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Effect of Exogenous Juvenile Hormone III and Precocene II on Agonistic Behavior and the Corpora Allata *in Vitro* Activity in the Male Lobster Cockroach *Nauphoeta cinerea* (Dictyoptera: Blaberidae, Oxyhaloinae)

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Ying-Ru Chen , Shu-Chun Chen, Huan-Wen Chang, Gaoge Sun, and Rong Kou (2005) Effect of exogenous juvenile hormone III and precocene II on agonistic behavior and corpora allata *in vitro* activity in the male lobster cockroach *Nauphoeta cinerea* (Dictyoptera: Blaberidae, Oxyhaloinae). *Zoological Studies* **44**(3): 409-416. In this study, the pharmacological effects of exogenous juvenile hormone III (JH III) and precocene II on agonistic behavior and corpora allata (CA) *in vitro* activity in the male lobster cockroach *Nauphoeta cinerea* were investigated. The probability of any control adult male adopting a dominant or subordinate status was 50: 50, and the average onset of agonistic behavior occurred on the 9th day post emergence. Topical application of exogenous JH III, at a dose of 200 or 300 μg/male, did not affect the determination of social status. The proportions of dominants resulting from JH treatment and the acetone-control were 42%~47% and 53%~58%, respectively. Compared with the control insects, the onset of agonistic behavior was significantly delayed for 3 d in the JH-treated dominants and for 1 d in the acetone-treated dominants. Contrary to the results of JH treatment, a propensity for inhibition of a dominant status was induced by precocene II treatment. The proportion of dominants resulting from precocene II treatment was about 38% of that at a dose of 100 or 200 μg/male. Precocene II treatment also showed an acceleration effect on the onset of agonistic behavior, which was about 1 d earlier in the 200 μg precocene-treated dominants than in the control group. The corpora allata from dominants exhibited a significantly higher JH *in vitro* release rate than those of subordinates, regardless of whether they were JH-treated, precocene-treated, or non-treated controls. http://zoolstud.sinica.edu.tw/Journals/44.3/409.pdf

Key words: Agonistic behavior, Juvenile Hormone III, *Nauphoeta cinerea*, Precocene II.

The lobster cockroach *Nauphoeta cinerea* is an insect species well known for its male conspecific agonistic behavior (Kramer 1964, Ewing 1967 1972, Moore 1990, Moore et al. 1997, Sirugue et al. 1992). Two 1st-encountered adult males usually exhibit obvious agonistic behavior which includes kicking, biting, and chasing; while the subordinate retreats and stands still and flat. Depending on the population density, these encounters determine either hierarchy (in a high density) or territoriality (in a low density) (Ewing 1972). The primary mediator of this agonistic behavior is the sex pheromone, which is only produced in the sternal glands of males and is composed of 3 major components: the genetically and developmentally correlated 2-methyl-thiazolidine and 4-ethyl-2-methoxyphenol, plus the genetically and developmentally independent 3-hydroxy-2-butanone (Sreng 1990, Sirugue et al. 1992, Moore et al. 1995). Later studies suggested that both cuticular hydrocarbons and sex pheromones are involved in establishing and maintaining social status (Everaerts et al. 1997, Moore et al. 1997, Roux et al. 2002).

Although the evolutionary relevance of *N. cinerea* agonistic behavior and the underlying

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pheromonal system are well established as mentioned above, physiological correlates under which *N. cinerea* display agonism have seldom been investigated except for a few earlier contradictory works on the corpora allata (CA). Hartman and Suda (1973) suggested that pheromone production does not seem to be controlled by the CA. Schal and Bell (1983) also reported that removal of the CA does not affect the formation of hierarchies. Later Sreng et al. (1999) indicated that an allatectomy performed at 2~3 d after emergence caused a decrease in sex pheromone levels, and concluded that JH III is involved in the differentiation of sternal glands. In the context of insect behavior, juvenile hormone (JH) is well known for its decisive role in behavioral development (Hartfelder 2000, Sullivan et al. 2000). Previous studies have suggested the involvement of JH in the agonistic behavior of adult insects, such as in the primitive social wasps *Polistes annularis* (Barth et al. 1975) and *P. gallicus* (Roseler 1991), bumblebees *Bombus terrestris* (Van Doorn 1989, Larrere and Couillaud 1993, Bloch et al. 2000), and the highly social honeybees *Apis mellifera* (Breed et al. 1992, Huang et al. 1994, Pearce et al. 2001).

In *N. cinerea*, the only known release product from the CA is JH III (Baker et al. 1984). To reinvestigate the possible link between JH and agonistic behavior in this insect species, exogenous JH III or precocene II which possesses anti-JH activity in some insects was applied topically, and the pharmacological effects of these chemicals on both the agonistic behavior and the CA *in vitro* activity were examined. The results of this study can serve as preliminary work for further research on the endocrinological correlates between the hormone system and agonism in *N. cinerea*.

MATERIALS AND METHODS

Study animals

Mixed-age and mixed-sex mass colonies of *N. cinerea* (Blaberidae: Oxyhaloinae) were reared in glass aquaria (30 x 30 x 20 cm) at 27 ± 2°C and 70% relative humidity, under a reversed 16L: 8D photoperiodic cycle. Insects had free access to dry dog food and fresh water. Newly emerged adult males were collected daily and marked on the pronotum with a small white dot (Tipp-Ex fluid) if they were treated with JH III or precocene II to facilitate behavioral observations. The day of emergence was adopted as day 1.

Topical application of JH III and precocene II

JH III or precocene II (6,7-dimethoxy-2,2-dimethyl-3-chromene), purchased from Sigma Chem. (Natick, MA, USA), was dissolved in acetone and topically applied onto the soft skin under the forewing on the day of emergence at the time they were marked with the white dot (Tipp-Ex fluid) on the pronotum. All males (almost all of the same size) were treated without CO_2 anesthetization, since our pretest found that CO_2 treatment causes a delay in the onset of agonistic behavior. For JH III treatment, each male received 200 or 300 μg of JH III in 2 μl of acetone (our pretest showed no obvious effects when the dose of JH III was below 150 μg). For precocene II treatment, each male received 100 or 200 μg in 2 μl of acetone (our pretest showed no obvious effect when the dose of precocene II was below 100 μg). Each JH III- or precocene-treated male was paired with a 2 μl acetone-treated male in a 11 x 20 x 4 cm transparent plastic cage, immediately after the topical application, with free access to dry dog food and fresh water. Since more than 90% of males in the control group initiated their agonistic behavior before the age of 20 d old, the onset of agonistic behavior was observed and recorded daily from the day of treatment, consecutively for a total of 20 d. Once the agonistic behavior was initiated, the CA from the dominant and subordinate male pair were respectively incubated on that day to investigate the JH III *in vitro* release rate as described in a later section.

Behavioral observations

Ewing (1967 1972) provided a basis for the characterization of the repertoire of agonistic acts in *N. cinerea*. Schal and Bell (1983) grouped these behaviors into the following categories: (1) preliminary acts such as *approaching* and *antennating*; (2) threat actions such as *stilt-walking*; (3) overt aggression (e.g., *lunging*); and (4) submission (e.g., *crouching*). In our experiment, only obvious overt aggression and submission were considered as the criteria of agonistic behavior. Agonistic behavior in the control group (n = 103 male pairs), JH III-treated group (n = 96 and 43 male pairs for the 200 and 300 μg JH III treatment, respectively), and precocene II-treated group (n = 52 and 53 male pairs for the 100 and 200 μg precocene II treatment, respectively) were observed for at least 20 consecutive days. Observations were conducted at 3~4 h into the scotophase

under a dim red light. Each male pair was observed for at least 10 min to decide whether agonistic behavior was occurring. The insect producing at least 5 agonistic interactions within 10 min was considered dominant.

Assay of CA *in vitro* activity

JH III release by the corpora allata (CA) incubated *in vitro* was determined using a radiochemical assay (Pratt and Tobe 1974, Tobe and Pratt 1974), as modified by Feyereisen and Tobe (1981). Males were anesthetized for a few seconds just before dissection. Individual pairs of the CC-CA complex were dissected in roach saline (10.3 g NaCl, 1.46 g KCl, 0.36 g $NaHCO_3$, 0.21 g $NaH_2PO_4 \cdot H_2O$, 1.34 g Na_2HPO_4, and 3 g/L glucose) (Kurtti and Brooks 1976), and each pair was incubated in 30 μl methionine-free medium 199 supplemented with 125 μM L-[*methyl*-^{14}C] methionine (NEN, with a specific activity of 50 mCi/mmol). Components of TC 199 and other reagents were purchased from Sigma Chem. as described before (Kou and Chen 2000a b, Kou, 2002). After 3 h of incubation at 28°C, JH III was extracted from the medium after removing the CC-CA by adding 30 μl isooctane (Sigma-Aldrich, HPLC grade) as described before (Ho et al. 1995, Kou and Tu 1998). Following extraction, the JH III was separated by thin-layer chromatography (Silica gel 60 F_{254} plates; Merck, Darmstadt, Germany) using ether: hexane, 1: 3 (v/v), as the solvent system as described before (Kou and Tu 1998, Kou and Chen 2000a b). Synthetic standards of JH III were obtained from Sigma Chem. ^{14}C radioactivity was determined by liquid scintillation counting in a Ready Safe™ liquid scintillation cocktail.

Statistical analysis

Since all data sets obtained for the JH *in vitro* release rate passed normality tests, one-way ANOVA (SAS Institute 1990) was used for the statistical analysis ($p < 0.05$). For the analysis of agonistic behavior initiation, Wilcoxon's rank sum test was employed ($p < 0.05$).

RESULTS

Effects of exogenous JH III and precocene II on the onset of agonistic behavior

In this experiment, dominants usually occupied the space inside the cap area of the transparent plastic cage, while the subordinates stayed beside the transparent water vial; this phenomenon is consistent with a previous report that the higher the rank of an animal the more likely that animal is to have a permanent territory (Ewing 1972); once they meet, the agonistic interaction occurs. The effects of topical application of JH III and precocene II on the agonistic behavior are shown in Fig. 1. Since our previous observations showed no significant difference in either average onset age of agonistic behavior or determination of social status between acetone-treated and non-acetone-treated males, only white-dot controls were used in this experiment. In the control test (Fig. 1A), the average onset age of agonistic behavior was around 9 (range, 5~19) d post-emergence, and the likelihood of any male being dominant or subordinate was almost equal (about 50%). Our results also showed that the small white-dot (Tipp-Ex fluid) treatment made no difference to the average onset age of agonistic behavior (8.9 ± 0.5 and 9.3 ± 0.5 d post-emergence for the white-dotted group and the non-white-dotted group, respectively, $p > 0.05$).

In the experiment of JH treatment, each JH-treated male was paired with an acetone-treated male. The results (Fig. 1B, C) of this experiment showed that the proportions of dominants to subordinates resulting from 200 and 300 μg JH III treatment were about 42%~47% and 53%~58%, respectively. In the 42% of dominants resulting from 200 μg JH III-treatment (Fig. 1B), the average onset age of agonistic behavior was significantly ($p < 0.001$) delayed for 3 d compared to that of the control (i.e., 12.8 ± 0.6 vs. 9.3 ± 0.5 d post-emergence). A significant ($p < 0.05$) delay (i.e., 10.1 ± 0.5 vs. 8.9 ± 0.5 d post-emergence) on the onset of agonistic behavior was also found in the other 58% acetone-treated dominants (their opponents were subordinates resulting from JH treatment). The agonistic behavior in these 42% of 200 μg JH III-treated dominants was also initiated (at 12.8 ± 0.6 d post-emergence) significantly ($p < 0.001$) later than that of the 58% acetone-control dominants (10.1 ± 0.5 d post-emergence). The pattern of this delayed effect on the onset of agonistic behavior in the 300 μg JH III treatment was similar to that of the 200 μg JH III treatment (Fig. 1C).

In the precocene II treatment, each treated male was also paired with an acetone-treated male. The results of this experiment showed that contrary to the effect of JH III treatment, only 38%

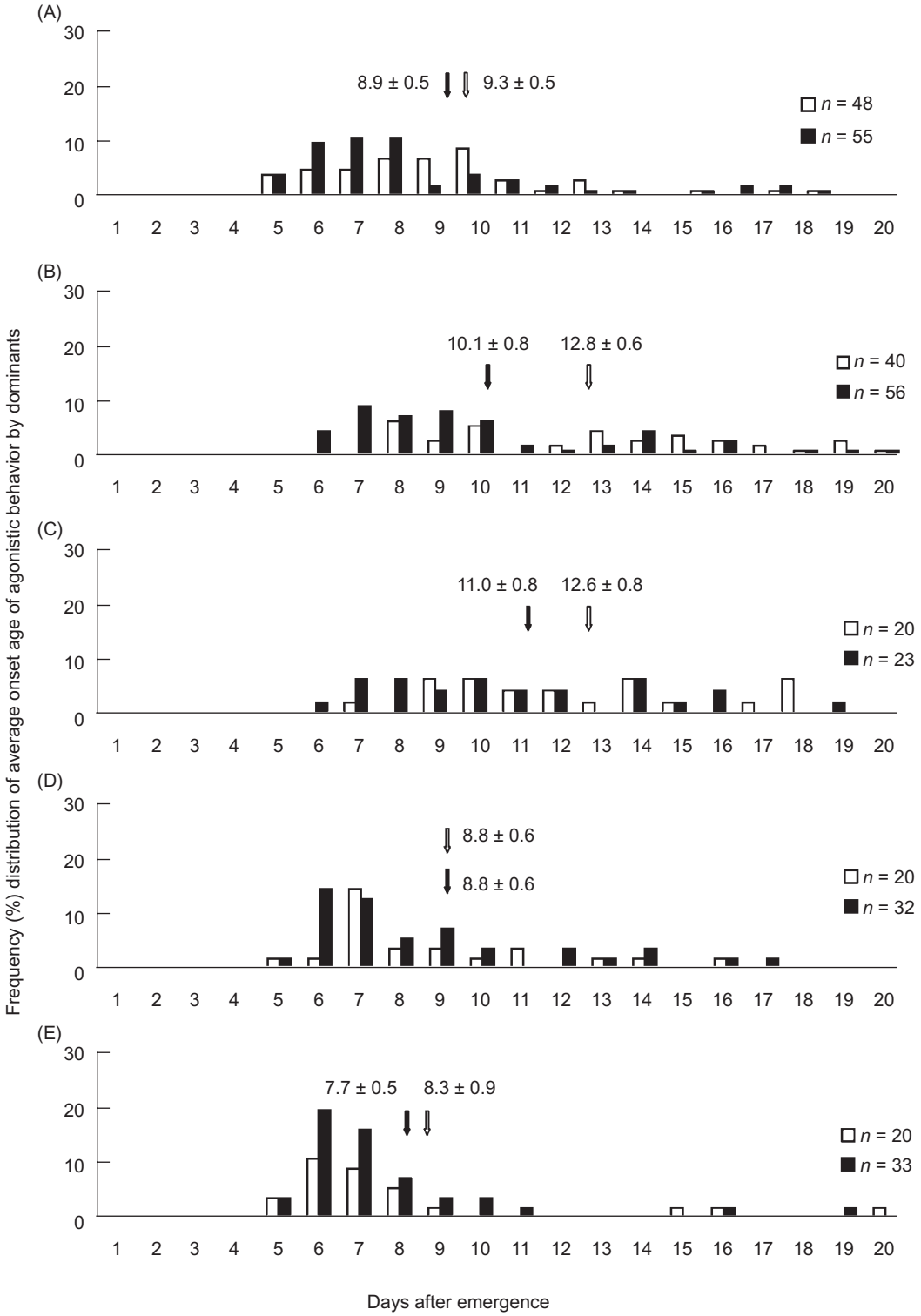

Fig. 1. Frequency distribution of the onset age of agonistic behavior (i.e., adopting a dominant status) in Nauphoeta cinerea adult males. *n*, numbers of male pairs. Males were paired on the day of emergence, regardless of whether they were treated or not. (A) Control; ■, non-white-dot (Tipp-Ex fluid) treatment; □, white-dot treatment on the pronotum. (B) 200 μg JH III treatment: ■, acetone-control, without a white dot on the pronotum; □, JH-treated, with a white dot on the pronotum. (C) 300 μg JH III treatment: ■, acetone-control, without a white dot on the pronotum; □, JH-treated, with a white dot on the pronotum. (D) 100 μg precocene II-treated: ■, acetone-control, without a white dot on the pronotum; □, precocene-treated, with a white dot on the pronotum. (E) 200 μg precocene II- treated: ■, acetone-control, without a white dot on the pronotum; □, precocene-treated, with a white dot on the pronotum.

of the precocene-treated individuals adopted a dominant status (Fig. 1D, E). The average onset age of agonistic behavior in the 38% of 200 μg precocene-treated dominants was significantly (p < 0.05) accelerated 1 d earlier than that of the control (i.e., 8.3 ± 0.9 vs. 9.3 ± 0.5 d post-emergence); also a significant (p < 0.05) acceleration effect was found in the other 62% of dominants (their opponents were subordinates resulting from precocene treatment) resulting from acetone treatment (i.e., 7.7 ± 0.5 vs. 8.9 ± 0.5 d post-emergence) (Fig. 1E). The average onset age of agonistic behavior did not significantly differ between the precocene-treated dominants and the acetone-treated dominants (i.e., 8.8 ± 0.6 vs. 8.8 ± 0.6 d post-emergence and 8.3 ± 0.9 vs. 7.7 ± 0.5 d post-emergence for the 100 and 200 μg precocene II treatments, respectively).

Effects of exogenous JH III and precocene II on CA *in vitro* activity

As described above in "Methods", once the agonistic behavior was initiated after JH III or precocene II treatment, CA from dominants and subordinates of the same male pair were respectively incubated on the 1st fighting day, and the JH III *in vitro* release rate was investigated. Results of this experiment are given in Table 1. In the control group, the probability of a male in a pair being dominant or subordinate was about 50: 50 (Fig. 1A), with dominant males possessing a significantly (p < 0.01) higher JH III *in vitro* release rate (0.95 ± 0.04 pmol/3 h/pair CA) than that of their opponent subordinate males (0.66 ± 0.03 pmol/3 h/pair CA).

In the 200 μg JH III-treated group, about 42% and 58% of JH-treated individuals resulted in a dominant and subordinate social status, respectively (Fig. 1B), and these are referred as 200 JH-D and 200 JH-S hereafter. For the 200 JH-D, the JH III *in vitro* release rate (0.86 ± 0.03 pmol/3 h/pair CA) was significantly (p < 0.05) higher than that of the acetone-treated subordinate opponents (0.67 ± 0.03 pmol/3 h/pair CA). On the other hand, the JH III *in vitro* release rate for the 200 JH-S (0.64 ± 0.03 pmol/3 h/pair CA) was significantly (p < 0.05) lower that than of its acetone-treated dominant opponents (0.85 ± 0.03 pmol/3 h/pair CA). In the 300 μg JH III-treated group, about 46.5% and 53.5% of JH-treated individuals resulted in a dominant and subordinate social status, respectively (Fig. 1C), and these are referred as 300 JH-D and 300 JH-S hereafter. For the 300 JH-D, the JH III *in vitro* release rate (0.85 ± 0.04 pmol/3 h/pair CA) was significantly (p < 0.05) higher than that of the acetone-treated subordinate opponents (0.71 ± 0.03 pmol/3 h/pair CA). On the other hand, the JH III *in vitro* release rate for the 300 JH-S (0.66 ± 0.03 pmol/3 h/pair CA) was significantly (p < 0.01) lower than that of its acetone-treated dominant opponents (0.91 ± 0.03 pmol/3 h/pair CA).

In the 100 μg precocene II treatment, 38.5% and 61.5% of precocene-treated individuals resulted in a dominant and subordinate social status, respectively (Fig. 1D), and these are referred as 100 P II-D and 100 P II-S hereafter. For the 100 P II-D, the JH III *in vitro* release rate (0.83 ± 0.05 pmol/3 h/pair CA) was significantly (p < 0.01) higher than that of its subordinate acetone-treated opponents (0.58 ± 0.05 pmol/3 h/pair CA). For the 100 P II-S, the JH III *in vitro* release rate (0.68 ± 0.03 pmol/3 h/pair CA) was significantly (p < 0.01) lower than that of its dominant acetone-treated opponents (0.95 ± 0.05 pmol/3 h/pair CA). In the 200 μg precocene II treatment, also about 38%

Table 1. JH III *in vitro* release rate of CA from dominant and subordinate *Nauphoeta cinerea* males, after treatment with JH III or precocene II or the control (n = 9~12). * and **: JH III *in vitro* release rate from dominants was significantly higher than that of their subordinate opponents at p < 0.01 and < 0.05, respectively. Dom, dominant; Subor, subordinate; Ctr, control. For JH III or precocene II treatment, the Ctr was acetone-treated

Social status	JH III (pmol/3 h/pair CA) release rate detected from each Treatment				
	Control	JH III 200 μg	JH III 300 μg	Precocene II 100 μg	Precocene II 200 μg
Treat-Dom	-	*0.86 ± 0.03	*0.85 ± 0.04	**0.83 ± 0.05	**1.02 ± 0.08
Ctr-Subor	0.66 ± 0.03	0.67 ± 0.03	0.71 ± 0.03	**0.58 ± 0.05	**0.69 ± 0.04
Ctr-Dom	**0.95 ± 0.04	*0.85 ± 0.05	*0.91 ± 0.55	**0.95 ± 0.07	**1.23 ± 0.11
Treat-Subor	-	0.64 ± 0.03	0.66 ± 0.04	0.68 ± 0.03	0.78 ± 0.05

and 62% of treated individuals resulted in a dominant and subordinate social status, respectively (Fig. 1E), and these are referred as 200 P II-D and 200 P II-S hereafter. For the 200 P II-D, the JH III *in vitro* release rate (1.02 ± 0.08 pmol/3 h/pair CA) was significantly ($p < 0.01$) higher than that of its subordinate acetone-treated opponents (0.69 ± 0.04 pmol/3 h/pair CA). For the 200 P II-S, the JH III *in vitro* release rate (0.78 ± 0.05 pmol/3 h/pair CA) was significantly ($p < 0.01$) lower than that of its dominant acetone-treated opponents (1.23 ± 0.05 pmol/3 h/pair CA).

DISCUSSION

In *N. cinerea*, agonistic behavior between adult males and the resulting dominant-subordinate hierarchies have been investigated in detail since the 1960s (Kramer 1964, Ewing 1967, Bell and Gorton 1978, Schal and Bell 1983, Moore 1990, Sirugue et al. 1992). Laboratory tests showed that the outcome of an encounter involving an untried male, regardless of its aggressive potential, depends mainly on the status of the opponent, especially if the latter has already been dominant (Ewing 1967, Manning and Johnstone 1970, Breed et al. 1980), suggesting the involvement of pheromones. Although later studies showed that both the cuticular hydrocarbon profile and sexual pheromone blend are involved in establishing and maintaining a dominance status (Everaerts et al. 1997, Moore et al. 1997, Roux et al. 2002), physiological correlates underlying the agonistic behavior are not well understood.

In the context of insect agonistic behavior, the role of JH has always been suggested, such as in the primitive social wasps and bumblebees and the highly social honeybees. In the primitive social wasp *Polistes annularis*, repeated topical application of JH to workers resulted in disruption of the colony social structure as indicated by a sharp increase in the frequency of dominance interactions and ovarian maturation (Barth et al. 1975). In the paper wasp *Polistes gallicus*, social rank is correlated with the activity of the CA, with the dominant foundress having a higher JH titer (Roseler et al. 1980), and the initial differences in the JH titer becoming even more pronounced during association with the foundresses (Roseler 1991). In the bumblebee *Bombus terrestris*, the association of social status and JH titer also suggested JH involvement in the modulation of dominance behavior (Van Doorn 1989, Larrere and Couillaud 1993, Bloch et al. 2000). In highly social insects, such as the honeybee *Apis mellifera*, workers reared in isolation had elevated JH levels and showed higher levels of aggression toward other bees (Breed 1983, Huang and Robinson 1992). Older bees, which have higher JH levels, are generally more aggressive than younger bees with lower JH levels (Breed 1983, Huang et al. 1994). JH titers of guard bees are higher than those of all other middle-aged bees, and guards exhibit low JH thresholds for the expression of aggression (Breed et al. 1992, Huang et al. 1994). Pearce et al. (2001) also found that JH titers were correlated with aggressive behavior in different seasons.

But in *N. cinerea*, previous results about the role of CA in the agonistic behavior are contradictory. With an allatectomy 5 d prior to or within 12 h following the imaginal ecdysis, Hartman and Suda (1973) suggested that pheromone production does not seem to be controlled by the CA. Schal and Bell (1983), who conducted allectomies on newly emerged males, also suggested that the presence of the CA is not requisite for the development of agonistic behavior and sex recognition. But Sreng et al. (1999) indicated that a allatectomy performed 2~3 d after emergence caused a decrease in sex pheromone levels, and concluded that JH III is involved in differentiation of the sternal glands. As a reference for the role of JH, we began with a simple topical application method, and our results showed that exogenous JH III (applied on the 1st emergence day) may have caused several pharmacological effects on agonistic behavior in adult males. First, exogenous JH may delay the onset of agonistic behavior in both JH-treated males and their acetone-treated opponents. Agonistic behavior, which is generally initiated on the 9th day after emergence in the control group, was significantly delayed for 3 additional days in the 200 and 300 μg JH-treated groups (Fig. 1A-C). In this study, the concentrations of topically applied JH III was high (200 or 300 μg), so a negative feedback effect cannot be excluded. This negative feedback effect may first have inhibited the onset of the agonistic behavior, and then some of these JH-treated individuals may have recovered from the negative effect and turned out to be late dominant fighters. One other factor which also has to be considered is that all of the topically applied JH might or might not have entered the insect body in its original form. Determining what kinds of metabolites formed after the topical application, with what forms and how much of the exogenous JH entered the insect body and exerted a delaying effect still

needs further elucidation. The results of this study also showed that exogenous JH exhibited no obvious effect on determination of the dominance status, since the proportion of JH-treated individuals which adopted the dominant or subordinate status was about 50%, similar to that of the control (Fig. 1A-C). Although the JH III *in vitro* release rate in JH-treated dominants (about 42%~47% of the JH-treated individuals adopted a dominant status) was significantly higher than that of their acetone-treated subordinate opponents, the other JH-treated subordinates (53%~58% of the JH-treated group) showed significantly lower JH III *in vitro* release rates than their acetone-treated dominant opponents. These results indicated that CA activity in dominants is significantly higher than that in the subordinates, regardless of whether or not they were treated with JH. Determining whether exogenously applied JH exerted its effect on CA activity or some of the JH-treated individuals recovered from the feedback effect still needs further study. One aspect we have to keep in mind is that even though the CA *in vitro* activity (or JH titer) is significantly correlated with the social status, we still have to elucidate the "cause-and-result" relationship, i.e., whether CA's *in vitro* activity (or the JH titer) is the cause or the result of the agonistic behavior.

Contrary to the delayed effect of JH III treatment, precocene II treatment significantly accelerated the onset of agonistic behavior, at the higher dose (200 μg). Precocene II also exhibited a negative effect on determination of the dominance status; about 62% of precocene-treated individuals adopted a subordinate status, and these subordinates showed significantly lower JH III *in vitro* release rates than did their acetone-control dominant opponents. The other 38% of precocene-treated individuals adopted a dominant status, and these precocene-treated dominants showed significantly higher JH III *in vitro* release rates than did their acetone-control subordinate opponents. Again, just like the results of the JH-treated experiment, the JH III *in vitro* release rate in the dominants was higher than that of the subordinates, regardless of whether or not they were treated with precocene. Precocenes are naturally occurring chromene derivatives, isolated from the plant *Ageratum houstonianum*, and which possess anti-JH activity in some insects (Bowers et al. 1976). The *in vivo* allatotoxic effect of precocene II was proven to be direct, due to highly reactive 3, 4-epoxy intermediates which originate from precocene oxidative bioactivation within the CA (Socha and Hodková 1978).

In this study, a propensity was also found for the onset of agonistic behavior of acetone-control opponents to be delayed if that of the treated individuals (e.g., JH III-treated ones) was delayed (Fig. 1A-C). On the other hand, if the onset of agonistic behavior of the treated individuals (e.g., precocene-treated ones) was accelerated, then their acetone-control opponents also accelerated their onset of agonistic behavior (Fig. 1A, D, E). In blattodeans and coleopterans, pheromone production is already known to be induced by JH III (Tillman et al. 1999). Since pheromones of the opponent play important roles in the agonistic behavior of *N. cinerea* (Ewing 1967, Manning and Johnstone 1970, Breed et al. 1980), whether and how the topically applied JH or precocene II affects the pheromone system are interesting topics for further study.

To sum up, exogenous JH III and precocene II exerted opposite effects on the onset of agonistic behavior in *N. cinerea* adult males. Although exogenous JH III did not affect the outcome of an agonistic encounter, precocene II showed a propensity to inhibit the dominant status. Since dominants always exhibited significantly higher JH III *in vitro* release rates than the subordinates, determining the role of CA (or JH III) in agonistic-related behavior is worthy of further investigation.

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