Effect of Juvenile Hormone and Juvenile Hormone Mimics on Sperm Transfer from the Testes of the Male Cat Flea (Siphonaptera: Pulicidae)

SUSAN R. DEAN AND ROGER W. MEOLA

Department of Entomology, Texas A&M University, College Station, TX 77843


ABSTRACT Sperm transfer into the epididymis was completed without a blood meal, when newly emerged male cat fleas, Ctenocephalides felis (Bouché), were exposed to filter papers treated with juvenile hormone III or the juvenile hormone mimics fenoxycarb, methoprene, or pyriproxyfen. As the concentration of juvenile hormone or the time of flea exposure to juvenile hormone or the juvenile hormone mimics increased, the percentage of fleas that transferred sperm also increased. The percentage of pyriproxyfen-treated males that transferred sperm reached 100% after 3 d; whereas, 7 d exposure to juvenile hormone, fenoxycarb and methoprene was required for 100% of the males to transfer sperm. Although sperm were present in the epididymis of treated fleas, insemination of females did not take place off the host either on juvenile hormone-treated filter paper or on juvenile hormone-treated dog hair.

KEY WORDS Ctenocephalides felis, juvenile hormone, juvenile hormone mimic, male maturation

IN FLEAS, THE tightly coiled vas efferens near the entrance to the testis is called the epididymis (Fig. 1). In many of the species studied thus far, the walls of the epididymis form a plug consisting of a layer of tightly folded columnar epithelial cells that prevent sperm from leaving the testes (Rothschild et al. 1986). As a result, unfed male fleas are often incapable of impregnating females even though mature sperm are present in the testicular follicles (Rothschild et al. 1970, Bai and Prasad 1979, Akin 1984). Male fleas therefore are considered to be physically immature as long as the epidermal plug remains intact (Rothschild et al. 1970).

Maturation of the male flea usually is associated with blood feeding. Studies of blood-fed male fleas, Spilopsyllus cuniculi (Dale), showed that the epithelial cells of the epididymis become flattened and cuboidal, forming a lumen through which sperm leave the testes (Rothschild et al. 1970, 1986) (Fig. 2). According to Rothschild et al. (1970), Xenopsylla cheopis (Rothschild), Nosopsyllus fasciatus (Bosc), and S. cuniculi require at least one blood meal before sperm are released from the testes. Akin (1984) reported similar results for male cat fleas, Ctenocephalides felis (Bouché). In contrast, Bai and Prasad (1979) demonstrated that the act of feeding itself may play an important role in male maturation. These authors found that ≈50% of the X. cheopis and Xenopsylla astia (Rothschild) matured when fed on saline rather than blood.

Rothschild et al. (1970) demonstrated that host hormones directly influence maturation of the rabbit flea, S. cuniculi. Significantly greater numbers of male rabbit fleas raised on pregnant does or newborn rabbits matured than those raised on buck rabbits. In addition, maturation of these fleas was faster on pregnant does or newborn rabbits than on buck rabbits. Males required only 96 h to mature on newborn rabbits versus up to 9 mo on buck rabbits. Accelerated maturity of fleas feeding on the pregnant doe or newborn rabbit was shown to be associated closely with increased host titers of estrogens and corticosterones in the host. Conversely, fleas feeding on buck rabbits were exposed to much lower titers of these hormones. Maturation of males feeding on buck rabbits could be hastened either by injecting corticosterones or estrogen into the blood of the host, or by spraying the fleas directly with cortisol, estradiol, or synthetic juvenile hormone.

The objective of this study was to investigate the role of juvenile hormone and juvenile hormone mimics in maturation of the male cat flea by determining whether external exposure of fleas to these chemicals would stimulate sperm transfer from the testes in the absence of blood feeding.

Materials and Methods

Laboratory Insects. Fleas were obtained from a colony originally supplied by Zoecon (Dallas, TX)
in 1987 and maintained in culture at Texas A&M University (College Station). Adult fleas for the colony were fed on 5 domestic cats housed in the Laboratory Animal Resources and Research facility on campus. Eggs collected daily were brought to our laboratory at the Center for Structural and Urban Entomology where they were sifted and placed on 30 g of larval rearing media made from 1 part dried bovine blood, 5 parts finely ground cat chow, and 0.05 parts powdered brewers yeast in 200 ml of fine sand. Cocoons were sifted from the diet and stored in plastic containers in an environmental chamber maintained at 25°C, a photoperiod of 14:10 (L:D) h, and a range of 70–80% RH. Adult fleas that emerged ≥ 14 d after initial cocoon formation were harvested for experimental use to ensure that approximately equal numbers of males and females were obtained.

Experiments Using Unfed Adult Fleas. Fleas were maintained on 7-cm filter papers (Fisher, Pittsburgh, PA). Each paper was treated with 1 ml of reagent grade acetone, (controls), an acetone dilution of juvenile hormone, or acetone dilutions of the juvenile hormone mimics, fenoxycarb, methoprene, or pyriproxyfen prepared from technical grade materials. Because the surface area of the filter paper was 38.5 cm², compounds were dissolved in acetone (42.35 μg/ml) to achieve a final dilution of 1.1 μg (AI)/cm². Three additional concentrations of juvenile hormone were dissolved in acetone solutions at concentrations of 423.5, 1,270.5, and 4,235 μg/ml to achieve final treatment concentrations of 11, 55, and 110 μg (AI)/cm², respectively. Treated filter papers were dried in a fume hood for a minimum of 2 h to allow evaporation of the acetone before fleas were placed on them.

Newly emerged adult fleas were obtained by breathing on or agitating the cocoons, which stimulated adult emergence. Adults were collected by vacuum aspiration into 8 groups of ≥80 fleas each. Groups of fleas were shaken onto control or treated filter papers that were placed on the bottom of pint canning jars. Jars were covered with organdy and placed in an environmental chamber maintained at 27°C and 70–80% RH. Approximately 15 male and 15 female fleas were removed from the control and each treatment group on the 3rd and 7th d and fixed for histological study.
Fig. 3. Histological section of an unfed flea showing folded columnar epithelial cells lining the epididymis. Epithelial cells fill the lumen preventing sperm from leaving the testis.

Fig. 4. Histological section of an unfed pyriproxyfen-treated flea. Columnar epithelial cells lining the epididymis have been transformed into squamous epithelia. Newly formed lumen (*) in the epididymis allows sperm to leave the testis. Bar = 20 μm. E, epithelial cell; Ep, epididymis; T, testis.

To ensure that the environmental requirements favorable for mating were met, we held unfed fleas on 1-g increments of dog hair in plastic cages similar to those used by Pullen and Meola (1995). Fed fleas maintained in these cages at 37°C mated readily in previous experiments. Four groups of 40 fleas (2 cages of controls and 2 cages of treated fleas) were maintained in an environmental chamber at 27°C and 70–80% RH. Dog hair lining the cages was treated either with acetone (controls) or acetone containing 70 μg juvenile hormone III per gram of hair (70 ppm). At 3 and 7-d after treatment, =15 male and 15 female fleas were removed from the treatment and control groups and fixed for histological study.

**Histology of Adult Fleas.** Adults were fixed in a combo fixative (8 parts absolute ethanol, 1 part 37% formaldehyde, 1 part chloroform, 10 drops DMSO, 0.5 g picric acid) for at least 24 h. Insects were processed in 70% ethanol for 1 h, 95% ethanol for 1 h, and 3 changes of absolute ethanol for 1 h each. Tissue was infiltrated with Hemo-De (a xylene substitute, Fisher) 30 min, equal parts of Hemo-De and paraffin for 30 min at 60°C, one change of paraffin for 1 h, and fresh paraffin over-night. Specimens were then placed in a vacuum of 10 torr for 30 min and embedded in paraffin for sectioning. Sagittal sections were cut at 7 μm and stained using the Mallory–Heidenhain procedure of Cason (1950) for 1 min. Sections were examined microscopically to determine the number of male fleas with an epithelial plug in the epididymis in each treatment. The numbers of female fleas with sperm in the spermathecae also were noted for each treatment.

**Statistics.** Data reported in percentages were transformed by arcsine. The Duncan multiple range test was used for multiple comparisons between means (NCSS 1987).

**Results**

**Maturation of Unfed Fleas Treated with Juvenile Hormone or Juvenile Hormone Mimics.** Histological sections of control fleas showed that folds of columnar epithelial cells blocked the passage of sperm from the testis into the epididymis (Fig. 3). In contrast, the epithelial cells in the epididymis of treated fleas had become differentiated into the more flattened cuboidal or squamous cells forming a lumen that allows passage of sperm from the testis to the epididymis (Fig. 4). As fleas were exposed to increasing concentrations of juvenile hormone, increasing numbers of fleas matured at 3 and 7 d after treatment (Table 1). Percentage of maturation was significantly greater than the controls for all concentrations of juvenile hormone III tested. Observations of fleas treated with pyriproxyfen showed that there was 100% maturation in 3 d, whereas fleas treated with fenoxycarb, methoprene.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc, μg/cm²</th>
<th>No. and % fleas with sperm in the epididymis</th>
<th>After 3 d</th>
<th>After 7 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0/14</td>
<td>0/17</td>
<td>0/17</td>
</tr>
<tr>
<td>JH III</td>
<td>1.1</td>
<td>6/16a</td>
<td>38b</td>
<td>8/14</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>8/16</td>
<td>50b</td>
<td>8/12</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>9/15</td>
<td>60b</td>
<td>13/13</td>
</tr>
<tr>
<td></td>
<td>110</td>
<td>11/15</td>
<td>73b</td>
<td>16/16</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>1.1</td>
<td>14/14</td>
<td>100c</td>
<td>15/15</td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>1.1</td>
<td>10/16</td>
<td>63b</td>
<td>17/17</td>
</tr>
<tr>
<td>Methoprene</td>
<td>1.1</td>
<td>7/15</td>
<td>47b</td>
<td>16/16</td>
</tr>
</tbody>
</table>

Comparisons were made using a Duncan multiple range test. Values in the same column followed by the same letter are not significantly different (P > 0.05). JH, juvenile hormone.
Table 2. Percentage of female fleas that were inseminated by males held on juvenile hormone-treated dog hair at 37°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days</th>
<th>Males with sperm in the epididymis</th>
<th>No. inseminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0/15b</td>
<td>0/18a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0/12b</td>
<td>0/16a</td>
</tr>
<tr>
<td>JH III (70 ppm)</td>
<td>3</td>
<td>14/14c</td>
<td>0/15a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12/13c</td>
<td>0/16a</td>
</tr>
</tbody>
</table>

Comparisons were made using a Duncan multiple range test. Values in the same column followed by the same letter are not significantly different (P > 0.05). JH, juvenile hormone.

oprene, or juvenile hormone III required up to 7 d for 100% maturation.

Effect of Juvenile Hormone or Juvenile Hormone Mimic Treatment on Impregnation of Female Fleas. Histological examination demonstrated that none of the female fleas confined with males on juvenile hormone at any concentration or juvenile hormone mimic-treated filter paper contained sperm in the spermathecae. Likewise, no sperm were present in the spermathecae of female fleas held with males on juvenile hormone III-treated dog hair (Table 2).

Discussion

Our tests showed that juvenile hormone and juvenile hormone mimics stimulated differentiation of the columnar epithelial cells that line the walls of the epididymis of the cat flea. As the cells flattened and assumed the squamous shape of the physically mature adult, a lumen was created in the epididymis, which allowed sperm to leave the testes and pass into the vas deferentia. At least 50% of the male fleas transferred sperm to the epididymis when they were exposed to juvenile hormone III or juvenile hormone mimics over a period of 7 d, compared with no sperm transfer in the untreated controls. Juvenile hormone activity was related directly to the juvenile hormone concentration on filter paper. As the dosage of juvenile hormone increased, so did the number of males that transferred sperm to the epididymis.

Rothschild et al. (1970) observed a similar process taking place in S. cuniculi that were sprayed with synthetic juvenile hormone. They reported that elimination of the testicular plug was associated very likely with a change in shape of the epithelial cells in the epididymis such as we observed in the cat flea.

Of the various juvenile hormone mimics tested, pyriproxyfen was the most active in promoting differentiation of epithelial cells. All pyriproxyfen-treated males had transferred sperm by 3 d, whereas 7 d were needed for sperm transfer in 100% of the males exposed to the same concentration of fenoxycarb and methoprene.

Although not conclusive, these results suggest that juvenile hormone secretion in response to blood feeding stimulates physical maturation of the male cat flea. It is not clear whether juvenile hormone secretion also stimulates movement of sperm or mating behavior in the male flea. Juvenile hormone-treated females that were caged with treated males failed to mate, but they were unfed and may not have been receptive to insemination or been sexually attractive to the males. Therefore, physical maturation may be only one of several requirements for sexual maturation in the male cat flea.

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