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Enhancement of short wing formation and ovarian growth in the genetically defined macropterous strain of the brown planthopper, *Nilaparvata lugens*

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Abstract

When JH II, III or methoprene was applied in the nymphal stages to two different strains of the brown planthopper which were selected to produce long (macropterous) or short (brachypterous) wing forms, no effect was observed on the molting profile or metamorphosis. Brachypterization of a majority of the presumptive macropters was, however, observed by application of these chemicals, although there was no effect on wing form in the presumptive brachypters. The results show that the sensitive periods for the brachypterization of the presumptive macropters falls between early antepenultimate instar and within 1 or 2 days of the penultimate instar, and that the chemicals were effective, in the following order of potency: methoprene > JH III > JH II. Ovarian growth was greatly enhanced in the presumptive macropters when JH III or methoprene was applied twice, within 12 h of the 3rd or 4th nymphal instar and 6 h before adult emergence. JH II on the other hand had no effect on ovarian growth when applied to the presumptive macropters at any of the nymphal stages. None of the chemicals had any effect on ovarian growth in the presumptive brachypters.

Keywords: Wing polymorphism; Juvenile hormone; Ovarian growth; Methoprene; *Nilaparvata lugens*

1. Introduction

Wing form in delphacids is determined by a developmental switch that responds to environmental cues, and the sensitivity of the developmental switch to those cues is heritable (Denno et al., 1995). Although the genetic basis of wing polymorphism in insects generally is not well understood, it is presumed to be under polygenic control in this group (Denno and Roderick, 1990; Denno et al., 1995). It is assumed that genes determine the level of the juvenile hormone that is present above or below a certain threshold, leading to the production of short or long wing forms, respectively (Harrisson, 1980; Roff, 1986). The brown planthopper, *Nilaparvata lugens*, is no exception because wing polymorphism in this species is known to be a common and ecologically important trait. Population density experienced during the nymphal stage is known to be the most important environmental factor affecting wing determination in *N. lugens*. In major populations collected in Japan, it is known that in females, crowding increases the relative proportion of macropterous hoppers, while in the males, a moderate nymphal density promotes the maximum proportion of brachypterous forms (Kisimoto, 1973; Iwanaga and Tojo, 1986). Moreover, various populations of *N. lugens* showing wing-form responses to densities different from those mentioned above have been discovered in the fields. Such populations range from being predominantly brachypterous to being predominantly macropterous (Iwanaga et al., 1985; Morooka et al., 1988).

Even though unambiguous effects of exogenous juvenile hormone have clearly been shown on environmental polyphenism (facultative polymorphism) in *N. lugens* (Iwanaga and Tojo, 1986), the physiological mechanisms underlying genetic polymorphism in this species have not been studied. Investigating the physiological basis of genetic polymorphism is very important since wing polymorphism is a consequence of both environmental and genetic factors. Two strains predominantly exhibiting a specific wing form, brachypterous or

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macropterous at a wide range of densities have been generated by successive selection for adults with these specific wing form (Morooka and Tojo, 1992). In previous papers (Ayoade et al., 1996a, b), we demonstrated that precocene II, a juvenile hormone antagonist can induce not only precocious metamorphosis in both strains, but also long-wing formation and retardation of ovarian development in the presumptive brachypterous strain. This suggests different fluctuations of juvenile hormone during the penultimate instar between these two strains. The effects of exogenously applied juvenile hormones (JH) and methoprene, a JH-agonist/mimic on these strains is expected to elucidate further the endocrinological control of genetically mediated polymorphism in this species, as opposed to environmentally induced polyphenism that has been the focus of most previous studies.

The present paper reports on the effects of JH II, III and methoprene on metamorphosis, genetically mediated wing-dimorphism, and ovarian growth, in *N. lugens*.

2. Materials and methods

Two strains of *Nilaparvata lugens* that were obtained from the stocks generated by Morooka and Tojo (1992), by successive selection of adults with specific wing form and color obtained under highly crowded conditions over 100 generations, were used in this study: the brachypterous and macropterous strains, being selected for short-winged, yellowish-brown coloration and long-winged, blackish coloration, respectively. These two strains produced about 100% short-winged or long-winged individuals under a broad range of densities. Hereafter, individuals of the former and latter strains are called presumptive brachypters and presumptive macropters, respectively. The experimental insects, ca 500, were kept in cages (11 × 12 × 18 cm) containing rice seedlings (variety Reiho) under a 16 L:8 D photoperiodic regime at 25 ± 2°C (Morooka et al., 1988).

JH II (Sci Tech), JH III (Sigma Chemical Co.) and methoprene (Otsuka Pharmaceutical Co.) were dissolved in acetone to yield 1 μg/μl stock solution and stored at – 20°C. These were diluted with acetone at the time of each application and applied using a syringe (Terumo) and a micro applicator (Burkard). The chemicals were diluted at concentrations of 1 ng, 10 ng, 100 ng, 1 μg, 10 μg in 0.1 μl acetone, and applied to the abdomen of synchronized 2nd, 3rd, 4th or 5th nymphal stadium insects once or twice within 12 h of molt, unless otherwise stated, after anaesthetizing with CO_2 for 30 s. After treatment they were reared on a rice seedling in a glass cylinder (2 cm dia × 20 cm tall) at a density of five insects per plant. Control animals were treated with 0.1 μl of acetone. The molting profile of the treated insects was monitored by checking for ecdysis every 6 h. The rice seedlings were changed every 24 h. The wing form of emerged adults and the time to final molt were noted; adults with wing-tips up to the last abdominal segment were regarded as macropters, while adults with shorter wings were regarded as brachypters. Twenty-five individuals were treated at each dose (five replicates containing five insects per cylinder). After adult emergence, effect(s) of chemical treatment on ovarian growth was recorded and compared with the control. This evaluation was carried out by dissecting the emerged adult females under a stereomicroscope and visually quantifying the size of the ovaries by scoring on a scale of 0–3 from day 0 to day 6 after adult emergence. The rating categories of Iwanaga and Tojo (1986) were used in the evaluation as follows: score 0, stage before vitellogenesis: score 1, ovarioles enlarged and vitellogenesis had started in about 1/3 of the oocytes; score 2, vitellogenesis completed in 2/3 of oocytes and some of them had formed a chorion; score 3; complete eggs in most ovarioles. An average of seven insects was dissected each time to obtain an average. Thus, 49 insects were required for each treatment, i.e. ca seven female adults over 7 days; to make up for the shortage in treated insects, data were collected in batches from repetitions of the experiments. The χ^2 test of heterogeneity was then applied to the data to determine whether the data were homogenous; the very few data that failed the test were not included in the final average.

3. Results

3.1. JHs II, III and methoprene effects on molting and metamorphosis

Fig. 1 shows the molting profiles of presumptive brachypters to which 100 ng of JH III was applied once within 6–12 h of the 2nd, 3rd or 4th stadium; neither prothetely nor metathetely were observed in any of the hoppers as the treated hoppers went through the same number of molts as the control hoppers. The stadium at which methoprene was applied was usually slightly prolonged, leading to a shortening of the subsequent stadia when compared with the control hoppers. The same results were obtained when the presumptive macropters were treated with JH III once during the three larval stadia (Fig. 2). Treating the hoppers twice during the nymphal stadia did not cause any observable effects on the molting profile or metamorphosis except for a substantial increase in mortality and proportion of malformed individuals (data not shown).

3.2. Effects of JHs II, III and methoprene on wing formation

Treatment of presumptive macropters nymphs between 6 h old 3rd and 48 h old 4th nymphal stadia

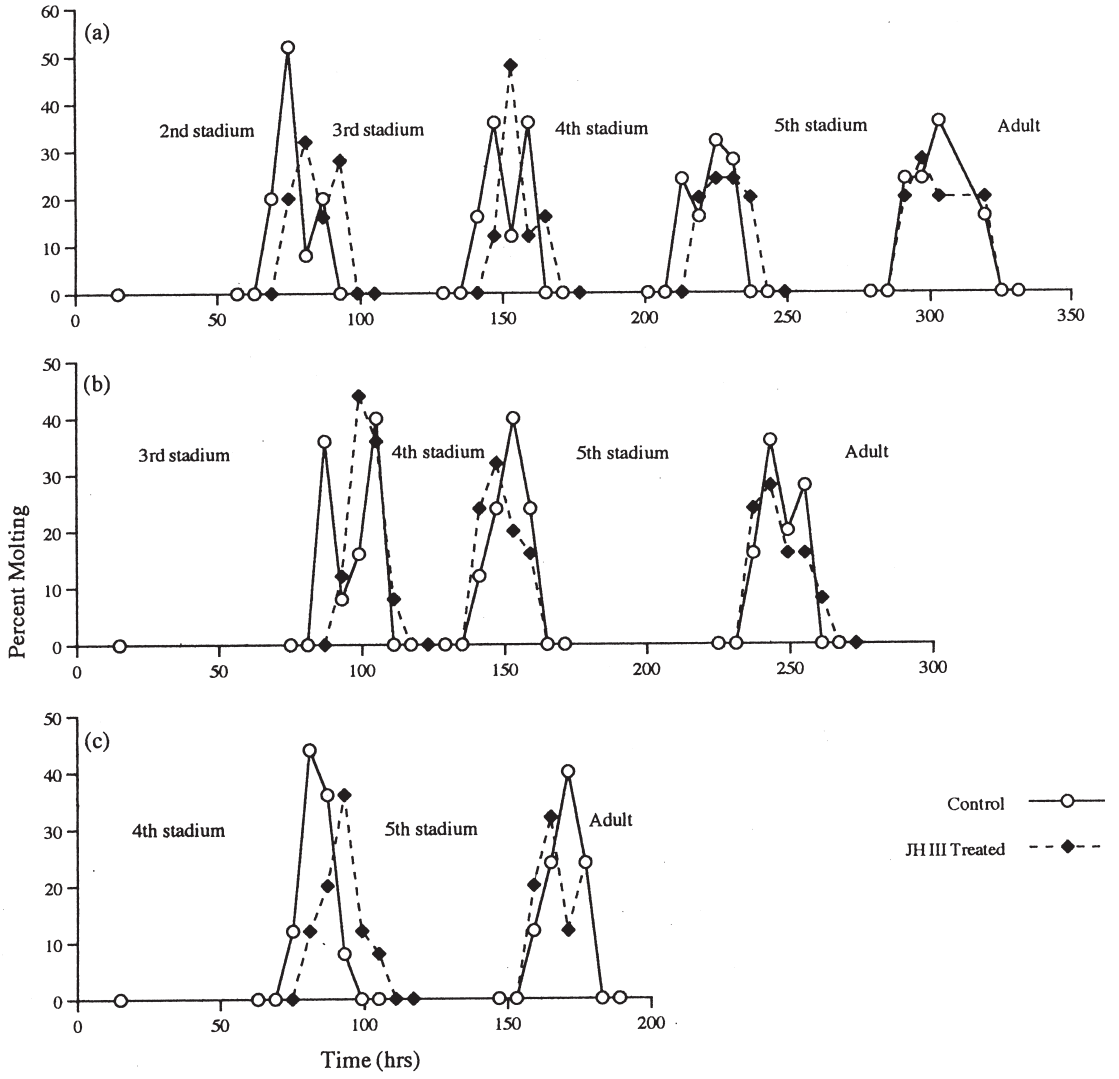

Fig. 1. Molting processes in the presumptive brachypters treated with JH III within 6–12 h after molting into 2nd (a), 3rd (b) or 4th (c) stadia. JH III was dissolved in 0.1 μl acetone, and topically applied to each animal. Control insects were treated with 0.1 μl acetone. Twenty-five animals were treated at each treatment. Five animals were enclosed in a test tube (2 × 20 cm height) with rice seedling, and reared at 25 ± 2°C under 16 hL:8 hD photoperiod. Molting was checked every 6 h. Abscissa is the time after treatment.

with JHs II, III or methoprene caused an overall decrease in the proportion of long-winged adults after treatment. However, the compounds were not equally effective; the order of potency was methoprene > JH III > JH II (Fig. 3). All the compounds were effective in reducing the proportion of long-winged progenies from about 100% in the control hoppers to 15–55% in the treated hoppers. These reductions were expressed in a dose-dependent manner. Methoprene was able to inhibit wing formation by 50% starting from the 1 ng dosage, and the natural JHs were able to inhibit wing formation by 50% starting from the 10 ng–10 μg dosage range, with JH II being the least effective. Generally, the males showed more sensitivity to treatment with both the JHs and methoprene than the females, and more males emerged as brachypters than did females in most of the treatments (Fig. 4). Treatment of JHs II, III or methoprene to 6–12 h old 1st, 2nd, and 5th nymphal stadium presumptive macropters did not induce brachypterous adults (see Fig. 3 although data for the 1st stadium is not shown). There was no significant difference between JHs II, III or methoprene-treated presumptive brachypters and the control in relation to wing form (data not shown), although there was 2–15% malformation in the wings of the treated presumptive brachypters and macropters. These malformed individuals sometimes lacked the fore wing or the wing protruded at an awkward angle to the thorax of the insects, making the wings apparently useless for flight.

3.3. Effects of JHs II, III and methoprene on ovarian growth

Ovarian growth differed between the presumptive macropters and brachypters, both for the control and the

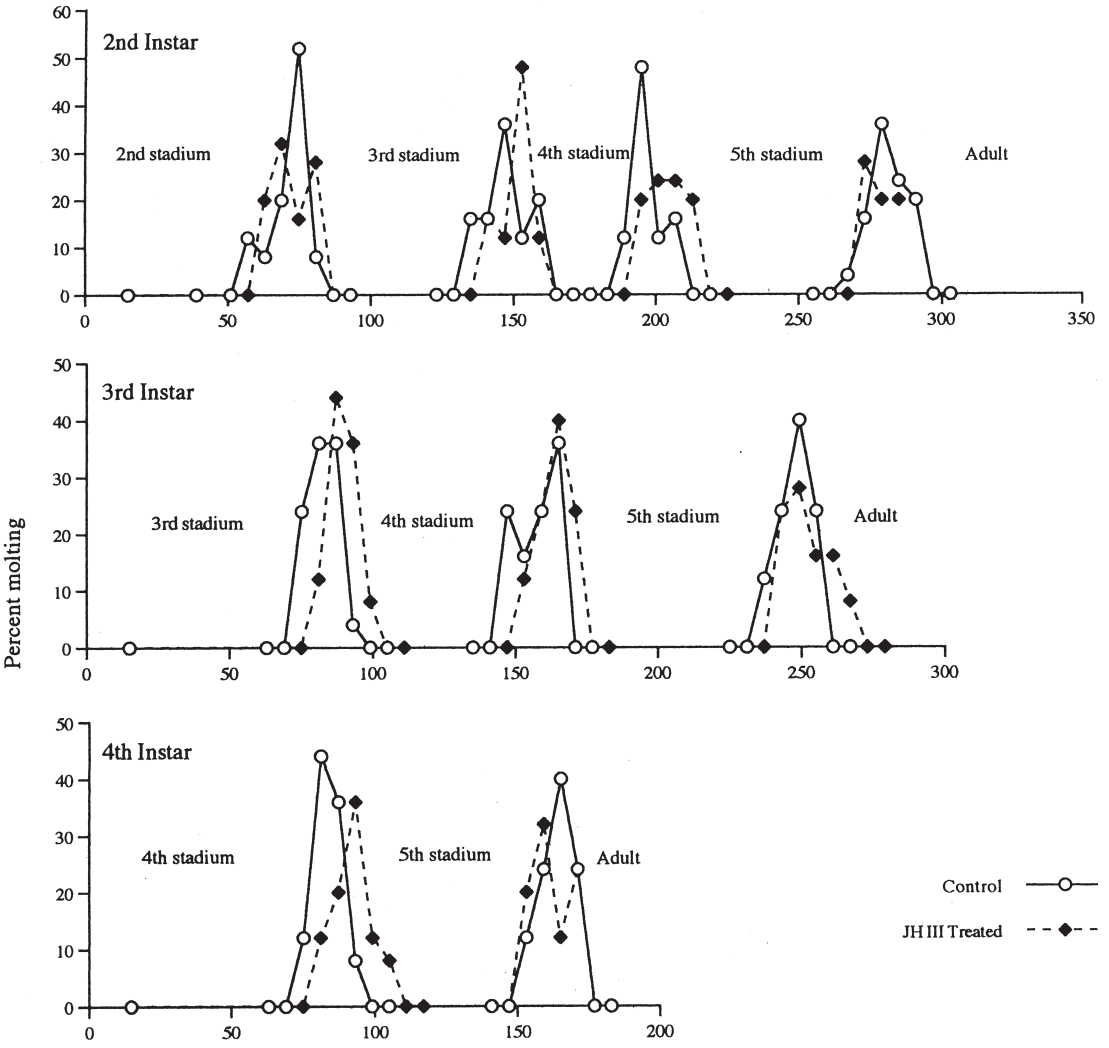

Fig. 2. Molting processes in the presumptive macropters treated with JH III about 12 h after molting into the 2nd (a), 3rd (b) or 4th (c). See Fig. 1 for details.

treatments. Ovarian growth started a day later in the acetone-treated control presumptive macropters than in their counterparts of the presumptive brachypter stock: ovarian growth in untreated controls from the latter strain surpassed that in the former (Figs. 5 and 6). JH III and methoprene were effective on ovarian growth in the presumptive macropters only when it was applied twice to the animals resulting in ovarian growth surpassing those of the controls at the 100–1000 ng dosage [Fig. 6(a) and (b) respectively]. None of the chemicals caused any significant difference in ovarian growth in the presumptive brachypters when the insects were treated twice (Fig. 5).

Of the two JHs, only JH III was effective in enhancing ovarian growth in the presumptive macropters, even when applied twice before adult emergence at a dosage of 100 ng. JH II was not effective when applied twice, even at the dosage of 10 μg (data not shown), and increase in dosage beyond this level caused such a high mortality that made further evaluation impossible.

4. Discussion

Apart from shifting the spacing of the molting profile, the results of the present experiments did not show any disruptive effects of JHs II, III and methoprene on metamorphosis in the brown planthopper, *N. lugens*. While very few studies have been done on the effects of JH on metamorphosis in hemimetabolous insects, particularly the Homoptera, our results are consistent with that reported in aphids. Lees (1978) reported the insensitivity of the fourth instar gynopara of the aphid, *Aphis fabae* (Hemiptera) to the juvenilizing action of JHs and their analogs, even though major morphogenetic effects were observed in the progeny. In the present experiments, JHs II, III and methoprene were effective on ovarian growth and wing formation although no effects on metamorphosis were observed. The present result also corroborates our previous results (Ayoade et al., 1996a, b) in which we reported different sensitive periods for precocene II effects on metamorphosis and wing formation in

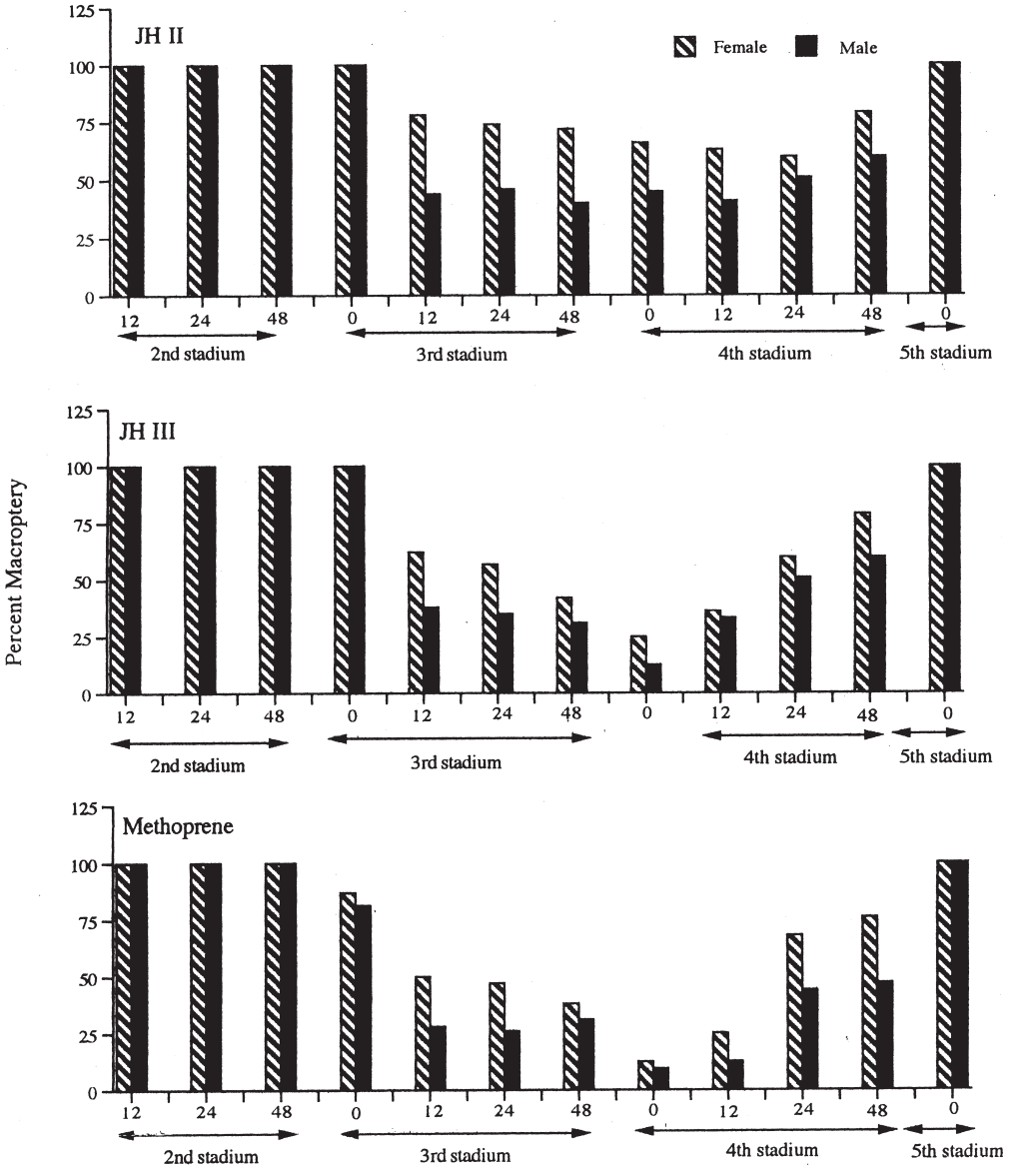

Fig. 3. Wing-form expression in the presumptive macropters treated with 100 ng of JH II, III or methoprene at the stages of 2nd, 3rd, 4th or 5th stadia given in abscissa. See Fig. 1 for details.

N. lugens. This observation promoted us to suggest that these processes go on independently in this species (Ayoade et al., 1996a, b). Although the reason for the lack of effect of the JHs and methoprene on metamorphosis (even though other major morphological processes were affected) remains unknown, it is not impossible that the JHs were acting as mimics, just like methoprene, with respect to *N. lugens* because its specific JH for this species has not been identified yet.

JHs II, III and methoprene strongly directed development from long to short wings in the presumptive macropters of *N. lugens* when applied between 6 h of the 3rd nymphal stadium and 48 h of the 4th stadium (Fig. 3). This is consistent with the sensitive stages observed for the effects of precocene II in this species: precocene II induced long-wing formation in the presumptive brachypters of *N. lugens* only when applied to 2nd or 3rd stadium nymphs (Ayoade et al., 1996a, b). Moreover, the present results show the first evidence of the effects of JHs II, III and methoprene on genetically mediated wing-dimorphism in *N. lugens*, even though Iwanaga and Tojo (1986) previously reported the brachypterizing effects of JHs I, II and methoprene in the 4th stadium on environmental wing-polyphenism in the same species. This evidence leads us to suggest that wing determination in the presumptive macropterous strain takes place under a reduced juvenile hormone titer within 1 or 2 days after reaching the penultimate stadium (Fig. 3). The insensitivity of the presumptive brachypters to JH and methoprene treatment in relation to wing deter-

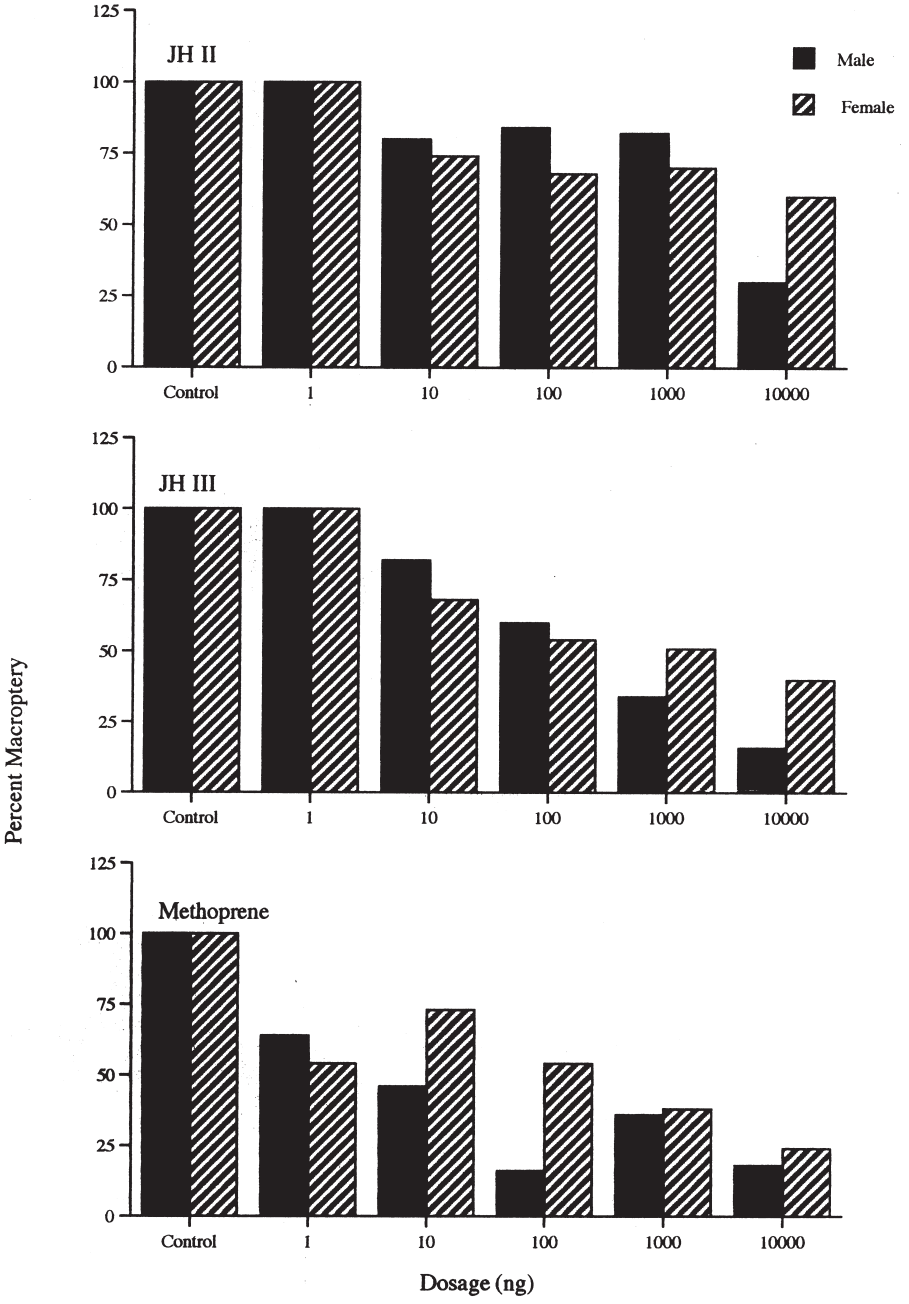

Fig. 4. Typical gender difference in wing-form expression in the presumptive macropters treated with JH II, JH III or methoprene within 6–12 h of emergence into the 4th nymphal stadium.

mination is expected as JHs and methoprene are known to induce brachyptery.

The brachypterizing effect of JHs II, III and methoprene was stronger in males than in females, as was observed in the presumptive brachypters which were induced to produce long wings by treating with precocene II (Ayoade et al., 1996a, b). Although the reason for this is unclear, differences in cuticle penetration, differential rates of synthesis and/or degradation in the haemolymph and the way the smaller male-size might affect sensitivity in relation to the larger females are all possibilities.

In pterygote insects, possession of wings by individuals of a wing dimorphic species is known to carry a fitness cost. This cost in females appears, in general, to be a reduced fecundity and a delay in the start of oviposition of the winged morph (Harrisson, 1980; Dingle, 1985; Roff, 1986). The foregoing is further confirmed by our results showing reduced ovarian growth and delayed oviposition in the untreated or acetone-treated presump-

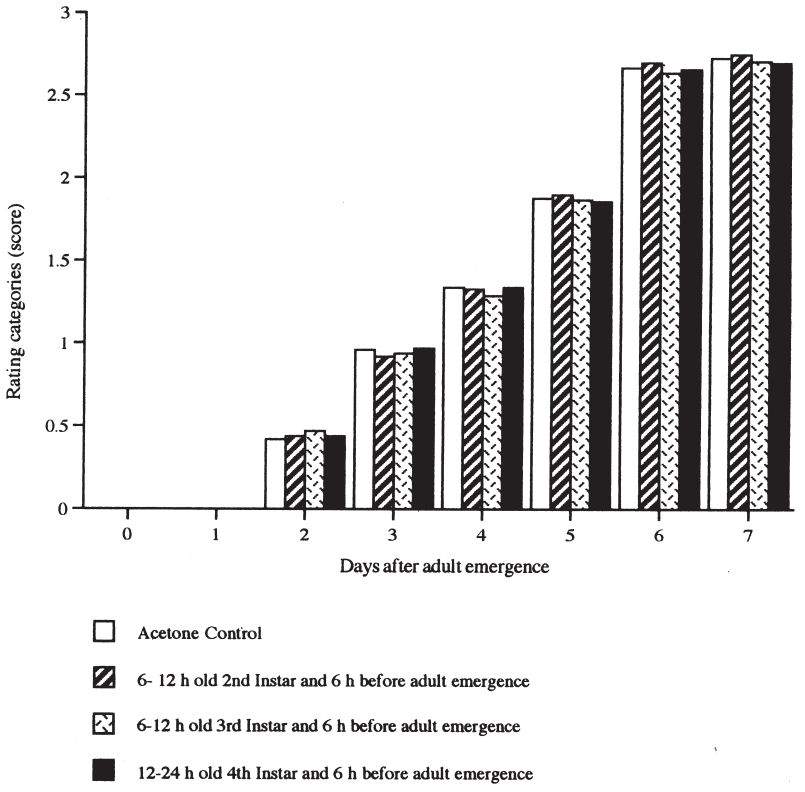

Fig. 5. Ovarian growth in presumptive brachypters treated twice with 100 ng JH III as given in the legend. The rating categories were adapted from Iwanaga and Tojo (1986): see Section 2 for details. No significant difference was obtained among the treatments when compared on daily basis (Duncan's multiple range test, $P = 0.05$).

tive macropters when compared with their presumptive brachypterous counterparts.

Our results show that JH application enhances ovarian growth and confirms that JH is required for oocyte maturation in *N. lugens* just like in many other insects. For example, we have reported that precocene II treatment within the sensitive nymphal stages and shortly before adult emergence caused retardation in ovarian development in the presumptive brachypters of *N. lugens* that are especially known for their high ovarian development (Ayoade et al., 1996a, b). Feinsod and Spielman (1980) also reported the restoration of normal previtellogenic follicular growth by juvenile hormone treatment in *Aedes aegypti* females which have been reared previously in crowded larval cultures and then starved as adults in order to induce ovarian diapause—a common phenomenon in this species when reared under this condition. Moreover, in aphids, JH and their analogs have been reported to increase reproduction (Kohno and Takaoka, 1977) and total fecundity (Hardie and Lees, 1985).

Works are now in progress to further support different stage-specific activities of corpora allata to secrete juvenile hormone between these two genetically defined strains, activities which are reflected in regulation of wing-formation and ovarian development.

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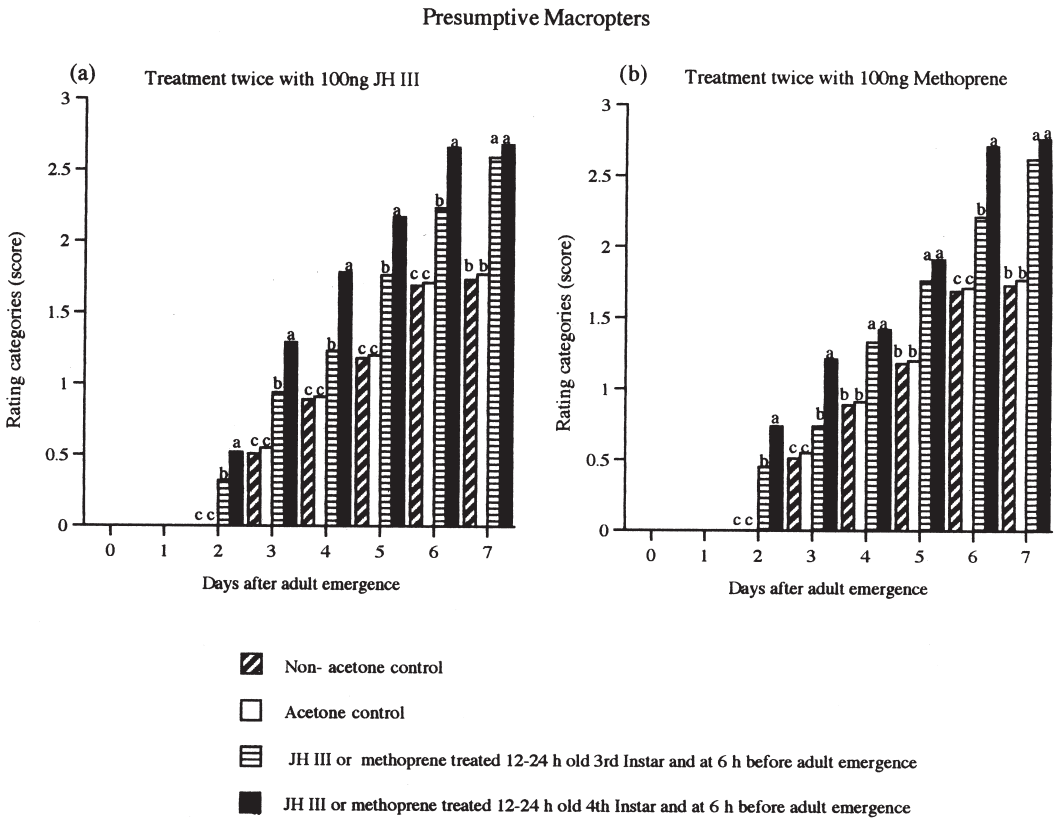

Fig. 6. Effects of JH III or methoprene treatment on ovarian growth in the presumptive macropterous strain. The hoppers were treated with 100 ng JH III (a), or methoprene (b) twice within 12 h of the 3rd or 4th nymphal stadium and 6 h before adult emergence as given in the legend. The rating categories were adapted from Iwanaga and Tojo (1986): see Section 2 for details. Values with the same letters are not significantly different (Duncan's multiple range test, $P = 0.01$).

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