Sexual difference in the effect of temperature on the larval development in *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae)

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Abstract

The effects of temperature on the larval developmental traits of the fall webworm, *Hyphantria cunea* (Drury), were investigated at 18, 20, 23, 25 and 27°C. In Japan, this species has two types of larval instars, the six-instar and seven-instar. The proportion of the seven-instar type was significantly higher in females than in males at all temperatures. In females, the proportion of the seven-instar type was high at higher temperatures. However, the effect of temperature on the incidence of larval instar types was not detected in males. The larval developmental time was significantly longer in the seven-instar type than in the six-instar type for both sexes at all temperatures. The difference in the developmental time between the instar types decreased as temperature increased. The pupae of the seven-instar type were heavier than those of the six-instar type at higher temperatures. These results suggest that the fitness advantage of the seven-instar type is greater in females than in males, and is relatively high at higher temperatures because the disadvantage of the long developmental period in the seven-instar type is reduced by higher developmental rates.

Key words: Body size; developmental time; larval-instar type; pupal weight

INTRODUCTION

The fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), was introduced from North America into central Europe and eastern Asia in the early 1940s (Warren and Tadic, 1970; Umeya and Itô, 1977). In Japan, the species has expanded its distribution area after the first finding in 1945 (Umeya and Itô, 1977; Gomi, 1996). The developmental traits of the species differentiated in local populations with the expansion of distribution (Gomi, 1996; Gomi and Takeda, 1996; Gomi et al., 2003).

The body size of insects generally correlates with fecundity (Honek, 1993) or developmental time (Roff, 1992). Thus, developmental time can be a function of fecundity through body size. Two types of *H. cunea* larvae, the six-instar and seven-instar, were reported in North America (Morris and Fulton, 1970) and Japan (Itô and Miyashita, 1968). In Japan, the incidence of the seven-instar type is lower in a trivoltine population than in a bivoltine population, and is not affected by photoperiod (Gomi, 1996). The incidence of the seven-instar type is higher in females than in males at 20°C (Gomi et al., 2003, 2005). The pupae of the seven-instar type are heavier than those of the six-instar type at both sexes at 20°C (Gomi, 1996; Gomi et al., 2003, 2005). There is a positive correlation between female body size and fecundity in *H. cunea* (Morris and Fulton, 1970; Gomi, 2000). Therefore, the seven-instar type of *H. cunea* has a fitness advantage of a larger body size for fecundity. On the other hand, the seven-instar type shows a longer larval developmental time than the six-instar type (Gomi, 1996; Gomi et al., 2003). In some insects, there is a disadvantage of prolonged development for survival because they are exposed for longer to natural enemies (Slansky, 1993; Atkinson, 1994). Therefore, the seven-instar type of *H. cunea* would have the disadvantage of prolonged development for survival compared to the six-instar type.

The developmental time of insects depends on the ambient temperature. The adverse effect of pro-
longed development for survival is reduced at higher temperatures because the difference in developmental time between the instar types probably shortens due to high developmental rates. Therefore, it may be expected that the incidence of the seven-instar type is high at higher temperatures in *H. cunea*. If the magnitude of a fitness advantage of larger body size is different depending on sex, the response to temperature may differ between sexes. These hypotheses were examined in the present study.

**MATERIALS AND METHODS**

The mid-instar larvae of *Hyphantria cunea* were collected on *Liquidamber stryculfa* L. and *Platanus* sp. in mid-June 2001 in Fukui city (36°04’ N, 136°13’ E), Honshu Island, Japan. The larvae originated from 12 nest webs occurring at two different sites. They were reared on an artificial diet, “Insecta LFS” (Nihon-Nosan-Kogyo, Yokohama, Japan), in transparent plastic cups (500 ml). The collected insects were reared at 25° ± 0.5°C and 16L-8D (16 h-light, 8 h-dark). All individuals avert pupal diapause in this photoperiod (Gomi, 1997). Adults that emerged within 24 h were randomly paired in the cup for oviposition. The egg-batches deposited were maintained in the cups with moist paper towels until hatching. Hatchlings from the eggs were used for experiments.

A few hundred larvae that hatched from several egg-batches on the same day were mixed and transferred to a leaf of *Populus nigra* L. and incubated at 18, 20, 23, 25 and 27° ± 0.5°C, 16L-8D in late July 2001. The number of larvae was halved at the third and fourth instars respectively, and the density of the fourth instars was 80–90 individuals per cup. A few leaves of *P. nigra* cut at the leafstalk were bound up in cotton and put in a glass vial (2 ml) filled with water. The leaves were replenished every 1–4 d depending on larval age and temperature.

In the field, larvae aggregate and construct nest webs until the fourth instar, and thereafter disperse and live individually. In the present experiment, 75 larvae were selected on the day of molting to the fifth instar from a single cup in each temperature and transferred individually to a small plastic cup (200 ml) with leaves of *P. nigra*. The number of subsequent molting and pupation days were recorded. The live weight of pupae was weighed at 5 d after pupation.

Statistical analyses for proportion of the seven-instar type were carried out using the chi-square test and Tukey-type multiple comparisons. Data of larval periods and live pupal weight were analyzed with the Mann-Whitney *U* test and Kruskal-Wallis test with nonparametric multiple comparisons. These statistical methods were mentioned in Zar (1999).

**RESULTS**

The proportion of the seven-instar type was compared between the sexes at each temperature and among the five temperatures in each sex (Table 1). The proportion of the seven-instar type was significantly higher in females than in males at all temperatures (chi-square test, *p* < 0.02). The proportions of the seven-instar type were significantly different at the five temperatures in females (chi-square = 19.21, *p* < 0.001). The incidence of the seven-instar type was high at higher temperatures (Tukey-type multiple comparisons for proportions, *p* < 0.05). In males, however, there was no significant difference in the proportions of the seven-instar type at different temperatures (chi-square = 3.83, *p* > 0.4).

Larval periods were significantly longer for the seven-instar type than the six-instar type in both

<table>
<thead>
<tr>
<th>Sex</th>
<th>18°C</th>
<th>20°C</th>
<th>23°C</th>
<th>25°C</th>
<th>27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14.2 (36 : 6)</td>
<td>22.5 (31 : 9)</td>
<td>28.6 (30 : 12)</td>
<td>15.6 (38 : 7)</td>
<td>16.7 (35 : 7)</td>
</tr>
<tr>
<td>Female</td>
<td>54.8 (14 : 17)</td>
<td>50 (17 : 17)</td>
<td>75.8 (8 : 25)</td>
<td>93.1 (2 : 27)</td>
<td>80 (6 : 24)</td>
</tr>
</tbody>
</table>

In parentheses, the number of the six-instar type is given in the left side and that of the seven-instar type in the right side. The proportion of the seven-instar type in males is not significantly different at different temperatures (chi-square test, *p* > 0.05). Values followed by the same letters are not significantly different using Tukey-type multiple comparisons for proportions (*p* > 0.05).
Effects of Temperature on Larval Development

Table 2. Larval period (d) shown by mean±SD in *H. cunea* reared on *Populus nigra*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Instar type</th>
<th>18°C</th>
<th>20°C</th>
<th>23°C</th>
<th>25°C</th>
<th>27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Six</td>
<td>49.2±2.4</td>
<td>38.9±1.6</td>
<td>28.9±2.0</td>
<td>24.6±0.7</td>
<td>22.7±0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(36)</td>
<td>(31)</td>
<td>(30)</td>
<td>(38)</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>53.5±1.7</td>
<td>44.0±2.4</td>
<td>32.1±3.0</td>
<td>27.1±0.7</td>
<td>25.3±1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td>(9)</td>
<td>(12)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Female</td>
<td>Six</td>
<td>50.3±2.3</td>
<td>39.8±1.8</td>
<td>30.4±1.9</td>
<td>25.0±0.0</td>
<td>24.0±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14)</td>
<td>(17)</td>
<td>(8)</td>
<td>(2)</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>54.8±1.6</td>
<td>46.0±2.9</td>
<td>33.6±3.0</td>
<td>29.7±2.5</td>
<td>26.3±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17)</td>
<td>(17)</td>
<td>(25)</td>
<td>(27)</td>
<td>(24)</td>
</tr>
</tbody>
</table>

1 Removed from statistical analyses because of the small sample size. The larval periods are significantly different between the instar types using the Mann-Whitney *U* test (*p*<0.01) in both sexes under all temperature conditions. Sample sizes are given in parentheses.

Table 3. Live pupal weight (mg) shown by mean±SD in *H. cunea* reared on *Populus nigra*

<table>
<thead>
<tr>
<th>Sex</th>
<th>Instar type</th>
<th>18°C</th>
<th>20°C</th>
<th>23°C</th>
<th>25°C</th>
<th>27°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Six</td>
<td>120.4±13.7**</td>
<td>121.3±7.9**</td>
<td>115.9±17.8**</td>
<td>134.2±12.8**</td>
<td>130.6±10.5**</td>
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<tr>
<td></td>
<td></td>
<td>(36)</td>
<td>(31)</td>
<td>(30)</td>
<td>(38)</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>126.6±15.8*</td>
<td>128.2±11.5*</td>
<td>133.8±10.6*</td>
<td>150.9±14.7*</td>
<td>147.8±4.4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td>(9)</td>
<td>(12)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Female</td>
<td>Six</td>
<td>170.8±17.1**</td>
<td>182.3±13.9**</td>
<td>173.1±20.1**</td>
<td>216.6±13.0*</td>
<td>216.9±18.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14)</td>
<td>(17)</td>
<td>(8)</td>
<td>(2)</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Seven</td>
<td>189.5±19.3**</td>
<td>185.6±20.3b*</td>
<td>188.9±18.4b*</td>
<td>246.3±29.8*</td>
<td>247.8±27.8**</td>
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<tr>
<td></td>
<td></td>
<td>(17)</td>
<td>(17)</td>
<td>(25)</td>
<td>(27)</td>
<td>(24)</td>
</tr>
</tbody>
</table>

1 Removed from statistical analyses because of the small sample size. * Significantly different between the instar types in each sex (Mann-Whitney *U* test, *p*<0.05). Values followed by the same letters in each sex and instar type are not significantly different at different temperatures using the Kruskal-Wallis test with nonparametric multiple comparisons (*p*>0.05). Sample sizes are given in parentheses.

sexes at all temperatures (Mann-Whitney *U* test, *p*<0.05), although females of the six-instar type at 25°C were excluded from all analyses because of the small sample size (Table 2). The difference of the larval periods between the instar types increased as temperature decreased, except for 18°C, although it was similar between at 27°C and at 25°C in males.

The pupae of the seven-instar type were significantly heavier than those of the six-instar type at higher temperatures (Mann-Whitney *U* test, *p*<0.05) (Table 3). However, differences between the instar types were not significant at lower temperatures, except for females at 18°C (*p*>0.05). The pupal weight was heavier in individuals raised at the two higher temperatures than in those at the other temperatures (Kruskal-Wallis test with non-parametric multiple comparisons, *p*<0.05), except for males of the seven-instar type, probably because of the small sample size.

**DISCUSSION**

In some lepidopteran insects, prolonged development is a disadvantage for survival because of the lengthening of exposure time to natural enemies (Fizgerald et al., 1988; Isenhour et al., 1989; Loader and Damman, 1991). In the present result, the seven-instar type of *H. cunea* showed longer larval periods than the six-instar type. Therefore, the seven-instar type probably suffers a greater disadvantage of prolonged development than the six-instar type in the field. It is inferred that natural enemies influence the difference of larval periods between the red-headed and black-headed forms of *H. cunea* in North America through feeding behavior (Takeda, 2005), although a relationship between larval periods and the frequency of attacks by natural enemies has not been proved in the species.

The proportion of the seven-instar type in females was higher at high temperatures in the pres-
ent study. The present results support the above hypothesis that adverse effects of prolonged development for survival are reduced at higher temperatures, and the incidence of the seven-instar type is high at higher temperatures. The difference in developmental time between the instar types was small at higher temperatures, which reduces the disadvantage of prolonged development. At higher temperatures, consequently, the fitness advantage of larger body size would be relatively valuable compared to the disadvantage of prolonged development in females.

The body size of insects decreases with an increase in rearing temperature in most cases, but increases in some cases (Atkinson, 1994). In the present study, the pupal weight was heavier at higher temperatures than at lower temperatures in both larval-instar types. Therefore, the present results were categorized to the latter minor cases. At higher temperatures, there may be other mechanisms to enlarge body size, for example extending the larval period in both instar types, in addition to increase the incidence of the seven-instar type. The pupal weight was significantly different between the instar types at higher temperatures, but the difference was obscured at lower temperatures. These results suggest that the advantage of larger body size is greater at higher temperatures than at lower temperatures. The increase in this advantage is another reason for the high proportion of the seven-instar type at higher temperatures.

In some insects, the developmental period and body size show geographically clinal variation within an invariable voltinism, where populations occurring at higher latitudes have shorter developmental periods and smaller body size than those at lower latitudes (e.g., Masaki, 1972; Obrycki and Tauber, 1981; Scott and Dingle, 1990). This type of local divergence is observed in the trivoltine population of *H. cunea* in Japan (Gomi et al., 2003). A southern population, which has long developmental periods and large body size, shows the higher incidence of the seven-instar types than a northern population (Gomi et al., 2003). Therefore, the relative advantage between the instar types geographically varies among populations, and consequently the temperature effect on larval development may be modified.

The proportion of the seven-instar type was significantly higher in females than in males at all temperatures in the present study. Sex-specific fitness variation in relation to body size has been reported in many animals, including insects (e.g., Austad and Sunquist, 1986; Sugiura, 1994; Rhen and Crews, 2001). In the present study, the six-instar type was male-biased and the seven-instar type female-biased. Females with a larger body size have a direct fitness advantage for depositing a larger number of eggs than those with a smaller body size in *H. cunea* (Morris and Fulton, 1970; Gomi, 2000). In males, there was no information regarding a positive relation between body size and fitness in the species. There has been no evidence that the disadvantage of prolonged development for survival differ between the sexes. Therefore, the fitness advantage of larger body size is relatively greater in females than in males. This would be a reason why the sexual difference was observed in the incidence of the seven-instar type in the present study.

ACKNOWLEDGEMENTS

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REFERENCES


