecdysteroids may act in conjunction with neuromodulators, such as biogenic amines (Harano et al. 2005), to induce the display of rank-specific behaviours. Submissive behaviours, foraging and nest maintenance are observed only in low rankers, while agonistic displays are associated with just the high rankers (Cuvillier-Hot et al. 2004a). JH and ecdysteroids have been implicated as behavioural modulators in other eusocial hymenoptera (reviewed in Nijhout and Wheeler 1982; Nijhout 1994; Robinson and Vargo 1997; Elekonich and Robinson 2000), although these behavioural effects vary as greatly between species as do the hormones' physiological effects. As an individual's rank changes, shifts in either one or both of these hormones might precipitate activational or

organizational effects on behaviour. Currently, there is little direct evidence that can be used to define the role of either hormone, but our results are suggestive. When alpha females were removed from colonies, many high rankers became more aggressive within 24 h, and some individuals showed the distinctive gaster curling behaviour of alphas in under 48 h. These behavioural changes are concurrent with equally rapid changes in the olfactory signals produced by the new alphas (Cuvillier-Hot et al. 2005). Because pheromone expression is also under endocrine control (reviewed in Blomquist et al. 2005), the changes in both behaviour and cuticular hydrocarbons in replacement alphas may be a direct response to the decrease in JH production that was observed after just 48 h. However, Cuvillier-Hot et al. (2004b) showed that application of a JH analogue did not diminish the agonistic displays of an alpha female despite decreasing her fertility and changing her hydrocarbon signature, suggesting that other factors are promoting her agonistic displays. The behavioural role of JH cannot be dismissed though, because prolonged exposure to an elevated JH titer might drive slower organizational changes that reduce the frequency of aggression and increase the frequency of foraging and nest maintenance. It is also possible that the titer can indirectly influence an individual's behaviour by modulating the sensitivity of chemoreceptors responsible for detecting differences in hydrocarbon profiles. Future studies to elucidate the effects of these hormones on both the behaviour and physiology of this queenless ant will have to discriminate between direct and indirect effects and will have to employ both short and long-term hormonal manipulation to ensure an accurate interpretation.

Acknowledgments We would like to thank the Department of Water Affairs and Forestry (Limpopo Province) for collecting permits. This work was partially supported by a grant from the National Research Foundation of South Africa. We thank Drs. L. Gilbert and J. Warren, University of North Carolina—Chapel Hill, for supplying ecdysteroid antibody, and Dr. C. Schal, Department of Entomology, North Carolina State University, for assistance with radiochemical analysis. All experiments were conducted in accordance the ''Principles of animal care'', publication No. 86-23, revised 1985 of the National Institute of Health, and with current United States statutes governing research.

References

Bloch G, Wheeler DE, Robinson GE (2002) Endocrine influences on the organization of insect societies. In: Pfaff DW, Arnold AP, Ettgen AM, Fahrbach SE, Rubin RT (eds) hormones, brain, and behavior, vol 3. Academic Press, San Diego, CA, pp 195–237

Blomquist GJ, Jurenka R, Schal C, Tittiger C (2005) Biochemistry and molecular biology of pheromone production. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 3. Elsevier, Boston, pp 705–751

Brent CS, Vargo EL (2003) Changes in juvenile hormone in maturing virgin queens of *Solenopsis invicta*. J Insect Physiol 49:967–974

Cuvillier-Hot V, Lenoir A, Crewe R, Malosse C, Peeters C (2004a) Fertility signaling and reproductive skew in queenless ants. An Behav 68:1209–1219

Cuvillier-Hot V, Lenoir A, Peeters C (2004b) Reproductive monopoly enforced by sterile police workers in a queenless ant. Behav Ecol 15:970–975

Cuvillier-Hot V, Renault V, Peeters C (2005) Rapid modification in the olfactory signal of ants following a change in reproductive status. Naturwissenschaften 92:73–77

Elekonich MM, Robinson GE (2000) Organizational and activational effects of hormones on insect behavior. J Insect Physiol 46:1509–1515

Fahrbach SE (1997) Regulation of age polyethism in bees and wasps by juvenile hormone. Adv Study Behav 26:285–315

Feyereisen R, Tobe SS (1981) A rapid partition assay for routine analysis of juvenile hormone release by insect corpora allata. Anal Biochem 111:372–375

Goodman WG, Granger NA (2005) The juvenile hormones. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 3. Elsevier, Boston, pp 320–408

Harano K, Sasaki K, Nagao T (2005) Depression of brain dopamine and its metabolite after mating in European honeybee (*Apis mellifera*) queens. Naturwissenschaften 92:310–313

Hartfelder K, Emlen DJ (2005) Endocrine control of insect polyphenism. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 3. Elsevier, Boston, pp 651–703

Hartfelder K, Engels W (1998) Social insect polymorphism: hormonal regulation of plasticity in development and reproduction in the honeybee. Curr Topics Dev Biol 40:45–77

Holbrook GL, Chiang AS, Schal C (1997) Improved conditions for culture of biosynthetically active cockroach corpora allata. In Vitro Cell Dev Biol 33A:452–458

Lafont R, Dauphin-Villemant C, Warren JT, Rees H (2005) Ecdysteroid chemistry and Biochemistry. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 3. Elsevier, Boston, pp 125–195

Monnin T, Peeters C (1999) Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav Ecol 10:323–332

Nijhout HF, Wheeler DE (1982) Juvenile hormone and the physiological basis of polymorphisms. Quart Rev Biol 57:109–133

Nijhout HF (1994) Insect hormones. Princeton University Press, Princeton, NJ

Peeters C (1993) Monogyny and polygyny in ponerine ants with or without queens. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 234–261

Pratt GE, Tobe SS (1974) Juvenile hormone radiobiosynthesised by corpora allata of adult female locusts in vitro. Life Sci 14:575–586

Raikhel AS, Brown MR, Belles X (2005) Hormonal control of reproductive processes. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 3. Elsevier, Boston, pp 433–491

Robertson HG (2002) Revision of the ant genus *Streblognathus* (Hymenoptera: Formicidae: Ponerinae). Zootaxa 97:1–16

Robinson GE, Vargo EL (1997) Juvenile hormone in the social hymenoptera: gonadotropin and behavioral pacemaker. Arch Insect Biochem Physiol 35:559–583

Sommer K, Hölldobler B, Rembold H (1993) Behavioral and physiological aspects of reproductive control in the *Diacamma* species from Malaysia (Formicidae, Ponerinae). Ethology 94:162–170

Swever BL, Raikhel AS, Sappington TW, Shirk P, Iatrou K (2005) Vitellogenesis and post-vitellogenic maturation of the insect ovarian follicle. In: Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive molecular insect science, vol 1. Elsevier, Boston, pp 87–155

Tobe SS, Pratt GE (1974) The influence of substrate concentrations on the rate of insect juvenile hormone biosynthesis by corpora-allata of the desert locust in vitro. Biochem J 144:107–113

Vargo EL, Laurel M (1994) Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. J Insect Physiol 40:601–610

Warren JT, Gilbert LI (1986) Ecdysone metabolism and distribution during the pupal-adult development of *Manduca sexta*. Insect Biochem 16:65–82

Warren JT, Smith W, Gilbert L (1984) Simplification of the ecdysteroid radioimmunoassay by the use of Protein A from staphylococcus aureus. Experientia 40:393–394

West-Eberhard MJ (1996) Wasp societies as microcosms for the study of development and evolution. In: Turillazzi S, West-Eberhard MJ (eds) Natural history an evolution of paper wasps. Oxford University Press, New York, pp 290–317

Zera AJ, Bottsford J (2001) The endocrine-genetic basis of life-history variation: the specific relationship between the ecdysteroid titer and morph-specific reproduction in the wing-polymorphic cricket *Gryllus firmus*. Evolution 55:538–549