Laboratory rearing of the ‘active’ phase of Callosobruchus maculatus F. (Coleoptera, Bruchidae).

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Polymorphism in C. maculatus has been noted and described. It has been seen that ‘normal’ individuals of C. maculatus are further distinguishable as ‘A’, ‘B’ and ‘C’ categories, and ‘active’ individuals as ‘X’ and ‘Y’ subphases. The present paper describes that, if stored legume seeds, containing second or third instar C. maculatus larvae, are exposed to the temperature of about 40°C with high [about 90%] RH, a number of adults emerging from the seeds are ‘active’, i.e. they belong to ‘X’ and ‘Y’ subphases. In such a generation ‘Y’ males are smaller in number than ‘X’ males, while ‘Y’ females outnumber ‘X’ females. This heat and high humidity treatment of later stages of bruchid development increases the total development period, decreases percentage of successful emergence of the bruchid from the seeds, and also prolongs the period of emergence in the culture.

Utida described dimorphism unconnected with sex in Callosobruchus maculatus. He mentioned the two forms among the adults as ‘flightless’ and ‘flight’ forms. Caswell chose to call the forms ‘normal’ and ‘active’ respectively. In the present study it has been found that ‘normal’/‘flightless’ are further distinguishable as ‘A’, ‘B’ and ‘C’ subphases, while ‘active’/‘flight’ are further identifiable as ‘X’ and ‘Y’ individuals. George and Verma have described in detail the distinguishing features of the subphases ‘A’, ‘B’, ‘C’, ‘X’ and ‘Y’.

At Durg in the MAPCOST Symposium on Chrysomelidae and Bruchidae in February 1988 Dr. Tarlok Singh of the Punjabi University, Patiala emphasized that ‘active’ forms could be located in cultures of C. maculatus only in the rainy season. Taking clue from this observation it was decided to culture C. maculatus in warm and humid conditions (so that Indian rainy season conditions are simulated) in an attempt to induce production of ‘active’ forms in the laboratory.

Though earlier workers Utida and Sano have experimentally shown that by larval crowding or by artificially heating the culture development of ‘active’ individuals could be induced, need was felt to reinvestigate this aspect and to arrive at inferences with reference to ‘X’ and ‘Y’ subphases.

Materials and Methods
For this study two sets of cultures of C. maculatus on ‘moong’ or green gram seeds (Vigna radiata (L.) Wilczek) with low larval density were used, each set of three cultures. One set was used as control and the other as experimental.

For both the sets low larval density cultures were prepared by the following method. Females were allowed to oviposit on fresh seeds for 24 hours. Then they were removed. Thus all eggs on the seeds were of nearly the same age. After hatching of the eggs 4000 seeds, each having only one egg, were taken in each of 6 rectangular open cardboard boxes, each measuring 17.5 cm X 30.0 cm. To this fresh seeds were added so as to make total of 500 g. In this way 6 cultures were prepared, each having 500 g. “moong” seeds, amongst which 4000 seeds were infested. In each culture proportion of fresh and infested seeds was approximately 3:1. The cultures, thus prepared were obviously of low density.

Shallow open cardboard boxes or trays were used for keeping the cultures so that seeds could be spread out into a thin layer, and they could be exposed to nearly uniform conditions of temperature and humidity. A hygrometer was included in each tray, which was covered with a nylon gauze.

The control set was kept in a thermostat oven at 30±2°C, and 70-75% RH throughout. The experimental
set was kept for the first 10 days from oviposition under similar conditions as the control set. On the eleventh day it was transferred for the rest of the developmental period to a chamber maintained at 40°C and RH above 90%.

The chamber for rearing at nearly 40°C was arranged as per the description of Verma. It was fitted with a 6V autobulb. The bulb was connected to the mains through a stepdown transformer and a rheostat. The rheostat was adjusted so as to get 40°C temperature more or less constantly. For RH of about 90%, cotton wool “saturated” with water was placed in a petridish in the chamber.

When adults emerged in both the sets, their numbers were counted after sorting them out on basis of their phase status. Pre-emergence period and total length of time of emergence were also recorded. Adults, emerged, were removed and counted every day.

Results
From the results, summarised in Table 1, it is obvious that, when developing larvae were transferred

![Fig. 1—Pre-emergence period and emergence period of C. maculatus in control cultures (C1) and in experimental cultures (C2). □ pre-emergence period. ■ emergence period, i.e. the duration when adults kept on emerging from seeds. The vertical line over each bar shows ± SE.]

<table>
<thead>
<tr>
<th>Table 1.—Results of experiments on rearing of C. maculatus in control cultures (C) and in experimental cultures (C2).</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Time (days)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pre-emergence period</td>
<td>35.0±0.5</td>
<td>35.0±0.5</td>
</tr>
<tr>
<td>Emergence period</td>
<td>10.0±0.5</td>
<td>10.0±0.5</td>
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</tbody>
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Pre-emergence period i.e. the duration when adults kept on emerging from seeds. The vertical line over each bar shows ± SE.
to a humid heating chamber during the second larval instar stage, the following changes could be made out.

1. Developmental period (i.e. pre-emergence period) increased in heat treated cultures. In control cultures adult emergence started after 18.00 ± 1.23 days from oviposition, but in heat treated cultures it started after 21.66 ± 0.41 days (Fig.1).

2. The duration of emergence also increased on heat treatment. In control cultures emergence continued for 11.33 ± 1.08 days, but in experimental cultures the corresponding period was 38.33 ± 2.17 days (Fig.1).

3. In experimental cultures percentage of successful emergence of adults decreased. In control cultures it was 87.11 ± 2.09%, and in heat treated cultures it was 31.45 ± 1.51% (Fig.2).

4. Among adults, emerged from a heat treated culture, some ‘active’, i.e. ‘X’ and ‘Y’ individuals could be made out. The proportion of ‘X’ and ‘Y’ individuals among emerged adults in such a culture was nearly 42.7%. But in a control culture proportion of ‘active’ was zero percent (Fig.3).
Discussion

From the present study it is obvious that ‘active’ (i.e. ‘X’ and ‘Y’) individuals of *C. maculatus* may be produced, if the rearing temperature is kept high along with high RH from the second or third larval instar. According to Utida the period from 11th to 15th day after oviposition at 30°C, which corresponds roughly to the third larval instar, was the critical stage, most susceptible to heat, when *C. maculatus* was reared on cowpea seeds. It was during this susceptible period that some individuals became ‘active’. According to Sano the susceptible period was from 9th to 11th day after egg laying. His cultures were made on azuki beans. In the present study, in which “moong” seeds have been used in culture preparation, the susceptible period has been found to be from 10th to 12th day after egg deposition. This period corresponds to second or third larval stage. (In preliminary experiments in the present study it was noted that, if *C. maculatus* larvae in “moong” below 11 day old from day of oviposition were exposed to 40°C, there was almost total mortality. It was also noted that exposure of infested seeds more than 12 days old did not yield any ‘active’ individuals).

As reported by Sano in the present study also it has been observed that control cultures have not yielded any ‘active’ individuals.

Different workers have expressed different opinions on the role of temperature and RH in the production of the ‘active’ phase in *C. maculatus*. General observation is that crowding leads to production of the ‘active’ phase. According to Utida crowding results in rise of temperature, which induces production of the ‘active’ morph. Sano has said that crowding generates not only heat but also increases humidity, and both the changes help induction of the ‘active’ phase. Sano-Fujii believes that in the production of the ‘active’ phase temperature has an important role, and the role of rise in RH is only secondary or supporting. Taylor has opined that increase in the initial population density is the main cause leading to the production of the ‘active’ phase. He has further pointed out that increase in the initial population density leads to larval crowding, accumulation of metabolic heat and rise of culture temperature. But he also points to the possibility of “behavioural and adult population interactions” resulting from increase in the initial population density as among the factors inducing production of the active form.

The present study has led to the inference that high temperature and high humidity induce production of the ‘active’ phase. Determination of the relative role of the two factors has not been possible, as at so high a temperature as 40°C, a culture of *C. maculatus* dies out unless high RH is maintained.

Tiwary and Verma have inferred that there is a genetic basis for development of polymorphism in *Callosobruchus analis*. F. Hence the situation that under similar abiotic / biotic conditions some ‘active’ individuals develop to become ‘X’ and some become ‘Y’, seems to be due to genetic variability among them.

In *C. analis* Verma has found that both increased temperature and increased humidity promote the production of ‘B’ and ‘B’-like ‘C’ individuals. In the present study in experimental cultures of *C. maculatus*, though percentage of ‘B’ and ‘B’-like ‘C’ individuals did not go up significantly, the percentage of ‘X’ and ‘Y’ individuals increased on increasing temperature and humidity. This difference between the two species is perhaps due to *C. analis* with a more restricted polymorphism, which does not include the subphases ‘X’ and ‘Y’.

In the present study in experimental cultures the pre-emergence period (i.e.the developmental period within the seed) was somewhat prolonged, while it was normal in control cultures. A similar situation has been described in *C. analis* by Verma. It seems that high temperature retards the normal development.

From Fig. 3 it is obvious that ‘Y’ males are always smaller in number than ‘X’ males. ‘Y’ males are clearly distinguishable from ‘normal’ (‘A’, ‘B’, ‘C’) males, but ‘X’ males are not much different from ‘normal’ males externally. Probably this is the reason why Taylor says that among males the two forms, ‘normal’ and ‘active’ are indistinguishable externally.

In the same way it is also obvious from Fig. 3 that ‘Y’ females are more numerous than ‘X’ females. ‘Y’ females are totally sterile. Probably it is due to this situation that Pajni says, “the abnormal morph in the Indian strain is completely sterile, unlike its counterparts in other countries which are less fecund”.

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