The Testis Development in 3rd- to 6th-Instar Nymphs of the Cricket, Gryllus bimaculatus

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[Short Communication]

The Testis Development in 3rd- to 6th-Instar Nymphs of the Cricket, *Gryllus bimaculatus*

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**ABSTRACT**—A simple method for determination of the early stages of nymphs of the cricket, *Gryllus bimaculatus*, was presented. The width of the head-capsules was found to be a useful value to determine the nymphal stages. On this evidence, the testes from 3rd- to 6th-instar nymphs were investigated and histologically analyzed. The number of spermatogonia rapidly increased between the 3rd- to 5th-instar. Primary spermatocytes entered into meiotic prophase at the latest 2 days after ecdisis into the 5th-instar.

**INTRODUCTION**

Insect testes have been used to study spermatogenesis, and more and more information has been accumulated (Schwalm, 1988). Our previous study on male germ cells of the cricket, *Gryllus bimaculatus*, revealed dramatic changes of the binding activities to many lectins during the primary spermatocyte stage. Various granular structures in specific germ cell stages exhibited certain lectin binding activities (Suzuki and Nishimura, 1995, 1997). For further study of the stage-specific phenomena, especially by biochemical analysis, it is indispensable to know how the differentiation of germ cells proceed according to nymphal development. We found, however, little informations about this. It is more difficult to determine the stages of cricket nymphs than holometabolous insects such as Lepidoptera, because of the lack of remarkable quiescent stages related to ecdisys. In this report we present a criterion for the determination of cricket stages and offer some descriptions on nymphal testis development.

**MATERIALS AND METHODS**

Crickets, *Gryllus bimaculatus*, were reareded on an artificial diet of mouse (Oriental Yeast Co. Ltd., Tokyo, Japan) at 29°C ± 1.5°C under 16L:8D photoperiod. After the day of hatching (day 0), twenty animals from the colony were sampled daily, and the width of their headcapsules measured using a digital caliper (Mitutoyo Co. Ltd., Tokyo, Japan) under binoculars. The cricket stages were designated by days after hatching or alternatively days after each ecdisys, e.g., III-0 denotes the day of ecdisis into the 3rd instar. For the sampling of V-2 and V-3 testes, V-0 nymphs were isolated on day 15 or day 16.

To observe the chromosomes of germ cells, testes were fixed in ethanol:acetic acid (3:1) for 1 hr at the room temperature and stained in 70% acetic acid at 4°C. The testis follicles were stained in acetic orcein and squashed in a drop of 45% acetic acid. For histological analyses, testes were fixed in Bouin’s fluid. They were then dehydrated in ethanol and 1-buthanol, embedded in paraffin, and sectioned at 5 μm. The sections were dewaxed and stained with hematoxylin and eosin.

**RESULTS AND DISCUSSION**

Under the rearing condition used in this study, most animals molted to be adult within 42 days after hatching, although individual differences in developmental rate increased after the 6th instar. They molted every 3-4 days up to the 6th instar. Ecdisys of cricket nymphs in these early stages were difficult to observe, and the growth of nymphs as body lengths is known to be gradual (Nishioka and Matsuura, 1977). The head-capsule, however, grows stepwise due to its hard scleritic nature. We tried to measure the width to examine whether this value could be utilized as an index of the nymphal stage. As shown in Fig. 1 and Table 1, head-capsule width proved useful to determine the early stages up to the 6th-instar. When sampling V-0 animals, we selected them from 15-day old animals by measuring their head width. Most of the rest of the animals also ecdisys on the next day into the 5th instar. Although the ranges overlapped after the 6th-instar, the stages of these later instars were easily determined by the developmental degree of the wings.

According to this criterion, we obtained testis preparations from III-0 to VI-0 animals. Testes at III-0 had already consisted of several spermatocytes but each cyst contained a few germ cells (Fig. 2a). The number of germ cells rapidly increased and frequent mitoses were observed at IV-0 (Fig. 2b) and V-0 (Fig. 2c). Primary spermatocytes at meiotic prophase apparently observed in the V-2 and V-3 testes (Fig. 2e, f), and consequently the VI-0 testes contained many large spermatocytes before the 1st meiotic division (Fig. 2d). Percy and Fletcher (1979) described that in the testes of the grass-
hopper, *Stauroderus scalaris*, the first meiotic metaphase I cysts appeared in 12-day-old nymphs. In *Locusta migratoria*, Dumser (1980) observed the first meiotic division in 10-day-old nymphs. The lateness of entering into meiotic prophase in *Gryllus* may be related to the prerequisite frequency of mitotic cycles, which number more than 9 (Suzuki and Nishimura, 1995), for spermatogonia of *Gryllus*, compared with 6 or 7 cycles in *Stauroderus* (Percy and Fletcher, 1979).

**REFERENCES**


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**Table 1.** The range of head Capsule width at each instar

<table>
<thead>
<tr>
<th>Instar</th>
<th>I$^a$</th>
<th>II$^a$</th>
<th>III$^a$</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>Adult</th>
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<td>Minimum</td>
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<td>1.31</td>
<td>1.72</td>
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<td>3.09</td>
<td>3.83</td>
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<tr>
<td>Maximum</td>
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<td>1.15</td>
<td>1.56</td>
<td>2.11</td>
<td>2.91</td>
<td>3.87</td>
<td>4.74</td>
<td>6.13</td>
<td>7.27</td>
</tr>
</tbody>
</table>

$^a$ Sexes not determined. After the 4th-instar, only the value of male are shown.

$^b$ All measurements in mm.
Fig. 2. Testes development of nymphs of *Gryllus bimaculatus*. (a-d) Bouin-fixed testis sections stained with hematoxylin and eosin. Sections of III-0 (a), IV-0 (b), V-0 (c) and VI-0 (d) nymphs are shown in the same scale. Scale bar, 100 µm. (e) V-2 (day 17) testis contained primary spermatocytes entering into meiotic prophase (asterisk). Scale bar, 100 µm. (f) Early pachytene spermatocytes observed in the squashed preparation from V-3 (day 18) nymph. Scale bar, 20 µm.