N O T E

Juvenile Hormone III Concentrations in Female Reproductives of *Solenopsis invicta* Buren[1]

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One of the roles attributed to juvenile hormone (JH) in many female insects is the control of reproductive development. Within eusocial Hymenoptera, behavioral and/or pheromonal mechanisms are used to establish and maintain reproductive dominance, which may involve JH mediation. Although juvenile hormone does not appear to function as a traditional gonadotropin in the honey bee, *Apis mellifera* L., it does play a major role in reproductive development in the primitively eusocial *Polistes* wasps and the bumble bee, *Bombus terrestris* L. (Robinson and Vargo 1997, Arch. Insect Biochem. Physiol. 35: 559-583).

Juvenile hormone III has been identified in the hemolymph of uninseminated female sexuals of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Burns et al. 2002, J. Insect Physiol. 48: 357-365; Brent and Vargo 2003, J. Insect Physiol. 49: 967-974). Studies (Fletcher and Blum 1983, J. Comp. Physiol. A 153: 467-475; Vargo and Laurel 1994, J. Insect Physiol. 40: 601-610; Vargo 1999, Physiol. Entomol. 24: 370-376) suggest that JH is important in maintaining reproductive dominance in queens, in that a queen primer pheromone suppresses JH titer in winged females, thereby inhibiting dealation, wing muscle histolysis, and rapid oogenesis. Normally female sexuals shed their wings and become reproductively active following a mating flight; however, dealation also may occur after the death of the queen (Hölldobler and Wilson 1990, The Ants, Belknap/Harvard University Press, Cambridge). Alates topically treated with methoprene have been found to dealate and undergo vitellogenesis in the presence of the queen (Vargo and Laurel 1994). Alates administered JH III also shed their wings in queenright colonies. The use of precocene II inhibited alates from shedding their wings in queenless

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colonies, but subsequent application of JH III reversed the effect of precocene (Burns et al. 2002). Furthermore, applications of increased JH concentrations decreased the time in which alates were induced to shed their wings (Kearney et al. 1977, Ann. Entomol. Soc. Am. 70: 699-701; Burns et al. 2002). The purpose of the current study was to isolate and quantitate JH from hemolymph of virgin and established mated female sexuals.

Hemolymph was collected from the thorax of sexually mature female alates and inseminated queens from established polygyne red imported fire ant colonies using techniques reported by Burns et al. (2002). Five-microliter samples of hemolymph from each set of females were centrifuged at 18,000 g for 2 min. Afterward, 45 μL of methanol and 100 μL of hexane containing 10 pg/μl of farnesyl acetate (internal standard) were added to each sample (Teal et al. 2000, Anal. Biochem. 277: 206-213). The samples were vortexed vigorously for 2 min and centrifuged for 5 min at 18,000 g before removing the organic layer. The aqueous layer was extracted two additional times with hexane, and organic layers were combined and analyzed.

Gas chromatography-mass spectroscopic (GC-MS) analysis was performed with a Finnigan-MAT ITS 40 ion-trap MS operated in chemical ionization (CI) mode (isobutane reagent gas) and interfaced to a Varian Star 3,400 GC equipped with a cool-on column injector. The analytical column in the GC, a DB5-MS capillary column (30 m × 0.25 mm, id.; 0.1 μL film thickness, J&W Scientific, Folsom, CA), was interfaced to a 10 m × 0.25 mm (id) uncoated, deactivated fused silica retention gap and a 10 cm × 0.5 mm (id) length of uncoated, deactivated fused silica in the GC injector (Teal et al. 2000). Farnesyl acetate (FA, internal standard), JH III, JH II, and JH I eluted at 32.3, 33.8, 35.4, and 37.3 min, respectively. Prior to analysis of natural products, we conducted a concentration gradient study in which we analyzed amounts of synthetic JH I, JH II, and JH III and farnesyl acetate, the internal standard, ranging in concentration from 0.01-5.0 pmol. Linear regressions were calculated for each of the diagnostic ions used for routine quantitative purposes (JH I- m/e = 263, 245, 217, 161, 153, 111; JH II-m/e = 249, 231, 203, 147, 139, 111; JH III- m/e = 235, 217, 189, 147, 125, 111; FA-m/e = 205, 149, 135, 121, 109, and 81; see Teal et al. 2000 for description of cleavage patterns and use of the internal standard). We then analyzed natural product samples. Identification of JH in natural samples was based on comparison of fragmentation patterns and retention indexes of compounds eluting during analysis of natural product samples with those of synthetic standards. Quantification of amounts of JH was accomplished as described by Teal et al. (2000).

Juvenile hormone III was the only JH homolog identified in hemolymph extracts from both uninseminated (n = 5) and mated (n = 9) *S. invicta* female sexuals. The mean (±SEM) of JH III found in hemolymph was 0.34 ± 0.059 pmol/μl in alates and 0.83 ± 0.14 pmol/μl in mated females. Inseminated queens had a significantly higher JH titer than female alates from queenright colonies (t = 2.54, df = 12, P = 0.95; Fig. 1). Whereas in their mother colony, female alates are inhibited from shedding their wings or developing their ovaries to maximum capacity by a queen-produced primer pheromone (Fletcher and Blum 1983, Vargo 1999). Results from the current investigation provide further support of JH's role as a gonadotropin in *S. invicta* and queen suppression of JH production in nestmate virgin female sexuals.

Under laboratory conditions, female alates were found to increase production of JH following isolation from their colony queen but in the presence of workers (Brent and Vargo 2003). Dealation is one of the most noticeable indicators that a sexual is

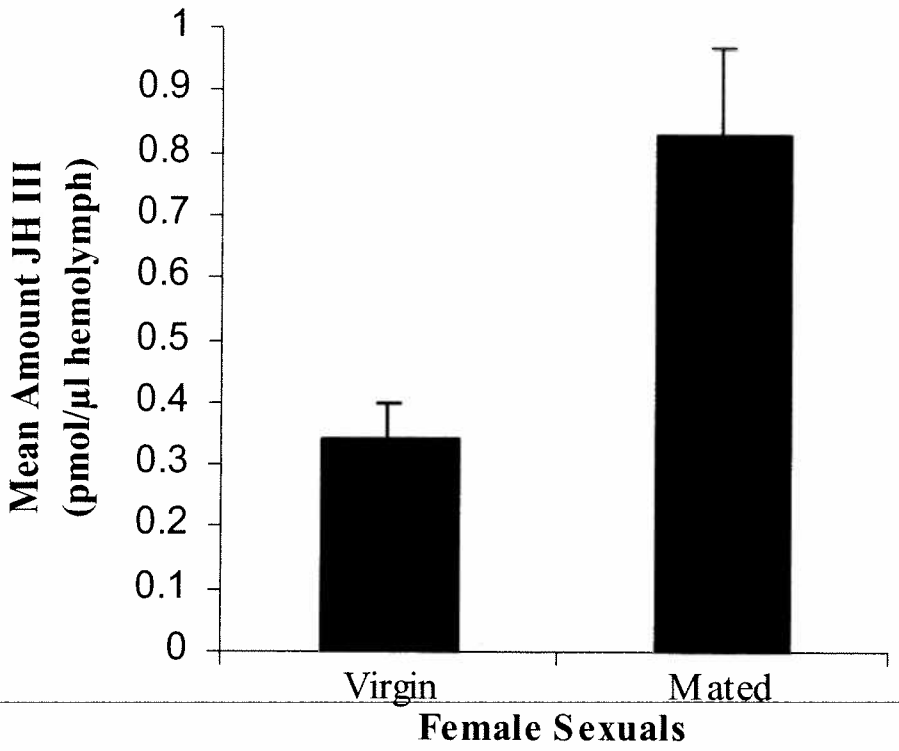

Fig. 1. Mean juvenile hormone III concentrations in hemolymph of virgin (n = 5) and mated (n = 9) *Solenopsis invicta* female sexuals (bar represents SEM).

no longer under queen pheromonal control, thereby removing a restraint on the corpora allata's capacity to produce JH. The stimulation to shed wings may take several days once the queen is removed from the colony (Fletcher and Blum 1983); however, mated females dealate within 30 min of landing from a mating flight (Markin et al. 1971, J. Georgia Entomol. Soc. 6: 145-156). Once alates engage in a mating flight, it is unknown when JH titers increase. Analysis of hemolymph extracts from newly-inseminated females may reveal a surge in JH production immediately after the flight, possibly induced by mating—by itself or in conjunction with other behavioral/ environmental cues associated with the flight (Vargo and Laurel 1994). Whereas increased topical JH III treatments did decrease the time in which dealation occurred, the effect became nonlinear and reached a plateau following topical treatments greater than 3.8 pmol, with maximum dealation after 48 h (Burns et al. 2002). This suggests that while JH is likely involved in dealation after a mating flight, other as yet unknown factors may be important. The data presented here clearly show that functional polygyne colony queens maintain elevated JH titers compared with JH concentrations from nonmated female sexual alates. Previous investigations (Fletcher and Blum 1983, Vargo 1999) have proposed that the reduction in JH levels in alates is the result of queen pheromonal control. An increase in JH could then stimulate dealation and ovarian development.

In summary, this investigation resulted in identification of JH III from uninseminated and mated *S. invicta* females. We found that JH titer is substantially higher in egg-laying queens than in pheromonally-inhibited alates. Juvenile hormone production appears to correlate with ovarian development, and the queen may inhibit dealation and reproduction of nestmate female alates by suppressing JH titer.

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