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Juvenile hormone and aggression in honey bees

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Abstract

We determined whether defense by individual bees against non-nestmates in honey bees (*Apis mellifera*) is correlated with their juvenile hormone (JH) titers, which are known to vary developmentally and seasonally. We bioassayed winter and summer bees for aggressive and non-aggressive individuals. Bees in winter could not be distinguished by task group, but bees in summer were segregated into nurses and guards. JH titers were correlated with aggressive behavior at two levels. First, winter bees and summer nurses, known to have lower JH titers, both showed less aggression toward foreign bees than did summer guards. Second, aggressive individuals had significantly higher JH titers than did non-aggressive bees within each colony. Inter-colonial variation in aggressiveness was maintained during summer and winter, suggesting a genetic basis for these differences. An alarm pheromone test further substantiated the existence of inter-colonial differences. We found significant variation in JH titers among different colonies, but this variation was not significantly associated with colony-level aggressiveness. The correlation between JH and levels of aggressiveness within a colony suggests a regulatory role for JH, but variation among colonies involves factors other than JH.

Keywords: Colony defense; Juvenile hormone; Division of labor; *Apis mellifera*; Honey bee

1. Introduction

Honey bees, *Apis mellifera* L., vary substantially in the degree of their aggressive response. This can be attributed to geographic variation among populations (Collins et al., 1989), differences among colonies within populations (Breed, 1991; Breed and Rogers, 1991), and differential expression of defensive behavior among bees within the same colony (Breed et al., 1992). Although a genetic component, which explains a portion of this variation in aggressiveness, is well established (Collins, 1991), the physiological mechanisms regulating levels of aggressiveness are not well understood. In this study, we address the hypothesis that juvenile hormone (JH) is an endocrinological correlate of aggression in honey bees.

Previous studies suggest that JH might be involved in the regulation of aggressiveness. Older bees, which have higher JH levels (Huang et al., 1994), are generally more aggressive than younger bees with lower JH levels (Breed, 1983). Bees treated with a JH analog exhibited an earlier response to alarm pheromone (Robinson, 1987), and the proportion of bees acting as guards also increased (Sasagawa et al., 1989). Workers reared in isolation showed higher levels of aggressiveness toward other bees (Breed, 1983), and recently Huang and Robinson (1992) found that isolated bees have elevated JH levels compared to bees reared in groups or in a colony. JH titers of guards are higher than all other middle age bees except undertakers (Huang et al., 1994); again, this links JH with aggression because guards exhibit low thresholds for the expression of aggressiveness (Breed et al., 1992). Giray et al. (1999, 2000) suggest that differences in division of labor between European and African strains of honey bees may result from the effects of JH on the rate of behavioral development (Robinson and

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Vargo, 1997). This pacing effect applies to aggressive behavior, as well as other behaviors such as foraging.

Studies on JH or aggressive behavior have focused mostly on bees in the summer, their active season. Investigations of winter bee behavior are less extensive and have typically dealt with longevity rather than aggressive behavior or other tasks (Fluri et al., 1977). JH titers vary seasonally in bees, with summer foragers having higher JH levels than foragers in early spring or late fall, and winter bees having JH levels lower than summer nurses (Huang and Robinson, 1995).

In this study, we determined seasonal differences in aggressiveness in honey bees and their association with JH levels. Guards vary little in their aggressiveness either within or between colonies (Breed et al., 1992), but the number of guards differs substantially among colonies. To further understand the mechanisms of aggression, we measured aggressiveness in winter bees and in summer nurses and guards, as well as colony-level aggression. This approach allowed us to explore subtle differences among individuals and colonies that would have been overlooked if only guards were sampled. Based on our understanding of the relationship between JH titers and task specialization, we tested the following hypotheses: (1) colonies vary in their levels of aggressiveness seasonally, so winter bees are less aggressive than summer guards; (2) summer nurses behave less aggressively than summer guards; and (3) intra- and inter-colonial variations in aggressiveness are associated with differences in hemolymph JH.

2. Materials and methods

2.1. Aggressiveness of winter bees

During January and February, we collected bees from colonies at apiaries on the East Campus of the University of Colorado in Boulder, Colorado. Each test consisted of a donor bee introduced into a recipient container with 10 bees from another colony, as described by Breed et al. (1992). As we wanted to compare seasonal differences between winter bees and summer guards, we selected bees for the recipient containers that behaved similarly to summer guards; they investigated the disturbance when the hive was opened and attempted to chase the forceps used to collect the bees. Fifteen groups of 10 of these bees were placed in cardboard cups (0.47 l), supplied with food and water ad libitum, and transported into the laboratory for testing. We also randomly selected 15 bees from a separate colony to be used as treatment donor bees. No group of recipient bees was used more than once, and the observer was blind with respect to the source of the donor bee. We repeated this procedure for five pairs of colonies so that a total of 75 donor bees were tested.

For each test, we marked one of the 75 donor bees on the thorax with a dot of fast-drying enamel for identification, then introduced it into the cup containing 10 non-nestmate bees and observed their behavior. We recorded a rejection when biting or stinging occurred, but if neither of these aggressive acts were observed over a period of five minutes, we scored the test as an acceptance. In controls, bees from the same colonies were used as the donors.

2.2. Aggressiveness of summer nurse bees

We tested the aggressiveness of summer nurse bees and guards from May to July, using the same five donor–recipient colony pairs tested in winter. We identified nurses as those bees with their heads in cells containing larvae and collected them using the same protocol as above, gathering cups of 10 nurses from each colony. Guards were identified using the criteria of Moore et al. (1987). Recipient groups consisted of 10 nurses or guards, following the protocol outlined above for winter bees.

2.3. Colony-level aggressiveness

An aggression assay was carried out at the University of Colorado to determine if there is a correlation between individual tests of aggressiveness and colony-level aggressiveness. We used isopentyl acetate (IPA), one of the primary components of alarm pheromone (Boch et al., 1962) for this purpose. Although many other compounds have been identified in the honey bee alarm pheromone (Blum et al., 1978; Shearer and Boch, 1965), IPA elicits a response similar to the full blend of alarm pheromone under experimental conditions (Boch and Rothenbuhler, 1974). Each treatment consisted of 50 μl IPA placed on a cotton ball placed at the colony entrance. We assessed the change in the number of bees by comparing Polaroid photographs taken immediately before and 90 s after exposure to IPA.

2.4. Measurement of JH titers

Hemolymph samples were taken from bees in experiments 1 and 2. Samples came from either ‘aggressive’ bees: recipient bees that attacked the introduced bee in the behavioral tests, or ‘non-aggressive’ bees: randomly chosen recipient bees when none of them showed aggressiveness in the behavioral tests.

After the behavioral assay, bees were immobilized on ice for 10–15 min until blood (hemolymph) was taken. Blood (0.8–4.25 μl per bee) was collected with a 5 μl capillary tube, measured to the nearest 0.1 μl, and stored in 0.5 ml acetonitrile at −20°C until shipped to the University of Illinois for analysis. The capillary tube and any other glassware that was used in the RIA was baked

at 500°C for 3.5 h to minimize JH adsorption (Strambi et al., 1981). All solvents were HPLC grade, obtained from either EM Science, Fisher Scientific, or J.T. Baxter Chemical Co.

A chiral-specific radioimmunoassay (RIA) (Hunnicutt et al., 1989) was used to measure JH III titers. JH III is the only JH homolog found in honey bees (Hagenguth and Rembold, 1978; Huang et al., 1994; Robinson et al., 1991). This assay has been validated for adult worker honey bees by Huang et al. (1994). Previous results (Huang et al., 1994; Goodman et al., 1993; Huang and Robinson, 1995) indicate that values from this RIA agree with two other recently developed RIAs, both of which have been validated with gas chromatography/mass spectroscopy (de Kort et al., 1985; Goodman et al., 1990).

The sensitivity of the RIA is about 5 pg 10-R-JH III per sample. Typical inter- and intra-assay variations for JH determinations were 9.2 and 10.6%, respectively (Huang and Robinson, 1996). A detailed description of the RIA can be found in previous studies on honey bees (Huang et al., 1994; Huang and Robinson 1995, 1996). JH titer differences among aggressive bees and non-aggressive bees were analyzed by two-way analysis of variance, with aggressiveness nested inside the colonies, using the SAS General Linear Model (SAS Institute, 1985).

3. Results

3.1. Aggressiveness of winter bees

Response to non-nestmate bees during January and February involved little aggressiveness. Only 25% (n=75) of the donor bees were subjected to aggression, as compared with 80% (n=75) of those introduced into cups containing summer guards. This difference was highly significant (χ^2=22.65, df=1, $p<0.0001$) (Fig. 1). No aggression was observed in winter controls; all introduced nestmate bees were accepted.

3.2. Aggressiveness of summer nurses

Of summer nurses, 48.8% (n=75) expressed aggressive behavior (Fig. 1); this is significantly less than summer guards (χ^2=7.1, df=1, p=0.008). Colonies varied substantially in aggressiveness (Fig. 2), and there was a significant correlation (r=0.93, n=5, $p<0.05$) between levels of aggressiveness for summer nurses and winter bees from the same colony. Within colony comparisons between summer nurses and winter bees showed only one significant difference (χ^2 comparisons of aggression rates, $p<0.05$).

In the IPA assay the change in the number of bees present at the colony entrance was consistent with the levels of aggressiveness observed in the previous two experiments. There was a trend for a positive correlation between the level of aggressiveness as measured in the aggression bioassay and the number of bees responding to IPA, but this correlation is not significant (r=0.765, N=5, ns) possibly due to small sample size.

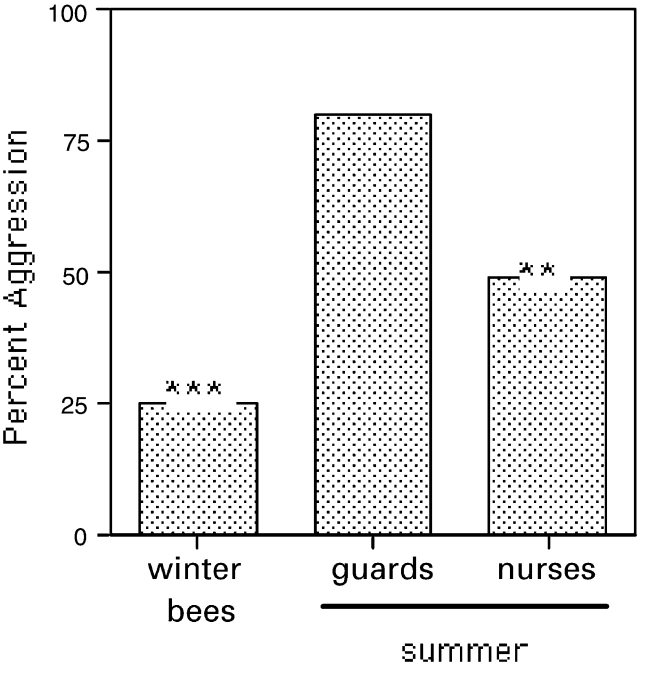

Fig. 1. Levels of aggression of summer guard bees are elevated, when compared with winter bees or with summer nurses. Each percentage is based on 75 replicates. Statistical comparisons are given in the text, ***differs from guards, $p<0.001$, **differs from guards, $p<0.01$.

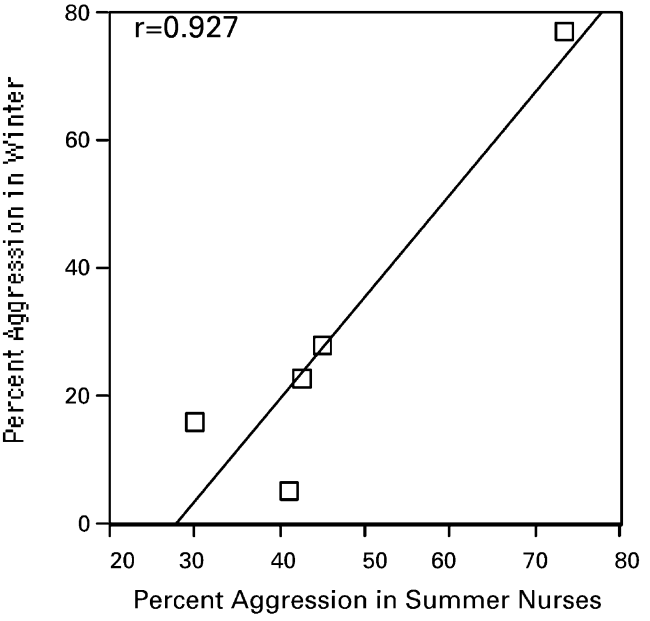

Fig. 2. Significant correlation between the percentage of bees showing aggression in winter bees and in nurses from the same colony.

3.3. JH titers

Colonies differed significantly in their JH titers (F=6.94, df=4,68, p=0.0001), and JH differed significantly between aggressive and non-aggressive bees (F=8.23, df=1,71, p=0.006, Table 1).

4. Discussion

Our results provide insight into the proximate factors that influence variation in aggressiveness within and between honey bee colonies. We conclude that: (1) aggressiveness varies seasonally within colonies because aggressiveness of winter bees is significantly lower than that of summer guard bees; (2) winter and summer levels of aggressiveness are correlated within colonies; (3) summer nurse bees display low levels of aggressiveness compared to guard bees, supporting previous findings that aggressiveness varies among castes within a colony (Breed et al., 1992); and, (4) JH titers are significantly higher in aggressive bees when compared to non-aggressive bees within the same colonies.

Our finding that JH levels are correlated with degrees of aggressiveness in honey bees is important in the context of understanding how JH, or JH correlated factors, may modulate behavior in the honey bee. JH titers are significantly lower in winter bees, even for bees that once foraged in late fall (Huang and Robinson, 1995), and in this study we found significantly lower aggression in winter bees. The comparison of guards and nurses also supports the correlation of JH titers with aggressiveness. Aggressive bees had higher JH titers than non-aggressive bees in the same colony.

It is not clear whether JH causes aggression directly or if other factors regulate aggression and JH is only one ‘symptom’ of aggressive bees. Guards and undertakers both have forager-like levels of JH, even though they are about one week younger than foraging aged bees and other middle aged bees have much lower JH levels (Huang et al., 1994), yet guards are the most aggressive bees towards non-nestmates (Breed et al., 1992). It would be interesting to know whether foragers that have guarded are more aggressive than foragers which have not guarded. Similarly, soldiers behave much more aggressively toward larger intruders (Breed et al., 1990) than do foragers, but their JH levels are similar to those of foragers (Huang et al., 1994). Further experiments are needed to explain the role of JH in regulating aggressiveness within a colony.

The strong correlation between summer and winter aggressiveness supports our hypothesis that variation between colonies would be consistent across seasons. Although hormone titers fluctuate, perhaps influencing the degree of aggressiveness of a colony, each colony displayed similar changes from one season to the next. The large range of aggressiveness among colonies was striking but consistent with other studies (Spivak et al., 1991). North American honey bees exhibit substantial variation in aggressiveness (Breed, 1991), and Breed and Rogers (1991) showed that this variation is correlated with genetic differences among colonies. Seasonal changes in the environment, coupled with genetic differences, alter the aggressiveness of honey bees throughout the annual cycle.

As aggressive bees showed higher JH within each colony, we expected to find that more aggressive colonies would have higher levels of JH. However, this relationship was not observed. A likely explanation for the lack of correlation between JH and colony-level aggressiveness is differences in stimulus thresholds. If each colony expresses different threshold levels to the JH stimulus, overall aggressiveness of the bees would vary among colonies. The nature of the threshold is unknown, but it could be either in response to JH, or to the stimulus leading to aggressive behavior. Our findings are similar to those of Robinson et al. (1987), who found no JH titer differences between Africanized and European bees despite their large difference in aggressive behavior.

Interactions between JH titers and seasonality combine to influence the variation in aggressiveness within and between honey bee colonies. While our results are correlational, we do show that JH is a proximate factor associated with aggressiveness in honey bees. JH may be a critical player in the chain of events that link the genetic variation in aggressiveness with expression of the behavior; further work will be required to determine what, if any, causative role JH plays.

Table 1
Comparison of JH titers between aggressive and non-aggressive bees. (means±S.E., numbers in parentheses indicate sample sizes)

Colony number	Aggressive bees	Non-aggressive bees
Colony 57	256.85±38.47 (11)	196.56±18.23 (4)
Colony 66	301.18±45.25 (6)	257.65±61.25 (9)
Colony 80	210.23±31.45 (8)	130.30±19.70 (7)
Colony 86	*	129.13±18.75 (15)
Colony 111	180.32±35.91 (7)	81.90±20.41 (8)

* This colony died prior to the collection of the aggressive bees.

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References

Blum, M.S., Fales, H.M., Tucker, K.W., Collins, A.M., 1978. Chemistry of the sting apparatus of the worker honeybee. Journal of Apicultural Research 17, 218–221.

Boch, R., Shearer, D.A., Stone, B.C., 1962. Identification of iso-pentyl acetate as an active component in the sting pheromone of the honey bee. Nature 195, 1018–1020.

Boch, R., Rothenbuhler, W.C., 1974. Defensive behaviour and production of alarm pheromone in honeybees. Journal of Apicultural Research 13, 217–222.

Breed, M.D., 1983. Correlations between juvenile hormone and aggressive behavior in worker honeybees. Insectes Sociaux 30, 482–495.

Breed, M.D., Robinson, G.E., Page, R.E., 1990. Division of labor during honey bee colony defense. Behavioral Ecology and Sociobiology 27, 395–402.

Breed, M.D., Rogers, K.B., 1991. The behavioral genetics of colony defense in honeybees: genetic variability for guarding behavior. Behavior Genetics 21, 295–303.

Breed, M.D., 1991. Defensive behavior. In: Spivak, M., Fletcher, D.J.C., Breed, M.D. (Eds.), The 'African' Honey Bee. Westview Press, Boulder, Colorado, pp. 299–308.

Breed, M.D., Smith, T.A., Torres, A., 1992. Guard honey bees: role in nestmate recognition and replacement. Annals of the Entomological Society of America 85, 633–637.

Collins, A.M., Rinderer, T.E., Daly, H.V., Harbo, J.R., Pesante, D., 1989. Alarm pheromone production by two honeybee (*Apis mellifera*) types. Journal of Chemical Ecology 15, 1747–1756.

Collins, A.M., 1991. Genetics of honey bee colony defense. In: Spivak, M., Fletcher, D.J.C., Breed, M.D. (Eds.), The 'African' Honey Bee. Westview Press, Boulder, Colorado, pp. 309–332.

De Kort, C.A.D., Koopmanschap, A.B., Strambi, C., Strambi, A., 1985. The application and evaluation of a radioimmunoassay for measuring juvenile hormone titres in Colorado beetle hemolymph. Insect Biochemistry 15, 771–775.

Fluri, P., Wille, H., Gerig, L., Lüscher, M., 1977. Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees (*Apis mellifera*). Experientia 33, 1240–1241.

Giray, T., Huang, Z.Y., Guzman-Novoa, E., Robinson, G.E., 1999. Physiological correlates of genetic variation for rate of behavioral development in the honeybee, *Apis mellifera*. Behavioral Ecology And Sociobiology 47, 17–28.

Giray, T., Guzman-Novoa, E., Aron, C.W., Zelinsky, B., Fahrbach, S.E., Robinson, G.E., 2000. Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. Behavioral Ecology 11, 44–55.

Goodman, W.G., Coy, D.C., Baker, F.C., Xu, L., Toong, Y.C., 1990. Development and application of a radioimmunoassay for the juvenile hormones. Insect Biochemistry 20, 35–364.

Goodman, W.G., Huang, Z.-H., Robinson, G.E., Strambi, C., Strambi, A., 1993. A comparison of two juvenile hormone radioimmunoassays. Archives of Insect Biochemistry and Physiology 22, 147–152.

Hagenguth, H., Rembold, H., 1978. Identification of juvenile hormone 3 as the only juvenile hormone homolog in all developmental stages of the honey bee. Zeitschrift für Naturforschung 33C, 847–850.

Huang, Z.-Y., Robinson, G.E., 1992. Honeybee colony integration: worker–worker interactions mediate hormonally regulated plasticity in division of labor. Proceedings of the National Academy of Science of the United States of America 89, 11726–11729.

Huang, Z.-Y., Robinson, G.E., 1995. Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees. Journal of Comparative Physiology B 165, 18–28.

Huang, Z.-Y., Robinson, G.E., 1996. Regulation of honey bee division of labor by colony age demography. Behavioral Ecology and Sociobiology 39, 147–158.

Huang, Z.-Y., Robinson, G.E., Borst, D.W., 1994. Physiological correlates of division of labor among similarly aged honey bees. Journal of Comparative Physiology A 174, 731–739.

Hunnicutt, D., Toong, Y.C., Borst, D.W., 1989. A chiral specific antiserum for juvenile hormone. American Zoologist 29, 48a.

Moore, A.M., Breed, M.D., Moor, M.J., 1987. The guard honey bee; ontogeny and behavioral variability of workers performing a specialized task. Animal Behaviour 35, 1159–1167.

Robinson, G.E., 1987. Regulation of honey bee age polyethism by juvenile hormone. Behavioral Ecology and Sociobiology 20, 329–338.

Robinson, G.E., Strambi, A., Strambi, C., Paulino-Simões, Z.L., Barbosa, J.M.N., 1987. Juvenile hormone titers in Africanized and European honey bees in Brazil. General and Comparative Endocrinology 66, 457–459.

Robinson, G.E., Strambi, C., Strambi, A., 1991. Comparison of juvenile hormone and ecdysteroid hemolymph titres in adult worker and queen honey bees (*Apis mellifera*). Journal of Insect Physiology 37, 929–935.

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.

Sasagawa, H., Sasaki, M., Okada, I., 1989. Hormonal control of the division of labor of adult honey bees (*Apis mellifera* L.). I. Effect of methoprene on corpora allata and hypopharyngeal gland, and its a-glucosidase activity. Applied Entomology and Zoology 24, 66–77.

Shearer, D.A., Boch, R., 1965. 2-Heptanone in the mandibular gland secretion of the honeybee. Nature 205, 530.

Spivak, M., Fletcher, D.J.C., Breed, M.D. (Eds.), 1991. The 'African' Honey Bee. Westview Press, Boulder, Colorado.

Strambi, C., Strambi, A., De Reggi, M., Hirn, M., Delaage, M., 1981. Radioimmunoassay of insect juvenile hormone and of their diol derivatives. European Journal of Biochemistry 118, 401–406.