Decreased number of nymphal stadia in the lygaeid bug, *Dimorphopterus japonicus* (Heteroptera: Lygaeidae)

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Abstract

The lygaeid bug, *Dimorphopterus japonicus*, is a common species in Japan which feeds mainly on the eulalia, *Miscanthus sinensis*. It shows a marked wing dimorphism of brachyptery and macroptery. Almost all species of the Heteroptera pass through five nymphal stadia, although some exceptions have been noted. In the present study, we observed that *D. japonicus* had four nymphal stadia before eclosion to adults, while *Dimorphopterus pallipes*, a closely related species, had five. The number of stadia in *D. japonicus* did not vary irrespective of sex or wing morph in our observation. This is the first report of a four stadia life cycle in lygaeid bugs. In addition, phytophagous species having a four stadia life cycle are uncommon in the Heteroptera. Although the reasons why *D. japonicus* has only four nymphal stadia remain unknown, it may have evolved from an ancestral species with a five stadia life cycle like *D. pallipes*.

Key words: Lygaeidae, *Dimorphopterus japonicus*, *Dimorphopterus pallipes*, nymphal stadium

INTRODUCTION

The lygaeid bug, *Dimorphopterus japonicus*, is distributed in the Honshu, Shikoku and Kyushu regions and Tishima Islands in Japan, the Russian Far East and China (Hirashima, 1989). Nymphs and adults feed mainly on the eulalia, *Miscanthus sinensis*, which is a perennial grass that is widely distributed in Japan. They hide in interstices between the spindle and overlapping young leaves or inside the leaf sheaths of older leaves loosely adhered to the stalk. This bug overwinters in the adult stage in Nagoya, central Japan (Fujita, 1977). It also shows a marked wing dimorphism of brachyptery and macroptery (Fujita, 1977). However, the life history traits of this species have been barely studied.

In Heteroptera, there are ordinarily five nymphal stadia (Slater et al., 1963; Štys and Davidova-Vilimova, 1989; Schuh and Slater, 1995). However, our preliminary observation suggests that nymphs of *D. japonicus* have only four stadia. The closely related species, *Dimorphopterus pallipes*, which mainly feeds on reeds such as *Phragmites australis* and *Phragmites japonica*, also shows a marked

wing dimorphism, and goes through five stages (Fujita, 1977; Hirata et al., 1998). The objectives of the present study were to confirm our preliminary observation on the number of stadia and to compare the nymphal growth patterns between *D. japonicus* and *D. pallipes*. Although some exceptions in the number of nymphal stadia have been reported in the true bugs, these are few in number, and those with detailed data are even fewer (Štys and Davidova-Vilimova, 1989). Therefore, the research on basic characters such as the number of nymphal stadia would be not only indispensable for advancing studies of the life history strategy of *D. japonicus* but will also be useful to understanding the biology and evolution of the Heteroptera, which contains many important agricultural pests.

MATERIALS AND METHODS

Number of nymphal stadia in *D. japonicus* and *D. pallipes*. Laboratory cultures of *D. japonicus* were established with newly emerged adults of 157 females and 170 males collected from an *M. sinensis* patch at Notorohara, the northern district of Okayama Prefecture in western Honshu Island,

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Japan, on August 7, 26 and September 11, 1997. They were reared in groups in 900-ml transparent plastic containers (9.4 cm in diameter, 16 cm in height) under 16L8D and 25°C. Bugs were provided with several chopped stalks and leaves (15 cm long) of *M. sinensis* for food and substrate for oviposition. First-stadium nymphs of *D. japonicus* within 24 h after hatching were randomly collected from stock cultures in the 3rd generation. This experiment was carried out in the winter season when *M. sinensis* had already wilted in the field, thus nymphs were reared on sugar canes as an alternative food. About 50 individuals were reared on young sugar cane stalks (var. NCo310 cultivated in the greenhouse of Okayama University) placed in a 900-ml plastic container under 16L8D and 25°C. A total of five containers were used for the experiment. The stalks and containers were replaced with new ones every three days. Ten individuals from one of five containers were sampled at 3-d intervals and were frozen to death. The rearing density in the five containers was adjusted by adding the individuals from other containers set on the same date. The head width was measured using a micrometer under a microscope on the day after each sampling. The wing form of emerged adults was checked for insects in two particular containers.

The 1st-instar stadium of *D. pallipes* were collected randomly within 24 h after hatching from eggs that had been laid by overwintered female adults collected from a *P. japonica* patch in Kagamincho, the northern district of Okayama Prefecture, in June 1999. They were confined in 900-ml plastic containers under the same conditions as *D. japonicus* and provided with the stalks of *P. japonica*. Ten individuals were sampled at 3-d intervals and were frozen to death. The head width was measured using a video micrometer (OLYMPUS, VM-60) on the day after each sampling.

The growth rate index values were calculated as described by Oku (1970). This index measures a ratio of mean head width of a given stadium to that of the previous stadium.

Comparison of nymphal growth between *D. japonicus* and *D. pallipes*. Eggs obtained from cultures in the 2nd generation were used for the experiment. Newly hatched nymphs were individually reared on fresh chopped *M. sinensis* stalks (6 cm long) in a 50-ml glass vial (4 cm in diameter,

Fig. 1. Frequency distributions of head width of nymphs in *D. japonicus* and *D. pallipes*.

7.5 cm in height) under 16L8D and 25°C. The number of nymphal stadia was determined by confirming the exuviae daily. Sex and wing form of the individuals were determined after adult eclosion.

As for *D. pallipes*, newly hatched nymphs from the eggs laid by females collected from a *P. japonica* patch at Noichicho, the southern district of Kochi Prefecture in Shikoku Island, in August 1996, were used for the experiment. They were reared on fresh chopped *P. japonica* stalks (6 cm long) in 50-ml glass vials under 16L8D and 25°C at a density of 5 individuals per container. The number of nymphal stadia was determined by confirming the exuviae daily. Sex and wing form of the individuals were determined after adult eclosion.

RESULTS

Number of nymphal stadia in *D. japonicus* and *D. pallipes*

Figure 1 shows the frequency distributions of nymphal head width for both species. These figures

indicate that the nymphal stage is divided into four stadia in *D. japonicus* and five stadia in *D. pallipes*.

Table 1 shows the mean head widths at nymphal and adult stages for both species. Each value was based on pooled samples of brachypterous females and brachypterous males from group rearing. The growth pattern up to the 4th stadium was almost the same for the two species. However, in *D. pallipes*, individuals underwent one more nymphal ecdysis and the adult head width increased further. The growth rate indices tended to decrease as nymphal stadium advanced in both species.

The percentages of macropters among *D. japonicus* adults (emerged from two containers) were 11.9% (brachypter: 52, macropter: 7) for females and 8.3% (brachypter: 44, macropter: 4) for males.

Comparison of nymphal growth between *D. japonicus* and *D. pallipes*

The durations of the respective nymphal stadia

Table 1. The head width and growth rate index^a at nymphal and adult stages in *D. japonicus* and *D. pallipes*

Species	Stage	<i>n</i>	Head width (mm) ($\bar{x} \pm \text{SE}$)	Growth rate index
<i>D. japonicus</i>	1st	39	0.278 $\pm$ 0.002	—
	2nd	19	0.385 $\pm$ 0.003	1.38
	3rd	24	0.495 $\pm$ 0.004	1.29
	4th	43	0.626 $\pm$ 0.004	1.26
	Adult	34	0.702 $\pm$ 0.007	1.11
<i>D. pallipes</i>	1st	53	0.277 $\pm$ 0.002	—
	2nd	25	0.382 $\pm$ 0.003	1.38
	3rd	16	0.490 $\pm$ 0.004	1.28
	4th	22	0.622 $\pm$ 0.003	1.27
	5th	36	0.775 $\pm$ 0.005	1.24
	Adult	42	0.877 $\pm$ 0.005	1.14

^a See text.

were compared between *D. japonicus* and *D. pallipes* (Table 2). All adults molted into brachypters in *D. japonicus*, while adults of *D. pallipes* emerged as either brachypters or macropters. Daily examination for the exuviae also showed that *D. japonicus* eclosed to adult after four ecdyses. Durations of the first- and final-stadia did not differ between the two species for either sex. However, they differed in middle-stadia. The 2nd-stadium was longer in *D. japonicus* than in *D. pallipes*. The 3rd-stadium in *D. japonicus* was longer than 3rd- or 4th-stadium in *D. pallipes*, and was shorter than the combined 3rd- and 4th-stadia in *D. pallipes*. Moreover, the change in the duration of each stadium with advanced stadia was slightly different between the two species. In *D. japonicus* the stadium became increasingly longer from the 2nd- to 4th-stadia, while in *D. pallipes* each stadium from the 2nd- to the 4th was similar, and the 5th stadium became much longer. The total nymphal period did not differ significantly between two species for either sex ($p > 0.05$, Mann-Whitney *U*-test). The developmental period of females was significantly longer than that of males of both species ($p < 0.05$, Mann-Whitney *U*-test).

Figures 2 and 3 show nymphs and a brachypterous female adult of *D. japonicus* and *D. pallipes*, respectively. First- and 2nd-instars of *D. pallipes* (Fig. 3) were lacking in wing pads. Wing pads appeared first in the 3rd-stadium, where wing pads were very small and did not extend to the 1st-abdominal segment. In the 4th-stadium, wing pads extended up to the 1st-abdominal segment or beyond. In the 5th-stadium, wing pads exceeded the 2nd-abdominal segment. On the other hand, in *D. japonicus* (Fig. 2) wing pads were absent in the 1st- and 2nd-instars, and appeared first in 3rd-instars, where they extended to the 1st-abdominal segment. Wing pads in the 4th-stadium of *D.*

Table 2. Comparison of nymphal period between *D. japonicus* and *D. pallipes*

Sex	Species	<i>n</i>	Stadium (days $\pm$ SE)					Nymphal stage
			1st	2nd	3rd	4th	5th	
Female	<i>D. japonicus</i>	22	11.1 $\pm$ 0.5	7.8 $\pm$ 0.3	10.1 $\pm$ 0.6	14.0 $\pm$ 0.5	—	43.0 $\pm$ 0.9
	<i>D. pallipes</i>	17	10.5 $\pm$ 0.7	6.4 $\pm$ 0.5	6.1 $\pm$ 0.4	6.1 $\pm$ 0.4	13.5 $\pm$ 0.6	42.5 $\pm$ 0.9
Male	<i>D. japonicus</i>	26	11.2 $\pm$ 0.6	7.5 $\pm$ 0.3	8.5 $\pm$ 0.5	12.3 $\pm$ 0.4	—	39.5 $\pm$ 1.1
	<i>D. pallipes</i>	13	9.9 $\pm$ 0.9	6.2 $\pm$ 0.5	5.5 $\pm$ 0.2	5.6 $\pm$ 0.5	12.5 $\pm$ 0.7	39.7 $\pm$ 1.2

Fig. 2. Nymphs and a female adult of *D. japonicus*. From the left, the 1st-, 2nd-, 3rd-, and 4th-instars and the brachypterous female (body length: 4.75 mm).

Fig. 3. Nymphs and a female adult of *D. pallipes*. From the left, the 1st-, 2nd-, 3rd-, 4th-, and 5th-instars and the brachypterous female (body length: 6.08 mm).

japonicus exceeded the 2nd-abdominal segment similarly to those of the 5th-stadium nymphs of *D. pallipes*.

DISCUSSION

In general, nymphal development is divided by

each ecdyse. The number of immature stages displayed by a given insect is usually typical, within an extremely narrow range of variation, of the species (Beck, 1972). Several cases, specifically in Lepidoptera and Orthoptera, have been reported in which species show a variable number of stadia, depending on genetic, nutritional, or seasonal fac-

tors. In the cotton bollworm, *Helicoverpa armigera*, the number of larval stadia ranged from five to seven, depending on the nutritive value of the diet (Casimero et al., 2000). The egg-overwintering ground crickets, *Dianemobius nigrofasciatus* and *Dianemobius mikado*, emerge into adults after six nymphal stages under short days (12L12D or 13L11D), but require two or three additional molts during a prolonged nymphal stadia under long days (14L10D or 16L8D) (Masaki and Walker, 1987).

Although almost all species of Heteroptera pass through five nymphal stadia, some exceptions have been noted (Slater et al., 1963; Štys and Davidova-Vilimova, 1989; Schuh and Slater, 1995). Such exceptions were reported in Mesovelliidae, Veliidae, Nepidae, Reduviidae, Microphysidae, Miridae, Tingidae, Nabidae, Anthocoridae, Cimicidae, Polytentidae, Tesseratomidae, and Piesmatidae (Štys and Davidova-Vilimova, 1989). Most were predaceous (except tingids) or parasitic and only two species, *Agapophya bipunctata* (Tesseratomidae) and *Piesma quadratum* (Piesmatidae), were phytophagous. Moreover, only two species, *A. bipunctata* (Tesseratomidae) and *P. quadratum* (Piesmatidae), were likely to have four stadia in Pentatomomorpha.

In Lygaeidae, however, no species with four nymphal stadia have ever been reported (Štys and Davidova-Vilimova, 1989). In the present study, we observed that *D. japonicus* passed through four stadia before eclosion to adults, while *D. pallipes*, a closely related species, had five (Fig. 1). These results were obtained from both brachypter- and macropter-destined nymphs, because in the experiment where head widths were measured, macropters also emerged (see Results). Therefore, macropters had a four stadia life cycle, too. Moreover, when nymphs were reared under conditions of five individuals in a 50-ml glass vial under 16L8D and 28°C, only one nymph was emerged as a macropterous female after 4 ecdyses (Sasaki, unpublished data). These indicate that the number of stadia four, irrespective of wing form. However, the possibility that this is a characteristic specific to the population used in the present experiment can not be ruled out. In fact, in the predaceous mirid, *Cyrtorhinus lividipennis*, populations in India and Thailand have five nymphal stages, the Philippines population has four, the Hawaii population has from three to five (Liquido and Nishida, 1985), and

the Malaysia population has from four to six (Sivapragasam and Asma, 1985). Therefore, to examine this possibility in *D. japonicus*, the number of stadia among different geographic populations should be compared in the future.

The reasons why *D. japonicus* has only four nymphal stadia remain unknown. It is likely that *D. japonicus* has evolved from an ancestral species with a five stadia life cycle, such as *D. pallipes*. The results of the present study on wing pad development and the length of each stadium may imply that the 3rd-stadium of *D. japonicus* represents the combinative development of 3rd- and 4th-instars of a 5-stadia species such as *D. pallipes*. The evolutionary factors that led this species to a four stadia life cycle should be examined in the future.

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