Some Behavioural Changes Associated with Phase Variation in the Armyworm, *Leucania separata* Walker

I. Reaction of Larvae to Mechanical Stimuli

By Syun’iti Iwao

Entomological Laboratory, College of Agriculture, Kyoto University, Kyoto

INTRODUCTION

In a previous paper (Iwao, 1962) the author has reported that the degree of crowding in the larval population exerts many-sided influences on morpho-functional characters of both larva and adult of the armyworm, *Leucania separata*. The phenomenon is considered to be essentially similar to the phase variation in locusts. In regards to behavioural aspect, it has been shown that the black larvae appeared in crowded population are much more active than the pale ones predominated in sparsely populated condition. The difference in the behaviour pattern between both types of larvae is, however, assumed not to be quantitative merely but qualitative also, though it has not been revealed yet. Neither, differential behaviour in the adult stage has not been proved.

Experimental analyses have been undertaken to clarify these points, because it is the major importance in understanding biological significance of this sort of polymorphism and their direct relation to ecology of the species concerned.

This paper describes differential behaviours exhibited by pale larvae and black larvae when they are dropped onto an illuminated field and subjected to a strong wind. Other aspects of the phase difference in behaviour will be reported in the following papers.

RESPONSE EVOKED BY DROPPING SHOCK

Material and Method

The larvae used for the test were either reared in isolation or a crowd of 10 individuals per container (10 cm in diamètre and 3.5 cm in height) at a constant temperature of 27.5°C under natural daylight conditions. A grass leaf, *Bromus unioloides*, was supplied daily as larval diet. In this species, the degree of darkening of larval colour changes in parallel with the rearing density: the larvae reared in isolation were invariably pale in colour (colour types 1-2) and those reared in crowd were black (types 4-5). These two kinds of larvae were subjected to the test at their 5th and 6th instars. The previous paper should be referred for details in rearing method and characteristics of the colour types of larvae.

An eight-armed glass apparatus was used for the experiment (Fig. 1). The main body is made of a cylinder, 6 cm high and 12 cm in diamètre, and eight arms are projected radially from the side wall at the bottom. Each arm is led to a small bottle, 6 cm long and 3.7 cm in diamètre.

The author wishes to express his sincere thanks to Professor Syunro Utida for invaluable advice and criticism. Thanks are also due to Mr. Hirosi Okuda for assistance in rearing the insects used for the experiments.

Contribution from the Entomological Laboratory, Kyoto University, No. 366. This work was supported by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education.

(Received for publication, March 20, 1963)
Fig. 1. A glass apparatus used for the dropping experiment.

metre, through a narrow neck of 1 cm in inner diameter and 5.5 cm long. The upper side of the cylinder is covered by glass plate with a hole of 3 cm in diameter on the centre.

A 20-W fluorescent lamp was placed 20 cm above the apparatus. Four arms out of the eight were covered by a black board to shut off the direct light and the other four were exposed to the light. The two types of the arms were arranged alternately.

The test insects were dropped in the centre of the bottom of the cylinder, individually or in a group of 10 at a time. The former is referred as an individual test and the latter as a group test. The insects dropped usually remained immobile for some time, and then they commenced active movement in order to escape. In course of wandering, they eventually discovered the mouth of an arm and entered into it. For each larva tested, the time required from dropping to the recovery of its activity or to entering into an arm was recorded along with the type of an arm it went in. Observation was continued up to five minutes from the beginning of the test.

For the individual test, only the final (6th) instar larvae were used. Under the rearing temperature mentioned above, the larvae ceased their feeding activities on the 4th or 5th day after moulting and entered into the soil for pupation. Therefore, 1st, 2nd and 3rd day individuals were subjected to the test. Twenty or 30 individuals of each pale and black larvae at the given age were used.

For the group test, the 5th instar larvae were tested along with larvae of three-days old of their final instar. As larvae complete the 5th instar within 2 days, only one-day old individuals were suited for the test. Five replications were performed in each series of the tests.

Any individuals were never subjected to the test twice on the same day. The experiment was carried out under temperature, 27.5°C, and relative humidity, about 60 per cent.

Results

Immobilization and Running Away: Fig. 2
shows the result of the individual test, in which a cumulative frequency of the individuals that began to move or those that entered into the arm is plotted in percentage against the time since dropping. For example, on the 1st day of final instar, 60 per cent of pale larvae dropped began movement within one minute, and 80 per cent did so within two minutes, namely 20 per cent of the larvae recovered from inactive state between one and two minutes after being dropped.

From the Fig. 2, it can be seen that the black larvae commenced their locomotory movement much sooner than the pale ones in all three ages tested, and that the duration of the time being remained motionless after being dropped tended to increase with advancement of their maturity.

When dropped, some larvae curled up and remained motionless for some time, and their response has often been called "spiral reflex". This type of response was commonly found in both pale and black larvae, and the percentage was not very different from each other; for instance, 53 per cent of the pale larvae and 47 per cent of the black ones on the 2nd day of the final instar, and 63 and 37 per cent respectively on the 3rd day (Table 1). However, most of the black larvae once curled up stretched out their body within one minute and began to move away, while considerable proportion of the curled pale larvae remained in this pose as long as over five minutes.

Incidentally it was observed that the immobile state of the latter often extended even over a quarter of an hour.

The larvae which did not curl up when being dropped tended to move sooner than the curled ones, but same difference between two types of the larvae was observed too. Some of the black individuals began movement as soon as they were dropped.

It is interesting that the responses shown by the pale larvae, either curled up or not, had a tendency to be divided into two groups, one that resumed activities after a relatively short period of immobility and the other that showed a prolonged period of immobilization. It was not certain if two tendencies of their response was caused either by the individual variations in response among the pale larvae or by some fluctuation in the dropping procedure such as varying intensities of the stimulus added and the pose of the larvae on dropping.

When a group of 10 larvae was dropped simultaneously onto the floor, the result was quite similar to that of the individual test (Table 2). As compared with the latter, both the black and the pale larvae tended to shorten the duration of time of their inactive states and they entered rapidly into the arms. However, the proportion of the pale larvae which remained immobile over five minutes was not reduced so markedly. Difference between responses of the pale and the

<table>
<thead>
<tr>
<th>Stage of larvae</th>
<th>Kind of larval response</th>
<th>Type of larvae</th>
<th>No. of larvae</th>
<th>Time being immobile (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6th instar:</td>
<td>Curled</td>
<td>Pale</td>
<td>15</td>
<td>33.3 6.7 6.7 0 0 53.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>14</td>
<td>92.9 7.2 0 0 0</td>
</tr>
<tr>
<td>2nd day</td>
<td>Not curled</td>
<td>Pale</td>
<td>15</td>
<td>46.7 26.7 6.7 0 0 20.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>16</td>
<td>87.5 6.3 0 0 6.3</td>
</tr>
<tr>
<td>6th instar:</td>
<td>Curled</td>
<td>Pale</td>
<td>19</td>
<td>10.5 10.5 21.1 10.5 5.3 42.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>11</td>
<td>36.4 36.4 9.1 9.1 0 9.1</td>
</tr>
<tr>
<td>3rd day</td>
<td>Not curled</td>
<td>Pale</td>
<td>11</td>
<td>36.4 9.1 9.1 0 18.2 27.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>19</td>
<td>89.5 10.5 0 0 0 0</td>
</tr>
</tbody>
</table>
black types was also apparent in the 5th instar, though high percentage of the larvae remained immobile over five minutes on the centre part of the floor.

Rapid recovery from inactivity among black larvae may partly be related to some kind of habituation which is assumed to occur in crowded population, though it is not yet demonstrated.

Selection of Dark and Light Arms by Pale and Black Larvae: The larvae eventually entered into either a dark or a light arm, except those remained immobile over five minutes. According to the individual test, majority of the pale larvae entered into dark arms, while black ones showed this tendency in a lesser extent (Table 3, a). The difference between both types of larvae in selecting an arm is statistically significant on the 1st and 3rd-day of their final instar, and overall difference, calculated by the FISHER's method (1950, §21.1,) is highly significant (P<0.01).

According to the group test, on the other hand, the proportion of the individuals that entered into the dark arms was reduced
considerably among the pale larvae\textsuperscript{2}, but it did not differ among the black ones. Consequently, the difference in the arm selection between both types of larvae became insignificant. Generally, either type of the larvae entered into the dark arms more frequently, but the black larvae of the 5th instar showed entirely opposite tendency.

These results suggest that pale larvae show a strong photonegative behaviour while the black ones are rather indifferent to the light, and that the light reaction of the former is disturbed largely by crowding, whereas that of the latter does not.

**DISCUSSION**

Death feigning or reflex immobilization as a response to mechanical disturbance is common among insects (c.f. WIGGLESWORTH, 1950). It may be a device to escape from predators, though the experimental evidence is still lacking. On the other hand, rapid running-away movement may also serve the same purpose. As shown in this paper, both types of escape behaviour are not mutually exclusive but one is succeeded by the other. However, it is interesting that a passive response, immobilization, is well developed among the pale larvae (low-density phase) and an active running away among the black ones (high-density phase). In this connection, it may be worthy to note that the pale larvae are procryptic in colour while the black ones are rather con-

\textsuperscript{2} Overall difference between the individual and the group tests is significant at 5% level.

---

**Table 4. Frequency distribution of the number of larvae which were blown off by strong wind during 1-minute test**

<table>
<thead>
<tr>
<th>Stage of larvae</th>
<th>Type of larvae</th>
<th>No. of larvae tested</th>
<th>Time in seconds</th>
<th>Total No. dropped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pale</td>
<td>49</td>
<td>0~10</td>
<td>14 (28.6%)</td>
</tr>
<tr>
<td>4th instar</td>
<td>Black</td>
<td>49</td>
<td>10~20</td>
<td>27 (55.1%)*</td>
</tr>
<tr>
<td>1st day</td>
<td>Pale</td>
<td>30</td>
<td>20~30</td>
<td>4 (13.3%)**</td>
</tr>
<tr>
<td>5th instar</td>
<td>Black</td>
<td>34</td>
<td>30~40</td>
<td>5 (13.3%)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>40~50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>50~60</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from pale larvae at 2.5% level.
** Significantly different at 0.1% level.
spicuous (Iwao, 1962), for immobilized behaviour is said to be often associated with concealing colouration (Cott, 1957; Schneirla, 1953).

In a sense, these two kinds of escape behaviour bear some analogy with the two modes of escape mechanism for more persistent adversity in the environment as exemplified by diapause and migration. The high-density phase of locusts and armyworms is mobile and its physiological state is assumed to be opposite to diapause condition, whereas the low-density phase is sedentary and sometimes associated with diapause as seen in the brown locust, Locusta pardalina (Matthies, 1951; see also Kennedy, 1961 and Iwao, 1962). Thus, the former responds in active manners to the environmental adversity and the latter in passive ways.

The result of the second experiment revealed other aspect of behaviour difference. The fact that the pale larvae tend to cling more firmly to the plant than the black ones may be regarded as an expression of the station-keeping characteristics of the former. In this case, the observed difference is due to their difference of grasping power. On the other hand, it is likely that two types of the larvae also differ in their responsiveness to a given shock stimulus. The problem is left to future study.

Sharov (1953) observed that with a Notodontid moth, Exaereta ulmi, dark larvae (his type 1) typical in the outbreak area responded very actively to a disturbance to the tree on which they live, but pale larvae (his type 4) in the sparsely populated forest were sluggish in response and rarely dropped from the tree when disturbed. Though the detailed nature of the phenomenon is not clear, it seems likely that the differences in behaviour, similar to those observed in the armyworm, might exist in Exaereta larvae, also.

In the first experiment described here, it is suggested that there may be some difference in the light reaction between the pale and the black larvae. This problem will be further analyzed in a following paper.

**SUMMARY**

In the armyworm, Leucania separata, the black larvae appeared in crowded population are different in many respects from the pale larvae in sparse population. This paper deals with the different reactions of these two types of the larvae to some mechanical stimuli.

When the larvae were dropped onto an illuminated field, they remained immobile for some time and then began to escape away. Most of the black larvae resumed their activity within one minute since being dropped, while a considerable percentage of the pale larvae showed a prolonged immobilization over five minutes. The difference was similarly observed in both experiments in which the larvae were dropped individually in a group.

After the recovery from the inactive state, the pale larvae tended to move into dark place more than the black ones. The photonegative tendency of the former was, however, reduced considerably when a group of the larvae was dropped simultaneously.

The difference was also observed in their grasping behaviour; the pale larvae clung more firmly to the plant than the black ones when exposed to a strong wind.

These results are to be regarded as additional evidences of the fact that the pale larvae are passive and station-keeping, while the black ones are active and mobile in habits.

**REFERENCES**

Cott, H. B. (1957) Adaptive coloration in ani-
アワトウの相変異に関連した行動の変化

第1報 機械的制圧にたいする幼虫の反応

渡 俊 一
京都大学農学部昆虫学研究室

アワトウの相変異についてはさきに詳しく報告したが（渡, 1962), 高密度型と低密度型の行動上の相違については十分の分析がなされていなかった。本報では低密度の時みられる淡色型幼虫と高密度下で生ずる黒色型幼虫の、機械的制圧に対する反応のちがいについて報告した。

蛍光灯照明下において一定の高さから幼虫を落下させるといわゆる偽死反応を示し、ついて逃避行動を起こす。淡色型幼虫では偽死の時間が長くしばしば5分以上にも及ぶが、黒色型幼虫では大部分1分以内に逃避行動に至り、全く偽死反応を示さないものもある。この傾向は幼虫を1匹ずつ落下させても、数匹一回落下させた場合でもかわらなが、後者では偽死の時間がやや短縮される。偽死から回復した後淡色型幼虫は暗所に向う傾向がつよいが、黒色型幼虫は光条件にそれほど影響されない。しかし、数匹まとめて落下させた場合には、前者にみられる負の光反応は不明瞭になり両型の差がなくななる。

一方、植物上の幼虫に強度5.5mの強い風を作用させると、この風による機械制圧は偽死反応を誘起させるようになるが、かえって植物上につよく密着しようとする行動を起こさせる。この植物への付着能力の点では淡色型幼虫の方が優れている。

以上の結果は、低密度型（淡色）幼虫が不活発で非移動的であり、高密度型（黒色）幼虫が活動的で移動性に富むという特性のちがいを反映したものとみることができる。