ZOOLOGICAL SCIENCE 14: 637–639 (1997)

[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-instars. Primary spermatocytes entered into meiotic prophase at the latest 2 days after ecdysis 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 holometaborous insects such as Lepidoptera, because of the lack of remarkable quiescent stages related to ecdyses. 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 reared 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 head-capsules 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 ecdyses, e.g., III-0 denotes the day of ecdysis 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 chromosome of germ cells, testes were fixed in ethanol:acetic acid (3:1) for 1 hr at the room temperature and stored in 70% ethanol 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.

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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. Ecdyses 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 ecdysed 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 spermatocysts 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-

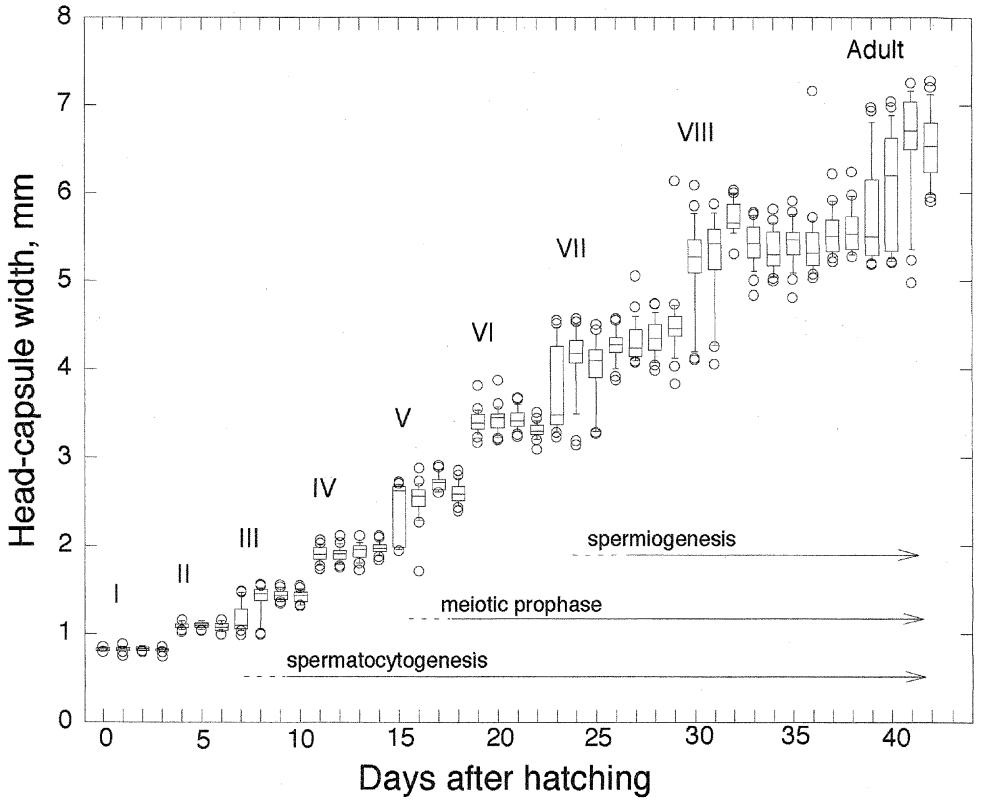

Fig. 1. Developmental change of the head-capsule of *Gryllus bimaculatus*. Twenty animals were sampled everyday and measured their head width. The boxes indicates the range between the 25th and 75th percentiles of the data. A line in each box marks the value of the 50th percentile. Capped bars indicate the 10th and 90th percentile points. Data outside 10th and 90th percentiles were shown with small circle. From day 0 to day 11, sampling were done without identifying the sex; on and after day 12, only the data of male crickets were shown. I - VIII, nymphal instar. Arrows indicate the appearance of the spermatogenic process.

Table 1. The range of head-capsule width at each instar

Instar	I[a]	II[a]	III[a]	IV	V	VI	VII	VIII	Adult
Minimum	0.74[b]	0.98	1.31	1.72	2.30	3.09	3.83	4.99	5.89
Maximum	0.88	1.15	1.56	2.11	2.91	3.87	4.74	6.13	7.27

[a] Sexes not determined. After the 4th-instar, only the value of male are shown.
[b] All measurements in mm.

hopper, *Stauroderus scalaris*, the first meiotic metaphase I cysts appeared in 12-day-old nymphs. In *Locusta migratoria*, Dumser (1980) observed the first meiotic devision 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).

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(Received April 18, 1997 / Accepted May 17, 1997)

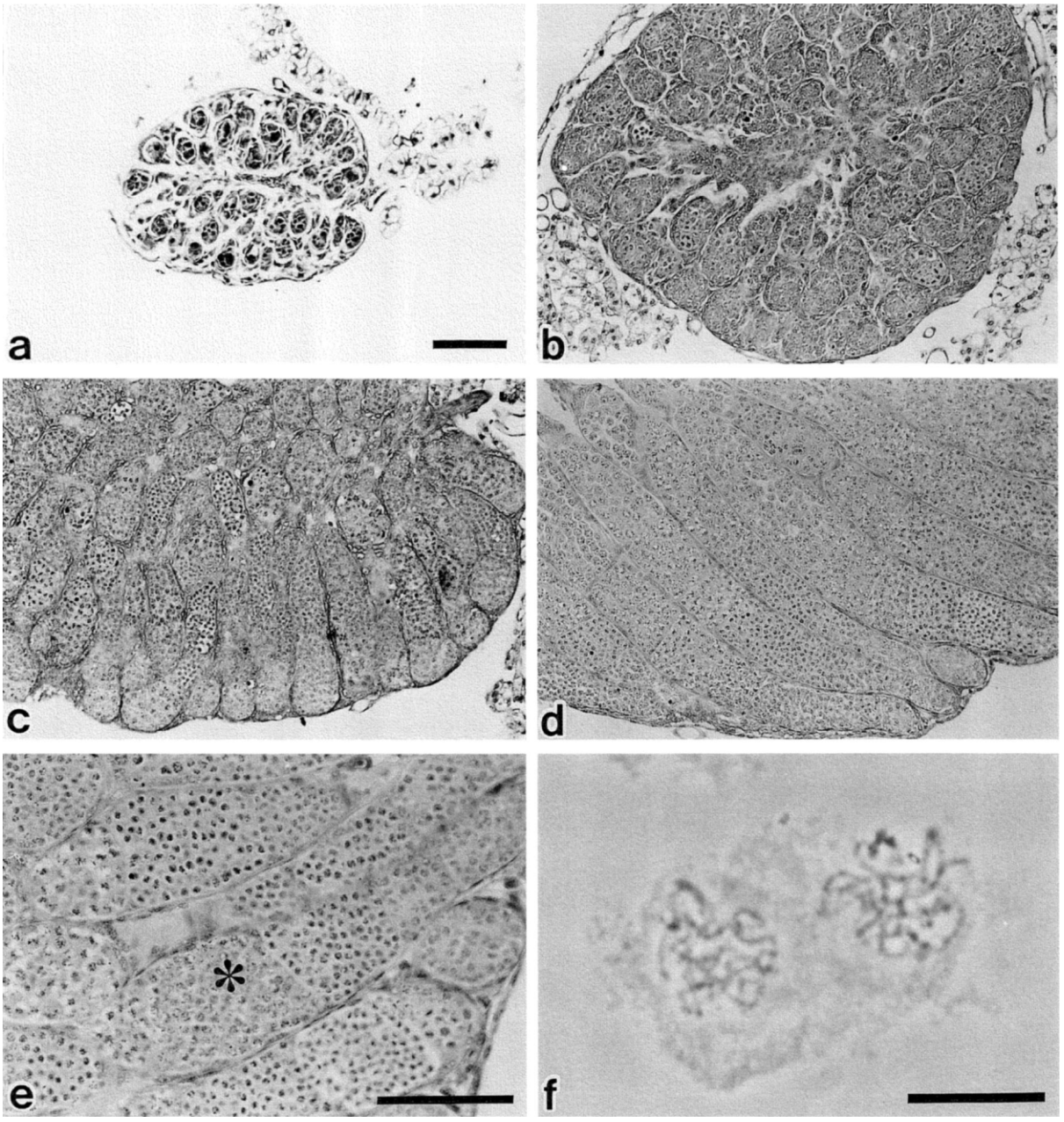

Fig. 2. Testes development of nymphs of *Gryllus bimaculatus*. (**a-e**) Bouin-fixed testis sections stained with hematoxylin and eosin. Sections of III-0 (**a**), IV-0 (**b**), V-0 (**c**) and VI-0 (**d**) nymphs are shown in the same scale. Scale bar, 100 μm. (**e**) V-2 (day 17) testis contained primary 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.