(Corning, Walpole, U.S.A.). The method has been described previously (INOUE and MITSUHASHI, 1988). The cell line was clearly distinguishable from other lepidopteran cell lines such as the ones derived from *S. seriatopunctata*, *Spodoptera litura*, *Papilio xuthus* and *M. brassicae* by their isozyme patterns (Table 1). The low electrophoretic mobility of PGI was a marked characteristic of this cell line.

The cell line was challenged with Si-NPV. The inoculum was prepared by dissolving polyhedra of the Si-NPV in 0.5% sodium carbonate (anhydrous), and passing it through a membrane filter with a pore size of 0.2 μm. Polyhedra were first recognized at the 4th day post-inoculation. The rate of polyhedra-containing cells increased with the advance of culture, and almost 100% of the cells had formed polyhedra by the 10th day post-inoculation. At this time many cells had already disintegrated, and the polyhedra were scattered all over the culture vessel. The cell line was found to be susceptible also to *Chilo* iridescent virus and *Sericesthis* iridescent virus. The infected cells showed iridescence at about a week post-inoculation. *Costelytra zealandica* iridescent virus also could replicate in this cell line, but the ratio of cells which showed iridescence was low. Examination of susceptibility of this cell line to other viruses is still being done. The cell line may be useful to propagate Si-NPV.

REFERENCES


Determination of Larval Instar Number in the Common Armyworm, *Leucania separata* (Lepidoptera: Noctuidae)

I. Temporal Patterns of Ecdysis in the Penuultimate Instar Larvae

Ken TATEISHI, Shigemi YAGI and Toshiaki SHIMIZU

Laboratory of Applied Entomology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

(Received April 13, 1988)

The typical course of larval exponential growth with moults at precise intervals has been explained by “Dyar’s law” or “Dyar’s rule” (DYAR, 1890). One exception to Dyar’s law is caused by the difference of the number of larval moults. As to the control mechanism of instar number, NHIOHT (1975) first demonstrated in the tobacco hornworm, *Manduca sexta*, that individuals with a head capsule wider than a defined threshold size pupate at the following moult whereas those with smaller head capsules undergo further larval moult. While, larvae of the common cutworm, *Spodoptera litura*, with head capsules wider than 1.65 mm are destined to become last instar larvae at the next moult (MORITA and TOJO, 1983). In *Leucania separata*, the period of the penultimate instar depends on rearing conditions such as the rearing density (TATEISHI, unpublished). In the present experiment, we investigated the relationship between

2 Present address: Central Research Laboratories, Hokko Chemical Industry Co., Ltd., Atsugi, Kanagawa 243, Japan
3 Present address: Department of Insect Physiology and Behaviour, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305, Japan
4 Present address: Department of Medical Entomology, The National Institute of Health, Kamiosaki, Shinagawa, Tokyo 141, Japan
5 To whom correspondence should be addressed.
Fig. 1. Relationships between live body weight (A), or head capsule width (B) and the time of the 4th larval ecdysis in the 5th instar larvae. Zero with an arrow shows light-off time. The 5th instar larvae became final instar larvae after 2 days (●, N=15, day 2 type), or 3 days of the instar duration (○, N=77, day 3 type), or became penultimate instar larvae (⊛, N=14, supernumerary moulting type), at the next ecdysis.

The period of the penultimate instar and body weight, or head capsule width of the penultimate instar larva at the time of ecdysis to this instar. The mechanism to control the period of the penultimate instar was discussed.

MATERIALS AND METHODS

Insect. The common armyworm, Leucania separata, was reared at 25°C on an artificial diet (Sumizuw and Fukami, 1983) under 16L-8D photoperiod (light off at midnight 24:00). We have termed the first 24 hr of each instar as Day 1, not as Day 0. A total of 106 individuals were used in the present experiments. Twenty to twenty-five 4th instar larvae were kept in each plastic container (20 × 14 cm, 5 cm height). We selected randomly 106 larvae before the head capsule slippage to 5th instar, and then they were reared individually on the artificial diet in a small cup (4 cm height, 10 cm diameter).

Measurements. The number of newly ecdysed larvae to the 5th (penultimate) instar were counted every hour from 3 hr before to 5 hr after light-off. Their body weights and head capsule widths were measured at the 4th ecdysis. Measurements during the time of light off were carried out under a dark-red light. Head capsule widths of the larva were measured using a calibrated ocular micrometer mounted on a dissecting microscope.

RESULTS AND DISCUSSION

Figure 1-A shows the body weight of the 5th instar larvae at the time of ecdysis. Among the larvae which ecdysed to the last instar at the next moult, day 2 type larvae (the period of penultimate instar was two days) weighed more than 47 mg and their ecdysis occurred nearly at the time of light-off. Fifteen out of 106 individuals were this type. Day 3 type larvae (the period of penultimate instar was 3 days) had ecdysed after 1 hr following light off; about 72% was this type. The period of the final instar was 5 days in both day 2 and 3 type larvae. In Manduca sexta larvae, the period of the final instar differs between types of “Gate” I and II depending on PTTH release (Truman et al., 1974). The larvae whose body weights were less than 30 mg at the 4th ecdysis developed to the 7th instar (N=14). About 13% was this type, the period of 6th instar of this type was 2 or 3 days. Figure 1-B shows the relationship between head capsule width of the 5th instar larvae and the time of 4th larval ecdysis. Average head capsule
Short Communications

Table 1. Comparison of body weight and head-capsule width at the time of the 4th larval ecydysis

<table>
<thead>
<tr>
<th>Type</th>
<th>Body weight (mg)</th>
<th>Head-capsule width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>55.2±5.3</td>
<td>2.36±0.05</td>
</tr>
<tr>
<td>Day 3</td>
<td>44.0±8.0</td>
<td>2.27±0.09</td>
</tr>
<tr>
<td>Supernumerary moulting</td>
<td>23.6±3.5</td>
<td>1.81±0.07</td>
</tr>
</tbody>
</table>

* Mean±S.D. (n=14-77).

Fig. 2. Fluctuation of live body weight at the time of the 4th larval ecydysis in the 5th instar larvae, which became day 3 (●) or day 2 type (●). See Fig. 1 for others.

Fig. 3. Comparison of the time of ecydysis among day 2 (●) and day 3 (●) types of the 5th instar larvae. Zero with an arrow shows light-off time. See Fig. 1 for others.

Hideharu Numata and Norio Matsui

Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi, Osaka 558, Japan

Received April 15, 1988

Many insect species show oviposition patterns controlled by circadian clocks and synchronized by environmental light-dark cycles (see Saunders, 1982, for review). Kadosawa (1982, 1983) reported that in *Riptortus clavatus*, some adult activities show marked daily periodicity, although no mention was given of the mechanism controlling this. This paper describes daily changes in oviposition activity under a 24 hr light-dark cycle, and examines whether the phenomenon is under a circadian clock control.

**MATERIALS AND METHODS**

Adults of *R. clavatus* were collected from legume fields in Kyoto City (35° N, 136° E), and their eggs, the first laboratory generation, were used for the experiment. Nymphs were reared under diapause-averting conditions, 16L:8D and 25±1°C, according to the method previously reported (Numata and Hidaka, 1982).

After adult emergence, each female was reared under the same conditions in a 200 ml plastic cup with 10 soybean grains, water and a piece of cotton wool for oviposition substratum. The females were mated once, 10–13 days after adult emergence. The observation of ovipositional rhythmicity was