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Induction of Macroptery, Precocious Metamorphosis, and Retarded Ovarian Growth by Topical Application of Precocene II, with Evidence for its Non-systemic Allaticidal Effects in the Brown Planthopper, *Nilaparvata lugens*

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Two strains of the brown planthopper, *Nilaparvata lugens*, exhibiting predominantly macropterous or brachypterous wing form in a broad range of population densities were topically treated with precocene II at different nymphal stages. The developmental profile, wing dimorphism and ovarian growth were investigated to know the effects of reduction/prevention of juvenile hormone synthesis by precocene II application on these characteristics. The results demonstrated that the sensitive periods to precocene II treatment for induction of precocious metamorphosis differed between the two strains: 2nd and early 3rd nymphal stadia in the presumptive brachypterous strain, while the 3rd and 4th (penultimate) nymphal stadia were sensitive in the macropterous strain. All of the precocious metamorphosis was induced in the 4th stadium and a dose-dependent response was observed for the disruption of metamorphosis. Changes in wing-form by precocene II treatment were observed only in the presumptive brachypterous strain. There was an overlap in the sensitive periods necessary for the disruption of metamorphosis and the induction of long wing forms in the presumptive brachypters, although the sensitive period for the disruption of metamorphosis was shorter. Macroptery induction was more prominent in males than in females. These results support that endocrinological events proceed differently from 2nd stadium between the two strains, and precocene II indirectly exerts its effects in the penultimate and last nymphal stadia to reduce juvenile hormone titer to change genetically moderated events; but the titer being too high in the penultimate stadium of the presumptive brachypterous strain to cause observable change(s) when precocene II was applied at this stadium.

Moreover, our results showed that a single application of precocene II at the 2nd, 3rd, 4th or 5th stadia failed to have any effect(s) on ovarian growth, although metamorphosis and wing dimorphism were disrupted. When the chemical was applied twice before adult emergence, ovarian growth was reduced drastically in the insects in which the two applications were carried out in the later stadia (4th and 5th). The lack of any effect(s) on ovarian growth in the single applications suggest that precocene II is not a systemic allatocidin but acts only to suppress the activity of the *corpora allata* and does not destroy it entirely.

Precocene Precocious metamorphosis Wing dimorphism Ovarian growth *Nilaparvata lugens*

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INTRODUCTION

Juvenile hormone has long been shown to be involved in metamorphosis (Novak, 1975), and to induce wingless or brachypterized wing forms when the titer is high at a specific period during development (Nijhout and Wheeler, 1982; Hardie and Lees, 1985). However, only a few cases of such effects of juvenile hormone on wing polymorphism have been reported such as in *Aphis fabae* by Hardie (1981), in *Nilaparvata lugens* by Iwanaga and Tojo (1986), and in *Gryllus rubens* by Zera and Tiebel (1988).

As a consequence of the discovery of the antiallatotropins better known as precocenes (a name derived from their ability to induce precocious development in insects) by Bowers (1976), it became possible to induce chemical allatectomy. The effects of the precocenes on processes such as sex pheromone synthesis inhibition, diapause induction, ovicidal activity and so on have been reported (Bowers 1976; Bowers *et al.*, 1976; Pratt, 1983), moreover, precocenes have also been reported to promote wing development in a few species of aphids (Mackauer *et al.*, 1979; Kambhampati *et al.*, 1984). However, because of the rather complicated effects of precocene on wing formation and metamorphosis in several aphids, (Hardie, 1986, 1987) pointed out the need to be wary of the presumption that precocene induces alate progeny because of anti-JH effects.

Wing dimorphism in the brown planthopper, *Nilaparvata lugens*, consisting of long-winged macropterous, and short-winged brachypterous adults is known to be caused by population density experienced during the nymphal stage (Kisimoto, 1965). In females, crowding increases the relative proportion of macropterous hoppers, while in the males, a moderate nymphal density promotes the maximum proportion of brachypterous form (Kisimoto, 1965). The sensitive period for the density effects has been shown to be in the 2nd, 3rd and also in the 4th (penultimate) larval stadia (Kisimoto, 1965; Iwanaga and Tojo, 1986).

Because of the inherent variation in wing-form response to density among field collected *N. lugens* populations (Iwanaga *et al.* 1985; Morooka *et al.*, 1988), it is imperative that strains in which wing-form expression is not greatly influenced by density are used in experiments designed to examine the involvement of juvenile hormone in wing formation. Recently, two strains predominantly exhibiting a specific wing form, brachypterous or macropterous, at wide range of densities have been generated by successive selections for adults with specific wing form (Morooka and Tojo, 1992). These special strains enable us to study genetically-based wing polymorphism as opposed to environmentally-induced polyphenism which has been the focus of most previous studies. This is the first time genetically-based polymorphism is being studied in *N. lugens*; the only other known species for which this has been done is *Gryllus rubens* (Zera and Tiebel, 1988, 1989).

The aim of the present paper is to investigate the effects of precocene II on metamorphosis, the hormonal basis of genetically-mediated wing dimorphism and ovarian growth in *N. lugens* and to establish the sensitive period.

MATERIALS AND METHODS

Insects

The experimental insects (*Nilaparvata lugens*), ca. 500, were kept in cages (11 x 12 x 18 cm) containing rice seedlings (variety Reiho) under a 16L:8D photoperiodic regime at 25 ± 2°C (Morooka *et al.*, 1988). The two strains used in the study were obtained from the stocks generated by Morooka and Tojo (1992), by successive selections of adults with specific wing form and color obtained under highly crowded conditions over 70 generations. The brachypterous and macropterous strains, selected for yellowish brown brachypters and blackish macropters, respectively, produced about 100% short-winged and 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.

Precocene treatment

Precocene II (Sigma Chemical Company) was dissolved in acetone to yield 10 μg/μl stock solutions. These were serially diluted with acetone at the time of each application and applied using a syringe (Terumo) and a micro applicator (Burkard) at concentrations of 1,10,100 and 1000ng dissolved in 0.1 μl of acetone to the abdomen of synchronized 2nd, 3rd, 4th and 5th larval stadium insects within 6h or 12h of the molt, unless otherwise stated, after being anaesthesized with CO_2 for 30s. After treatment they were reared on a rice seedling in a glass cylinder (2cm diam. x 20cm tall) at a density of 5 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. Some individuals that emerged as adults with fully developed *genitalia* but missing one of the nymphal stadia were noted as precocious. Twenty five individuals were treated at each dose; 5 replicates containing 5 insects per cylinder. After adult emergence, effect(s) of precocene II 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 7 insects were dissected each time in order to obtain an average. This meant that 49 insects were required for each treatment, i.e. ca. 7 female adults over 7 days; to make up for the shortage in treated insects, data was collected in batches from several repetitions of the experiment. 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.

RESULTS

Effects of precocene II on the development profile

When precocene II was topically applied to presumptive brachypters, the responses of the hoppers differed depending on the nymphal stage at treatment (Figs 1–3). Figure 1 shows a progressive prolongation of the 2nd nymphal stage from 6 to 18 h as the dosage treatment of 6 h old 2nd stadium nymphs increased, with a corresponding omission of the 5th stadium, starting from the 1ng treatment level, i.e. the hoppers molted from the 4th stadium straight into the precocious adult form. Although the treatments did not alter the duration of the 3rd stadium, the duration of the 4th stadium of these precociously metmorphosed animals became shortened with increasing dosage; by 16 h at the 1000 ng dosage level. As the total of these effects, precocene treatment led to a reduction in the average number of hours required for the insects to emerge as adults by about 24–36 h. Dosage above 1000 ng resulted in such a high percentage of mortality that made evaluation for effects impossible (data not shown). Similar results were obtained for the presumptive brachypters treated within 6 h of the 3rd stadium where the 5th nymphal stadium disappeared at the 100 ng dosage level with a corresponding prolongation of the 4th stadium duration (Fig. 2), while the treatment of 6–12 h old 3rd stadium nymphs did not induce any precocious metamorphosis, despite a progressive prolongation of the 3rd stadium duration (Fig. 3). The insects could not be induced to molt into the adult stage directly from the 4th stadium even when the dosage was increased to 10 μg; high mortality rates made further dosage increase impracticable (data not shown). No effects were observed on development profiles for the presumptive brachypters treated in the 1st, 4th and 5th stadia (data not shown).

Sensitive stages to precocene II for the interference with metamorphosis in the presumptive macropterous hoppers differed from those of presumptive brachypterous hoppers. No effects were observed on metamorphosis for presumptive macropters treated at the 2nd stadium (data not shown). On the other hand, the presumptive macropters treated with 1ng precocene II within 6–12 h of the 3rd nymphal stadium exhibited a prolonged 3rd stadium period with a corresponding shortening of the duration of the 5th stadium, and the 5th stadium eventually disappeared, resulting in emergence of precocious adults from the 4th nymphal stadium from the 10 ng dosage level (Fig. 4). The same results were obtained when 0–6 h old 3rd stadium nymphs of the presumptive macropters were treated with precocene II (data not shown). Figure 5 shows similar results for the treated 6 h old 4th stadium presumptive macropters, and a dosage of 10 ng was also sufficient to induce precocious metamorphosis, while in the treated 12 h old 4th stadium presumptive macropters, 100 ng was necessary for altering metamorphosis (Fig. 6).

Effect of precocene II treatment on wing form

Precocene II also induced macropterous wing form in the presumptive brachypterous hoppers, where the hoppers were treated at the 2nd or 3rd stadium (Figs 1–3). Even 1 ng applied to 12 h old 3rd stadium nymphs was effective and induced 20–60% macropters, while even 1000 ng dosage could not induce precocious metamorphosis in the treated hoppers (Fig. 3). In these two stadia, precocene II treatment caused a gradual reduction in the percentage of hoppers that emerged as brachypters, i.e. presumptive brachypterous hoppers were induced as macropters in a dose-dependent manner. In all the treatments (1–1000 ng), the males were more sensitive and more males emerged as macropters than females. Precocene treatment of presumptive brachypters in the 1st, 4th and 5th stadia shortly after ecdysis did not induce any macropterous adults. None of the presumptive macropters emerged as brachypters, moreover, there were no differences between the treated hoppers and the control in relation to wing form, except for the generally reduced body size in the precocious adults of the presumptive macropters. On the other hand, precocene-induced macropters from presumptive brachypters in addition to reduced body size also exhibited a darker body coloration when compared with the untreated hoppers of both macropterous and brachypterous strains (Figs 7a–d). Figures 7a and b show typical examples of untreated presumptive adult macropters and brachypters, respectively, while Figs 7c and d show typical examples of precociously emerged precocene II treated presumptive adult macropters and brachypters, respectively. As shown in Fig. 7e, another feature observed in less than 10% of the precociously emerged adults was the malformation of wings. In these hoppers, the wings seemed to develop but were so malformed that they were apparently useless for flight.

Effects of precocene II on ovarian growth

There was no significant difference in ovarian growth between the control and the precocene II treatments when the hoppers were treated at a dosage level of 100 ng once at any of the nymphal stages from the 1st to the 5th stadia for both the presumptive brachypters and macropters (Figs 8a and b). Ovarian growth started a day later in the

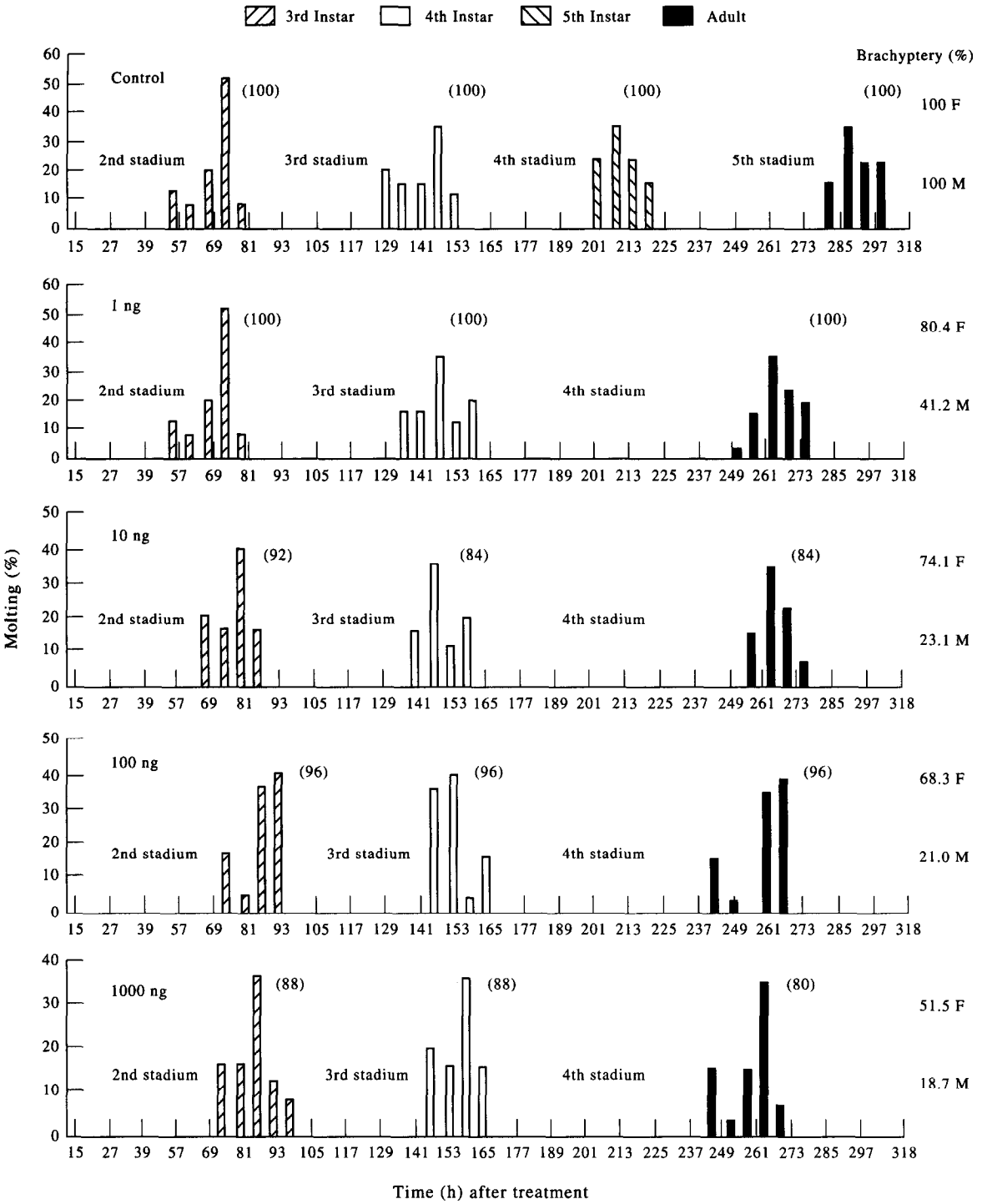

FIGURE 1. Molting processes and wing-form expression in the presumptive brachypters treated with precocene II about 12 h after molting to 2nd nymphal stadium. Precocene II was dissolved in 0.1 μl acetone, and topically applied to each animal. Control insects were treated with 0.1 μl acetone. 25 animals were treated at each dosage. Five animals were enclosed in a test tube (2 x 20 cm height) with a rice seedling, and reared at 25 ± 2 °C under 16 h L:8 h D photoperiod. Molting was checked every 6 h. M and F represent percentage of brachypterous male and female adults that emerged from the treated nymphs, respectively. Percentage survival for each treatment are given in parentheses.

macropters than in the brachypters, likewise the brachypters attained >2.5 score for ovarian growth by the 6th day after adult emergence, while the macropters had scores <2.5 even by the 7th day after adult emergence. It should be noted that some of the same hoppers used in this evaluation already exhibited the disruptive effects of the same precocene treatments on metamorphosis and wing formation (Figs 1–7). On the other hand, Figs 8c and d show that a second treatment of 100 ng precocene II to the hoppers approximately 6 h before adult emergence drastically reduced ovarian growth in the hoppers to which the previous application was carried out, especially in the later stadia (4th and 5th).

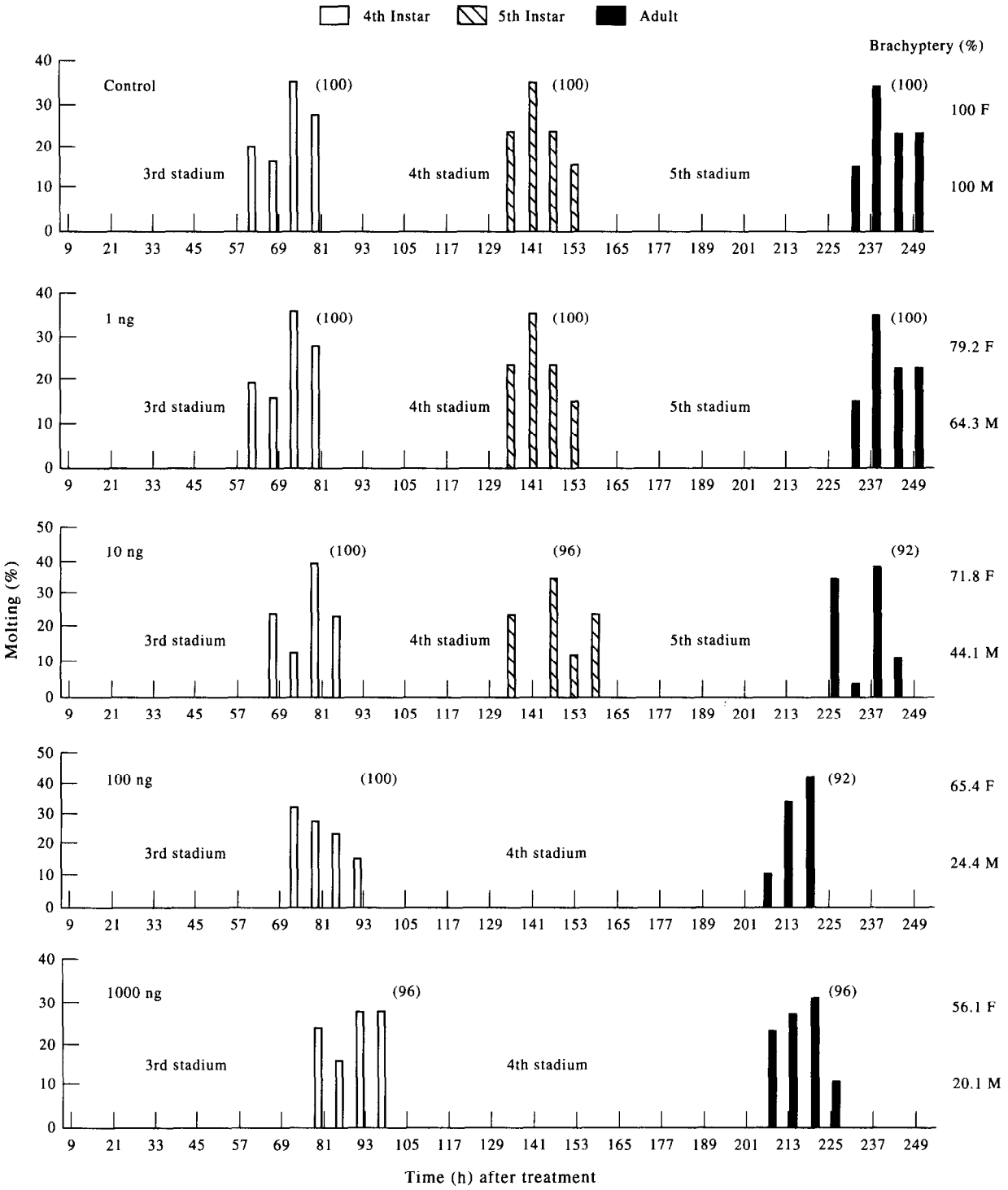

FIGURE 2. Molting processes and wing-form expression in the presumptive brachypters treated with precocene II about 6 h after molting to 3rd nymphal stadium. See Fig. 1 for details.

DISCUSSION

In this article we have demonstrated the induction of precocious metamorphosis in both strains, and also the long wing formation from presumptive brachypterous hoppers by topical application of precocene II. In the presumptive brachypterous strain, the formation of macropterous wing form was enhanced more prominently in males than in females, with increasing dosage even from 1ng up to 1000 ng of precocene II, in all cases when the nymphs were treated at 6 h of 2nd stadium, 6 h or 12 h of 3rd stadium (Figs 1–3), but no effects were observed when nymphs in other developing stages were treated. As no single treatment of precocene II could induce macropterization in all of the treated hoppers, a longer period of precocene II persistence around 2nd and 3rd stadia seems to be necessary to exhibit its overt effect on wing formation.

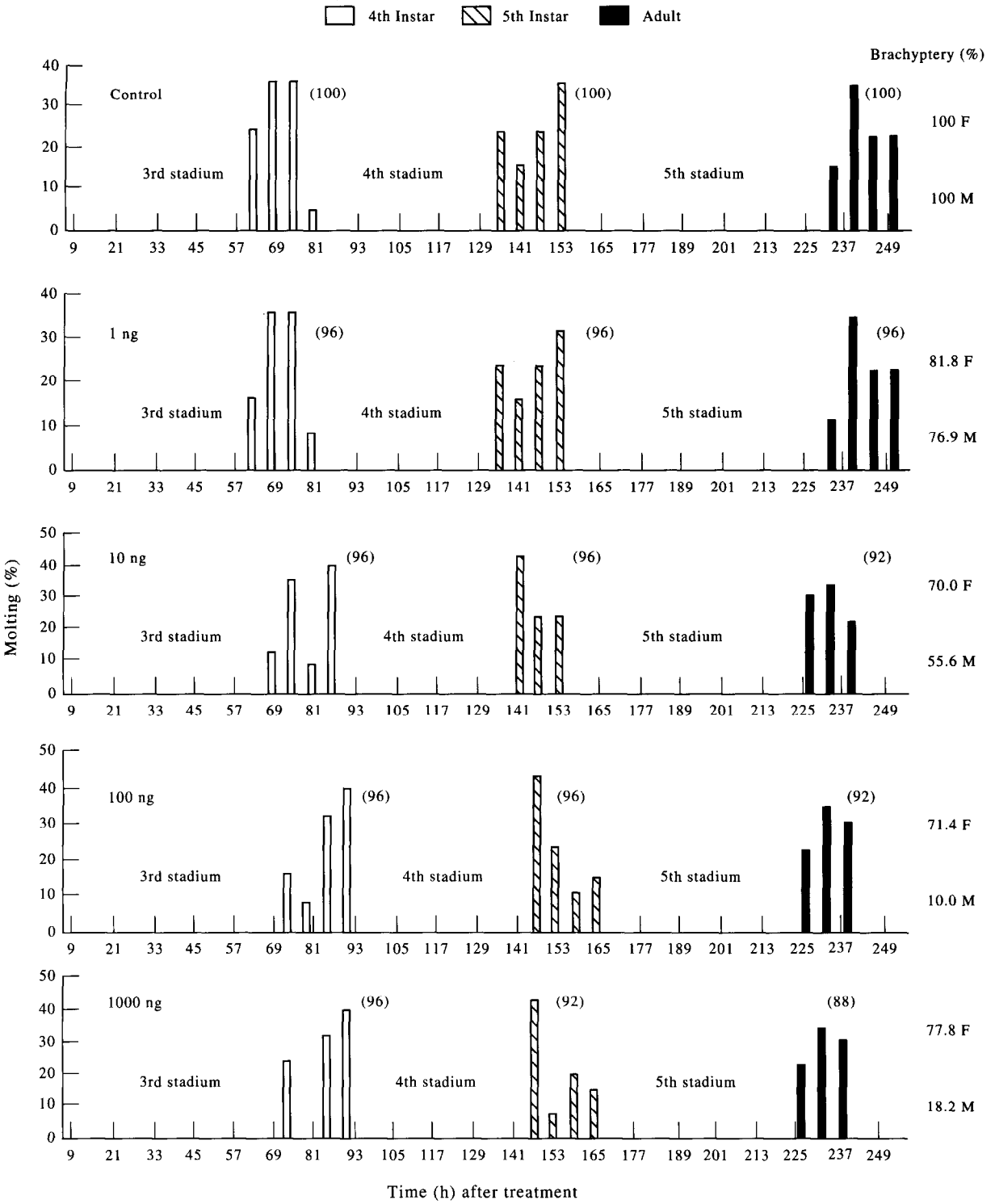

FIGURE 3. Molting processes and wing-form expression in the presumptive brachypters treated with precocene II about 12 h after molting to 3rd nymphal stadium. See Fig. 1 for details.

In a previous work (Iwanaga and Tojo, 1986), juvenile hormone was shown to increase the proportion of brachyptery in a strain of *N. lugens* that was mostly macropterous in the previous generations when nymphal density was increased during the 4th stadium. It was also demonstrated that isolated rearing starting from late 4th stadium prominently increased the ratio of brachyptery, but isolation from the 5th stadium did not show such influence (Iwanaga and Tojo, 1986). These facts support the idea that juvenile hormone titer above a threshold level shortly before the last nymphal ecdysis determines the induction of brachypterous wing form, and precocene II works to induce long wing forms by lowering the titer under a threshold level during this period. But the mechanism of juvenile hormone titer reduction during this period, probably indirectly caused by reduction of *corpora allata* activity around the 3rd stadium, remains unclear.

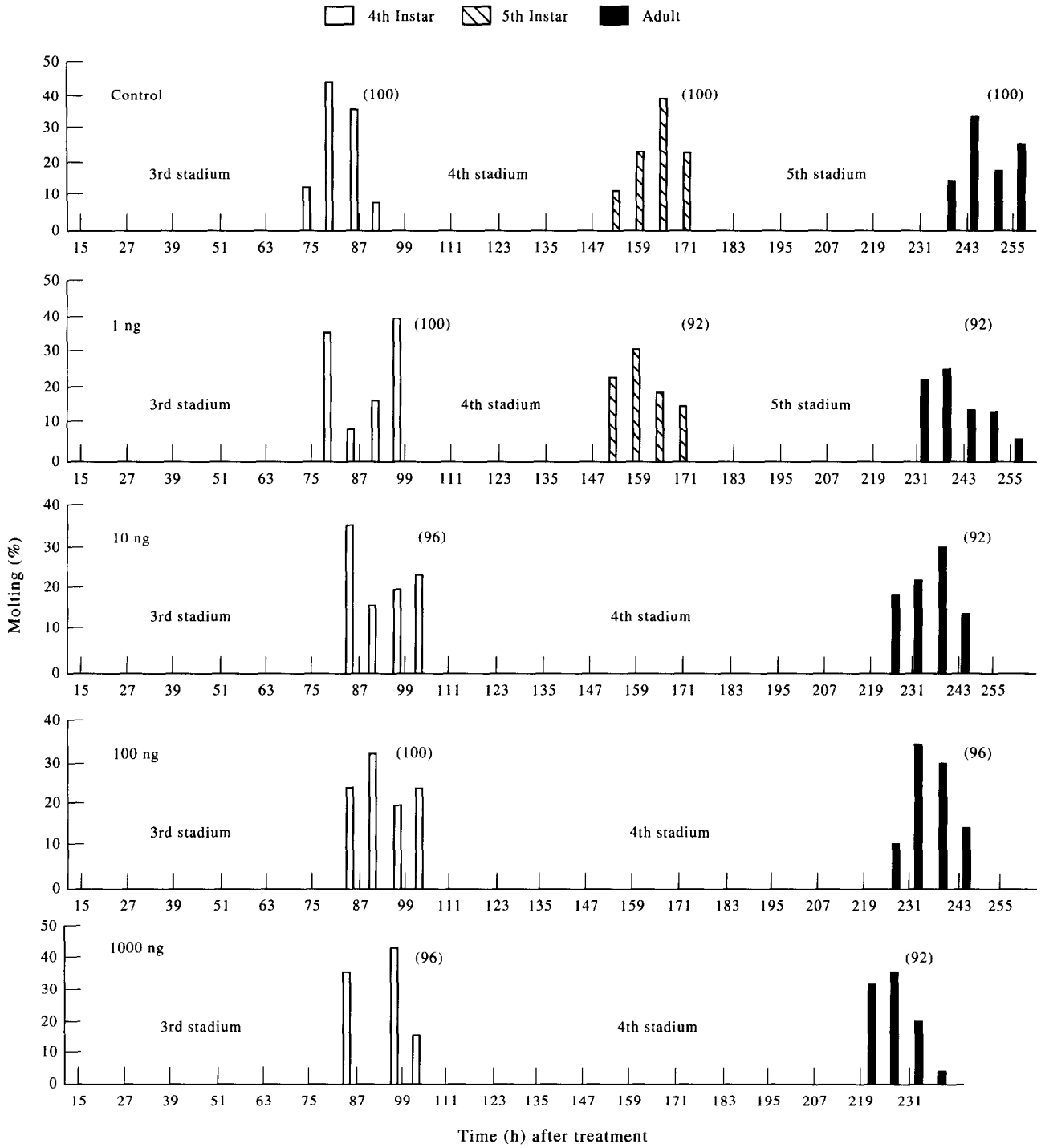

FIGURE 4. Molting processes in the presumptive macropters treated with precocene II about 12 h after molting to 3rd nymphal stadium. See Fig. 1 for details.

Although the genetic basis of wing dimorphism in insects is not well understood, studies on a wide variety of insects suggest that although it may in some cases be inherited as a simple Mendelian character, in others it is polygenic (Harrison, 1980; Roff, 1986). Morooka (1992) demonstrated in *N. lugens* that wing form is controlled by two sets of major genes; one on a locus in an autosome, and the other on the X sex chromosome and that the genes responsible for brachyptery function in an additive way. For example, if (b) and (m) represent the brachypterous and macropterous alleles, respectively, wing length becomes shorter with an increase in the proportion of b alleles. The sex chromosome type in *N. lugens* is known to be XX for females and XY for males; four b genes in females and three b genes in males are presumed to be involved in an individual hopper of the presumptive brachypterous strain used in the present study (Morooka, 1992); the extra b gene in the female hoppers possibly works to induce more active secretion of juvenile hormone from the *corpora allata*. This agrees with the pre-

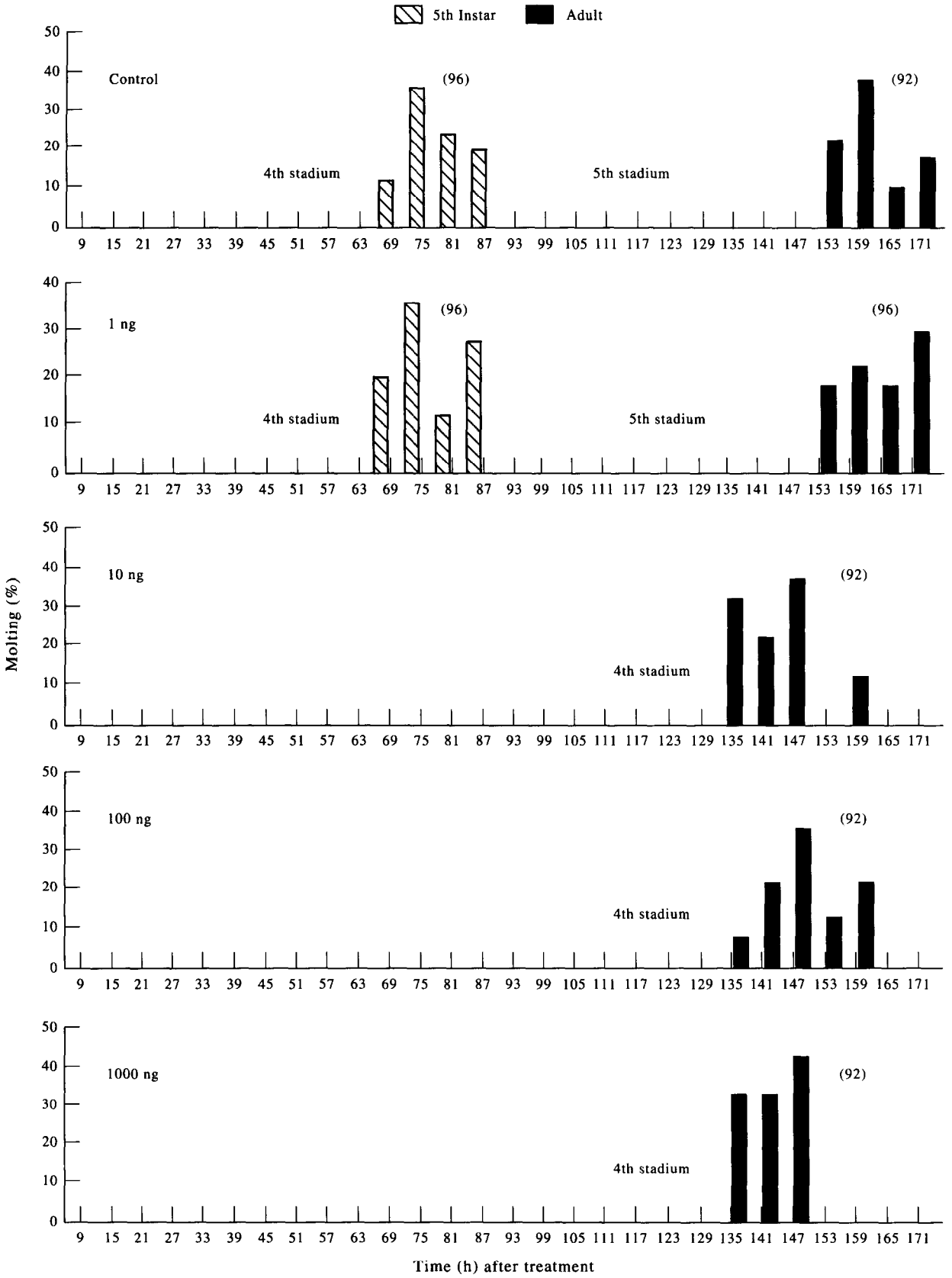

FIGURE 5. Molting processes in the presumptive macropters treated with precocene II about 6 h after molting to 4th nymphal stadium. See Fig. 1 for details.

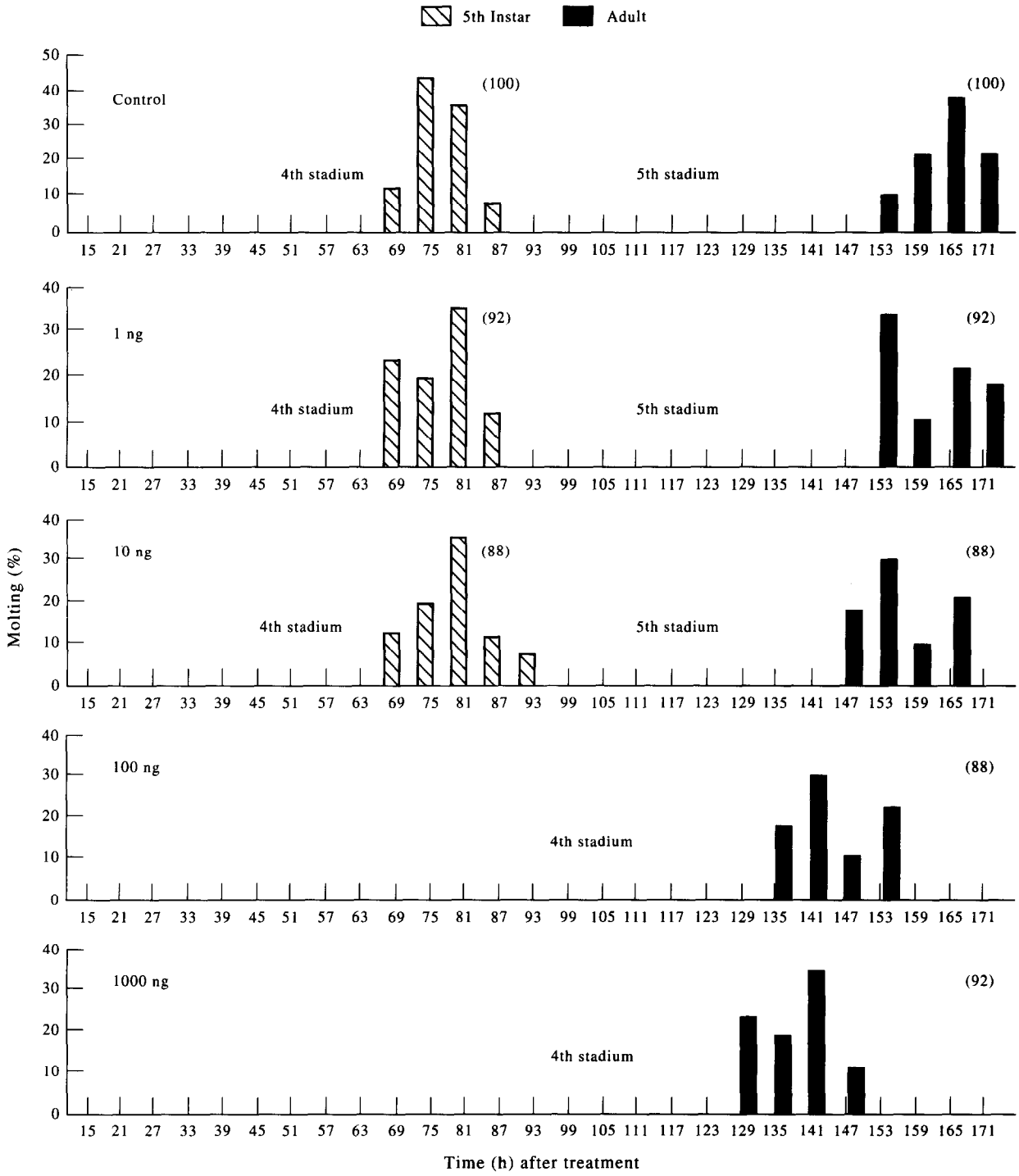

FIGURE 6. Molting processes in the presumptive macropters treated with precocene II about 12 h after molting to 4th nymphal stadium. See Fig. 1 for details.

sent results showing less sensitivity of the females compared to males of the presumptive brachypters to precocene II treatment for induction of long wing form.

According to Riddiford (1985), precocious metamorphosis results when ecdysone is released in the absence or during sub-threshold levels of juvenile hormone early in the feeding period of the penultimate larval stadium. Present results show that in the presumptive macropters, even 10 ng of precocene II treatment to the 4th stadium nymphs was effective to induce precocious metamorphosis (Figs 5 and 6); on the other hand, in the presumptive brachypters, no indication of disruption of metamorphosis neither by way of prolongation of stadium duration nor precocious metamorphosis was observed after precocene II application even at 10 μg (data not shown). These results strongly support the hypothesis that the brachypterous strain possesses such a high activity of *corpora allata* for juvenile hormone secretion

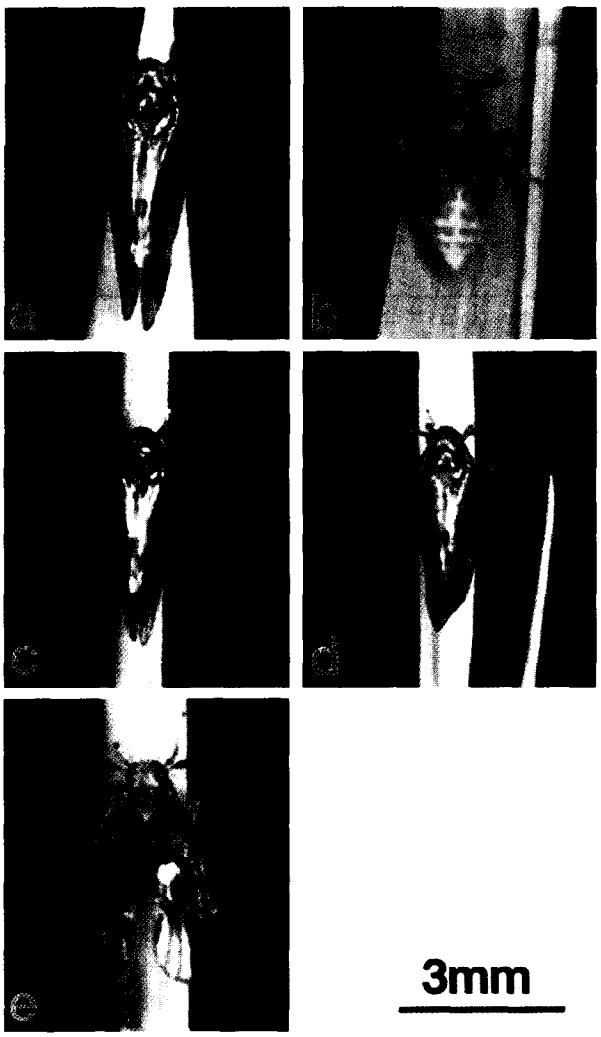

FIGURE 7. Photographs of *N. lugens* females showing non-treated and precociously emerged precocene II treated presumptive macropters and brachypters, and a malformed presumptive macropter. a: Acetone-treated presumptive macropter, b: Acetone-treated presumptive brachypter, c: precociously emerged presumptive macropter, d: precociously emerged presumptive brachypter, e: malformed presumptive macropter.

at the early penultimate nymphal stadium which could not be suppressed even by large doses of precocene II.

Precocene II-induced precocious metamorphosis in the presumptive brachypters when applied in the 2nd or early 3rd nymphal stadium, while in the presumptive macropters, precocene II caused precocious metamorphosis when applied in the 3rd or 4th nymphal stadium. The dose necessary to disrupt the metamorphosis increased as the hoppers were treated in a later nymphal stage (Figs 1–6). Thus, the sensitive periods to precocene II for disruption of metamorphosis differed between the two strains, supporting also that the endocrinological events proceed differently between the two strains even during the 2nd and 3rd stadia. The fact that all of the precocious metamorphosis was induced starting from the 4th nymphal stadium even when precocene II was used in earlier nymphal stadium is unexpected. The mechanism to induce precocious metamorphosis from the 4th stadium remains obscure, but it seems to be possible that precocene II functions to reduce *corpora allata* activity during the sensitive period, which indirectly triggers the organs to secrete juvenile hormone under a threshold level to allow larval-imaginal commitment in the penultimate stadium.

Alate progeny has been shown to be induced from apterous adults of several aphid species prenatally treated with precocene I, II or III (Mackauer *et al.*, 1979; Delislie *et al.*, 1983; Kambhampati *et al.*, 1984; Hardie, 1986, 1987). In some of these aphids, potencies of precocene analogues for induction of alate do not coincide with those for precocious metamorphosis; these results have raised doubts about the presumption that precocene promotes wing development because it decreases juvenile hormone titers (Hardie, 1986, 1987). The effects of precocene in these aphids seem to differ from those in *N. lugens*, where precocene II exhibited both effects to promote long wing formation and block metamorphosis in a dose-dependent way, with 1 ng providing an effective dose in some cases. In the aphids used in the above-mentioned studies, whose sizes were much smaller than those of *N. lugens*, minimum effective dosage to induce any effect was 1 μg or more. In *N. lugens*, there have been discrepancies: a single application of precocene II to early 2nd stadium presumptive brachypters could induce precocious metamorphosis in all of them, initiated from 1 ng dosage, but macroptery increased with increasing dosage (Fig. 1) as in the case when 12 h old 3rd stadium hoppers of this strain were treated, but the treatment failed to induce any precocious metamorphosis even with 1000 ng of precocene II (Fig. 3). These results showing non-coincidental induction of the two events by precocene II may be realized by stage-specific sensitivities to precocene II, which indirectly reflect in reduction of juvenile hormone titer to influence respective affairs in different degrees in later nymphal stages. Work is in progress to support this hypothesis.

It is generally accepted that precocene shows allaticidal effects in hemimetabolous insects (Bowers, 1976, 1985; Pratt, 1983). However, our results in *N. lugens* demonstrating the ability of the hoppers treated with precocene II once during any nymphal stages to undergo ovarian growth at non-significantly different levels from the control hoppers (Figs 8a and b) even though metamorphosis and wing formation were disrupted, strongly support that precocene II was only able to suppress the activity of the *corpora allata* in the treated insects just enough to disrupt physiological activities at the nymphal stages, and was able to recover its activity and secrete the juvenile hormone necessary for ovarian growth after adult emergence. On the other hand, a second treatment at about 6h before adult emergence to hoppers previously treated in the 4th or 5th stadium drastically reduced ovarian growth in both strains of the hoppers as opposed to the mild reductions in ovarian growth observed in the hoppers that were treated at the earlier stadia (2nd and 3rd stadia; Figs 8c and d). The results suggest that the

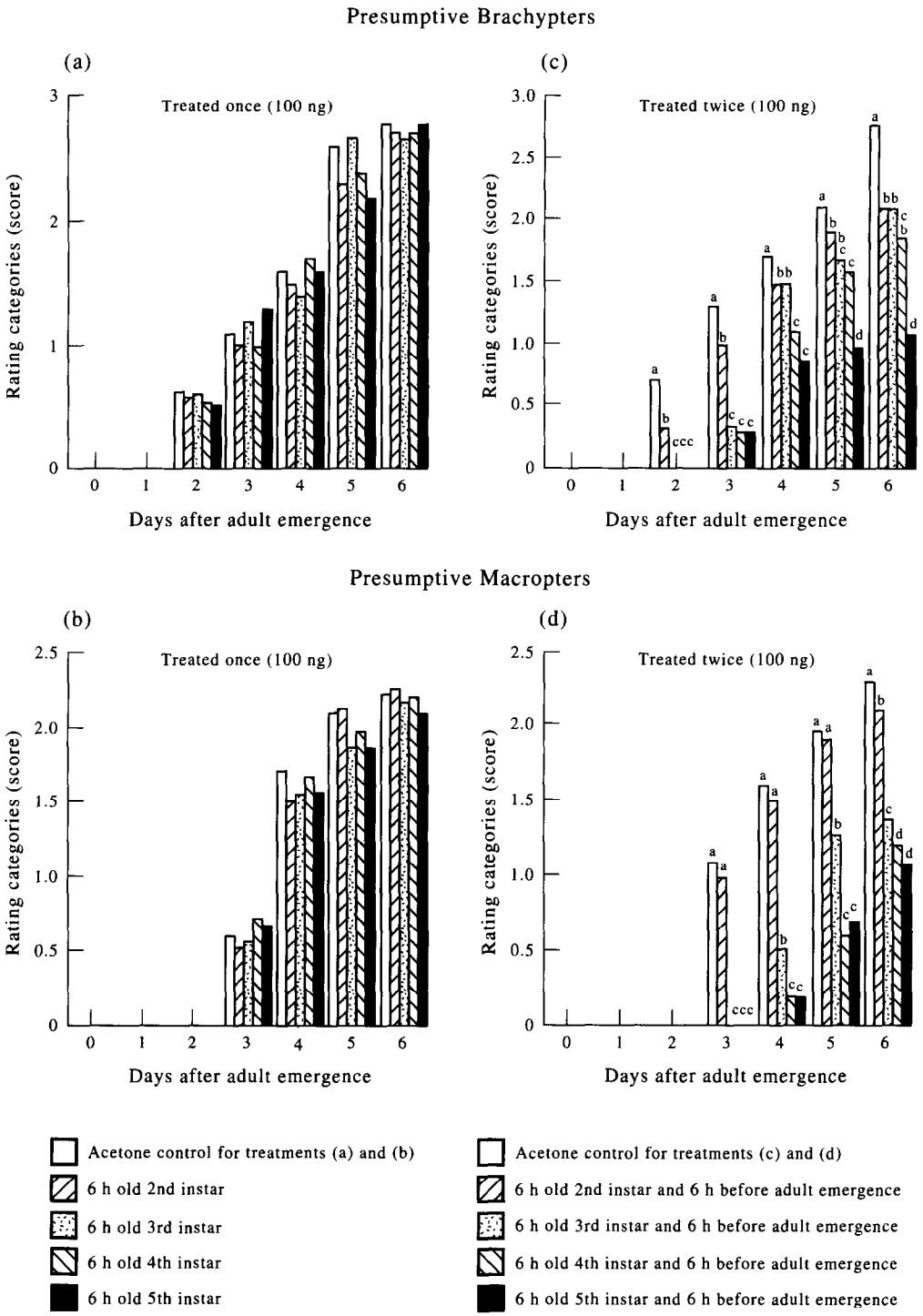

FIGURE 8. Effects of precocene II treatments on ovarian growth in the presumptive brachypterous and macropterous strains of *N. lugens* adults. The hoppers were treated with 100 ng precocene II, once (a, b) or twice (c, d). The times of treatments are given in the legend. The rating categories were adapted from Iwanaga and Tojo (1986): see MATERIALS AND METHODS for details. Values with the same letters are not significantly different (Duncan's multiple range test, $P = 0.05$).

corpora allata were able to recover partially when they were treated twice; first in the 2nd or 3rd stadium and then later 6 h before adult emergence, while they almost failed to recover when they were treated in the 4th or 5th stadium and subsequently 6 h before adult emergence. This evidence leads us again to be of the view that precocene acts only to suppress *corpora allata* activity but not as an allatocidin; the organs may revive after the precocene effects wear out by the time of adult emergence.

Topical application of a juvenile hormone analogue, methoprene, to the macropterous hoppers of *N. lugens* 24–36 h after adult emergence has been shown to enhance the ovarian growth up to the level in the bra-

chypterous hoppers (Iwanaga and Tojo, 1986). So, it seems to be reasonable to suggest that juvenile hormone titer during the adult stage differs between presumptive macropterous and brachypterous strains used in the present study; its earlier increase in the brachypters induced earlier growth of ovaries compared to the macropterous strain (Fig. 8), and precocene II treatment shortly before adult emergence retards the increase.

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