

METHOPRENE AFFECTS THE ROTATION OF THE MALE TERMINALIA OF *Aedes Aegypti* MOSQUITOES

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ABSTRACT. The rotation of the male terminalia of *Aedes aegypti* mosquitoes was investigated with chemical, surgical, and mechanical methods. Neither decapitation nor ventral nerve cord transection affected the post-emergence rotation, but methoprene administered to 4th-instar larvae and early instar pupae slowed rotation significantly and, in the case of pupal treatment, also interfered with successful insemination. Methoprene appeared to interfere with the developmental maturation of the cross muscles located between the 7th and 8th abdominal segment and postemergence nervous or humoral signals did not appear to affect this rotation.

INTRODUCTION

In *Aedes aegypti* (Linn.) mosquitoes, the terminalia of the male rotates 180° within 48 h after emergence, allowing "inverse correlation" to occur, in which the dorsum of the aedeagus becomes contiguous with the venter of the vagina when the genital conduit is formed (Lamb 1922, Roth 1948, Spielman 1964). Chevone and Richards (1976) described 2 pairs of opposing muscles found in the rotating region of *Ae. aegypti* that appeared to be the driving force of terminalia rotation. This rotation occurs in either a clockwise or counterclockwise direction, and apparently one or the other member of a set of muscles contracts, thereby determining the ultimate direction of rotation.

Spielman and Williams (1966) observed that when larval male *Ae. aegypti* were reared in the presence of sublethal doses of a juvenile hormone (JH) analog, they were unable to complete the 180° terminalia inversion that is required for successful mating as adults. In addition, Bouchard and Wilson (1987) demonstrated that a higher percentage of adult male *Drosophila melanogaster* (Meigen), when treated with sublethal doses of methoprene as larvae, were infertile, largely due to incomplete circumversion of the terminalia.

In this report, we examined the control of post-emergence terminalia rotation and demonstrate that sublethal doses of methoprene slowed this rotation and prevented successful mating with females when applied during a critical period in the pupal stage.

MATERIALS AND METHODS

Mosquito strain and rearing: *Aedes aegypti*, UGAL strain, was used in all experiments. All stages were maintained at 27°C and 70–80% RH under a 14:10 (L:D) photoperiod. For most experiments, larvae were reared on a standard diet of rat chow, brewers yeast, and lactalbumin hydrolysate (1:1:1 by weight). Adults ingested 10% sucrose solution through cotton wicks. Male and female pupae were separated by size, and males were select-

ed for treatment. For experiments involving larval methoprene treatment, 25 4th-instar larvae were transferred to 100 × 25-mm petri dishes with 50 ml of water (tap water deionized water, 1:1) and a dilution of methoprene. In these experiments, Tetramin® (TetraWerke, Melle, Germany) fish food was used as the nutrient source.

S-Methoprene (a gift from Sandoz Agro, Inc., Dallas, TX) was diluted in acetone and topically applied to pupae and adults in concentrations of from 6×10^{-9} to 8.2×10^2 pg. Fourth-instar larvae were treated with methoprene added to rearing water at concentrations of from 0.001 to 5.0 ppb. The larval median lethal concentration (LC₅₀) was 0.05 ppb. Controls were treated with acetone alone.

Measurement of terminalia rotation: Terminalia rotation was measured by placing the males under a stereomicroscope fitted with a video camera. The degree of terminalia rotation at various times after emergence was measured on the video monitor using a protractor placed against the screen.

Surgical manipulations: Newly emerged males were decapitated and the wound was sealed with paraffin wax. Controls were etherized only at 1 h after emergence. The rotation of the terminalia of treatments and controls was measured at 10 and 24 h.

The terminal abdominal ganglion was removed by inserting forceps into the ventral portion of the intersegmental membrane between the 7th and 8th abdominal segments, grasping it, and cutting all connectives. Controls were newly emerged males with only their intersegmental membrane cut. The intersegmental membranes were then sealed with wax. The degree of terminalia rotation was recorded after 24 h for both the treatments and the controls. The same procedure was followed when the ventral nerve cord was transected except that the entry point to sever the ventral nerve cord was the intersegmental membrane between the 2nd and 3rd abdominal segments.

To prevent the terminalia of newly emerged mosquitoes from rotating, a drop of molten wax was placed on the intersegmental region between the 7th and 8th abdominal segments 1 h after adult emergence. Controls had their terminalia waxed 48 h following emergence after full rotation was com-

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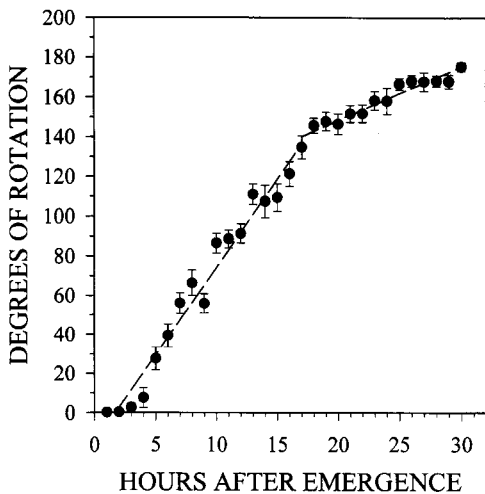


Fig. 1. Mean degree of terminalia rotation as a function of time after adult male emergence. Each point represents the average degree of terminalia rotation \pm sem from 1 to 30 h after adult emergence of a minimum of 20 mosquitoes. Both lines represent first-order regression correlating the average degree of terminalia rotation with time; 1–18 h: slope = 8.92, $r^2 = 0.97$; 18–30 h: slope = 2.50; $r^2 = 0.94$.

pleted. The drop was small enough so that it did not interfere with mating. Two males of either control or treatment groups were placed in a cage with one unmated female for 3 days and the female was examined for the presence of sperm in the spermathecae.

When 3 or more treatments were analyzed that

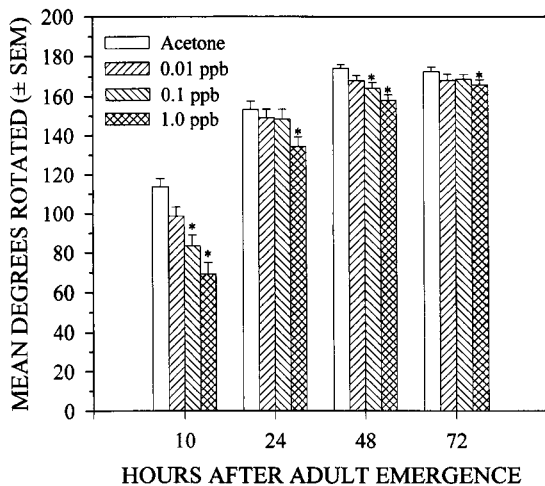


Fig. 2. Mean degree of terminalia rotation of males raised in the presence of methoprene during the 4th instar. Rotation was observed and recorded at 10, 24, 48, and 72 h. Each bar represents the responses of at least 50 mosquitoes. An asterisk above the bars represents a significant difference from acetone-treated controls.

were not normally distributed, the Kruskal–Wallis one way analysis of variance (ANOVA) on ranks was used. If this test found a significant difference among the treatment medians, a multiple comparison of the groups was performed with the Dunn's test either versus the control group or an all pairwise analysis among all the treatments. The Mann–Whitney rank sum test was used when 2 treatments were being compared and the groups did not have a normal distribution. If 2 groups had a normal distribution, the Student's *t*-test was used. The *z*-test was used when qualitative proportional data were compared. A *P* value of <0.05 was considered significant in all the above comparisons. Statistics were performed with the statistical program SigmaStat (Jandel Scientific, San Rafael, CA).

RESULTS

We first examined the rate of terminalia rotation in untreated *Ae. aegypti* males over a period of 30 h. Because the rotation occurred nonlinearly, 2 regression curves were computed. Between 1 and 18 h after emergence, rotation occurred at a more rapid rate (slope = 8.92; $r^2 = 0.97$) than from 18 to 30 h after emergence (slope = 2.50; $r^2 = 0.94$) (Fig. 1).

To examine the possibility that either hormones or a nerve impulse from the brain initiated rotation, we first decapitated males at emergence. However, the rotation of the terminalia of the decapitated mosquitoes did not differ significantly from that of controls at 10 h ($P > 0.15$) or 24 h ($P > 0.15$), suggesting that the brain was not involved either by nervous or humoral pathways. The possibility that the ventral nerve cord carried a neural signal from the thoracic ganglion that caused initiation of rotation was also considered. When the ventral nerve cord was transected shortly after emergence, the terminalia rotation of treated males did not differ significantly ($P > 0.49$) from that of the sham-operated controls.

The terminal abdominal ganglion innervates several structures in the posterior abdominal segments of the female, and we examined the potential role of this ganglion in the regulation of male terminalia rotation. However, both the terminalia of males that had their ganglia removed, as well as the sham-operated controls, failed to rotate. This experiment suggested that the intersegmental membrane between the terminal segments must be intact for rotation to occur. Although the terminal abdominal ganglion may innervate the muscles that cause rotation, its importance could not be determined.

Because prior experiments had shown that methoprene affects rotation, we treated 4th-instar larvae with sublethal concentrations of the juvenile hormone analogue. As shown in Fig. 2, a dose of 1 ppb significantly ($P < 0.05$) slowed the rotation of the terminalia at 10, 24, 48, and 72 h compared to controls. The lowest dose of 0.01 ppb had no sig-

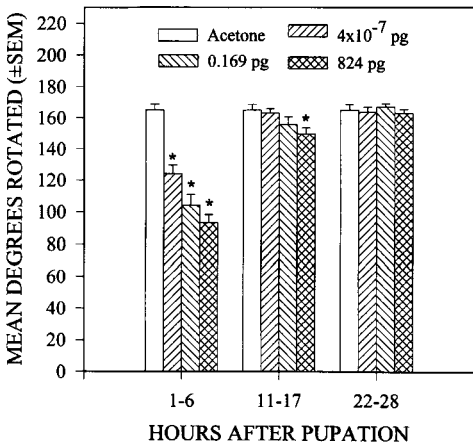


Fig. 3. Mean degree of terminalia rotation, recorded at 120 h postemergence, of male pupae of 3 age groups treated topically with methoprene. Each bar represents the responses of at least 50 mosquitoes. An asterisk above the bars represents a significant difference from acetone-treated controls.

nificant effect on rotation at any hour observed. At 10 and 48 h, 0.1 ppb had a significant ($P < 0.05$) effect on slowing the terminalia rotation of males.

We next topically applied methoprene during the pupal stage to determine whether the cross muscles involved in rotation were more sensitive to methoprene treatment at a particular time in their development. As shown in Fig. 3, when the pupae were treated early in their development (1–6 h), the rotation was significantly slowed ($P < 0.05$) at even the lowest dose compared to controls. When the

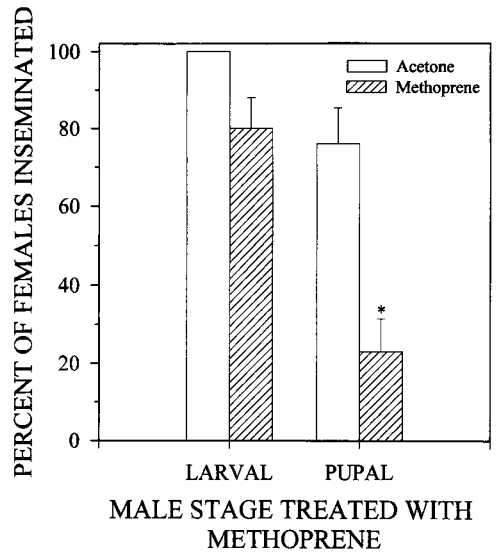


Fig. 5. Percentage of females inseminated by males that were treated with methoprene either as 4th-instar larvae (1 ppb) or as 1–6-h-old pupae (6×10^{-9} pg). Each bar represents the responses of at least 50 mosquitoes. The asterisk above the bar represents a significant difference from acetone-treated controls.

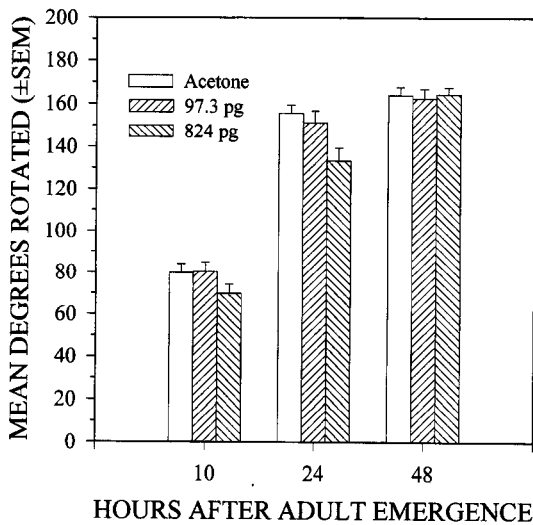


Fig. 4. Mean degree of terminalia rotation of males after methoprene was applied topically at 10, 24, or 48 h after adult emergence. Each bar represents the responses of at least 50 mosquitoes.

pupae were treated later in their development (11–17 h), after the cross muscles began to form, methoprene was less effective in slowing this rotation, with only the highest dose having a significant effect ($P < 0.05$). By 22–28 h, when the cross muscles were fully developed, rotation was unaffected. Consequently, it appeared that the early pupal stage is most sensitive to the methoprene treatment. When methoprene was topically applied to newly emerged adult males, there was no significant difference ($P > 0.05$) compared to the rotation of acetone-treated controls at 10, 24, or 48 h (Fig. 4).

To determine whether rotation was necessary for successful mating to occur, males treated at emergence with a drop of wax to fuse the abdominal segments were allowed to attempt to mate with previously unmated females. Although all control males waxed at 48 h after emergence successfully inseminated females ($n = 14$), none ($n = 14$) of the treated, unrotated males did so.

Having demonstrated that the prevention of rotation also prevented mating from occurring, we next examined whether the methoprene-mediated effects on rotation also affected mating. There was no significant reduction ($P > 0.05$) in the ability of males treated as larvae with as much as 1 ppb to inseminate virgin females at 72 h compared to controls (Fig. 5). In contrast, males treated with as little as 6×10^{-9} pg of methoprene were significantly less likely ($P < 0.05$) to inseminate females compared to controls.

DISCUSSION

Methoprene administered topically during the pupal stage significantly affected the rotation of the terminalia of newly emerged male *Ae. aegypti* and interfered with the insemination of females. There was a window of sensitivity of 1–6 h after pupation during which rotation was most affected by methoprene application. The time of methoprene sensitivity corresponded to the time at which the aggregation of myoblasts that form the rotational muscles during the early pupal stage occurred (Chevone and Richards 1976). Gordon and Burford (1984) proposed that methoprene disrupts the production of certain proteins in immature *Ae. aegypti* by altering the neuroendocrine activity of the insect, and it is possible that structural proteins involved in the development of these rotational muscles were affected by methoprene treatment. We did not examine the ultrastructure of the muscles in methoprene-treated insects to verify this. Treatment with methoprene after adult emergence, decapitation, or ventral nerve cord transection had no significant effect on postemergence terminalia rotation. Our decapitations and ventral nerve cord transections confirmed the conclusions of Hodapp (1960²), who also reported that terminalia rotation was independent of the head and thorax. Although the terminal abdominal ganglion innervates the genitalia, we could not determine its involvement in the initiation of rotation because sham-operated controls with a disrupted intersegmental membrane also failed to rotate.

We also demonstrated that males with unrotated terminalia, even in older males that were otherwise sexually mature, were unable to successfully copulate. We only maintained the positions of no rotation (0°) and full rotation (180°), and therefore could not conclude whether intermediate rotations might still allow insemination to occur. Roth (1948)

² Hodapp, C. J. 1960. The mechanism of terminalia rotation in male *Aedes aegypti* (L.). Masters thesis. University of Maryland, College Park, MD.

observed that *Ae. aegypti* males that rotated "about" 135° were usually not successful in copulating with females. Provost et al. (1961) reported that male *Aedes taeniorhynchus* (Wiedemann) that underwent a 90° rotation could still inseminate females when a forced mating technique was employed. We have demonstrated that although exposure to sublethal doses of methoprene in the larval stage slowed terminalia rotation of male *Ae. aegypti*, it was not sufficient to interfere with their mating. Topical application of methoprene to pupae during a sensitive period was more effective, but will unlikely play any role in control strategies.

ACKNOWLEDGMENTS

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