

A NEW TECHNIQUE FOR THE PROSPECTIVE SURVEY OF SEX CHROMATIN USING THE LARVAE OF LEPIDOPTERA

WINIFRED CROSS AND ALISON GILL

Department of Genetics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K.

ABSTRACT. A way of examining the heteropyknotic body is described, using cells from prolegs amputated from living larvae. Larval survival rate is high and the results are accurate. Prospective testing for the presence or absence of sex chromatin is particularly valuable in studying intersexes, e.g., in *Lymantria dispar*, where the adult phenotype is not necessarily an indication of the chromosome constitution of the larva.

It is well known that in Lepidoptera a heteropyknotic body may be found in the somatic cells of the female whereas it is lacking in the male (Smith, 1945). However, there are some exceptions to this rule (Traut and Mosbacher, 1968); for example, in *Papilio machaon* L. the male is polymorphic for the character (Clarke *et al.*, 1977). There is also good evidence that in the female the body is derived from the W(=Y) chromosome (Suomalainen, 1969; Traut and Rathjens, 1973) and that where it is present in the male it is associated with a particular autosome (Clarke *et al.*, 1977).

Testing for the "Smith" status has usually been carried out on freshly killed larvae or adults, but Daker (1977) showed that in *Hypolimnas bolina* L. it was possible to assess it from a spine taken from a living larva which thereafter usually developed normally.

In the present paper we show that it is also possible to obtain good preparations using a proleg of last instar larvae. We, in fact, found that our preparations from prolegs were of better quality than those from spines. Moreover, this method is particularly useful in dealing with larvae which have no spines.

MATERIALS AND METHODS

The species investigated were *Papilio glaucus* L., *Papilio dardanus* Brown, *Euploea core amymone* Cr., *Hypolimnas bolina* L. and *Lymantria dispar* L.

The *P. glaucus* stock was bred at Caldy, Wirral, and derived from two females from Virginia, U.S.A., kindly supplied by Prof. J. J. Murray. Mrs. Jennifer Maddison of Ibadan sent the butterflies from which all the Nigerian *P. dardanus* stock was bred and Mrs. Gweneth Johnston posted to us living *E. core* butterflies from Hong Kong which produced the tested larvae. In *H. bolina*, race hybrid stock was used, the parent forms coming from Sarawak (from Mr. Stephen Kueh) and Sri Lanka (from Mr.

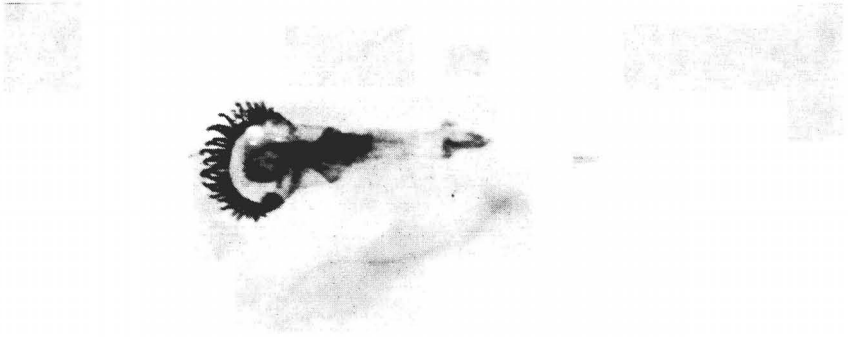


FIG. 1. Proleg tip of *H. bolina* larva showing tissue scraped out. (As seen under dissecting microscope.)



FIG. 2. Nuclei of tissue cells containing heteropyknotic body from a larva which developed into a female butterfly. (Using $\times 90$ —oil immersion—objective.)

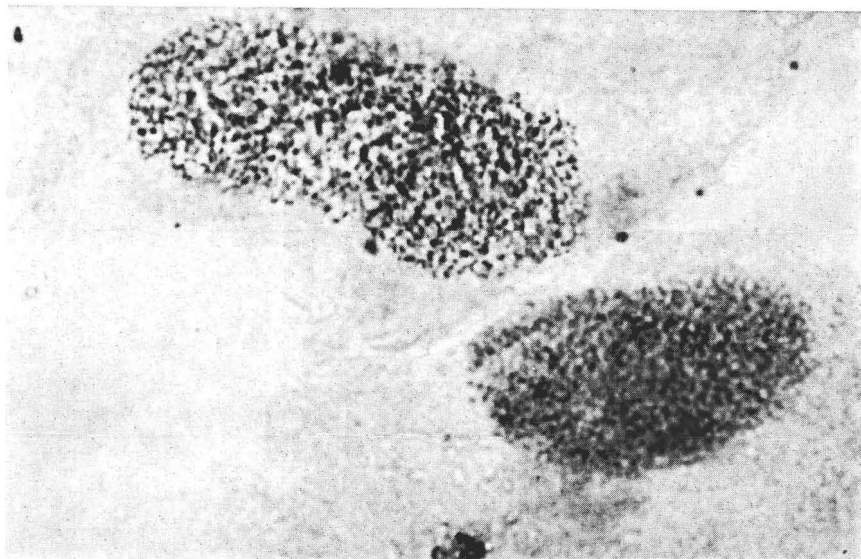


FIG. 3. Nuclei of tissue cells lacking heteropyknotic body from a larva which developed into a male butterfly. (Using $\times 90$ —oil immersion—objective.) Editor's note: photograph reduced to $\frac{3}{4}$ size of original.

P. B. Karunaratne. The pure Japanese broods of *L. dispar* originated from a wild Nagoya female supplied by Dr. Shigeru Ae, and the hybrid brood was from a mating between a German female (from Herr Willy Schultz) and a bred Nagoya male.

In the early experiments, the larva to be tested was lightly anesthetized, and then the extreme tip of one of the abdominal prolegs (Fig. 1) was removed with a sharp pair of dissecting scissors. Later it was found that better survival was obtained without an anesthetic. Enough tissue can be scraped from the inside of the proleg to make one good preparation; the material is teased out and spread as thinly as possible. After the amputation each larva was kept separately.

The cells are not fixed before staining. Two drops of 2% orcein in 45% acetic acid are placed over the tissue and a coverslip added immediately. After 10–15 minutes the coverslip is firmly pressed to make a "squash" preparation. The "Smith" body when present can be clearly seen under the $\times 40$ objective as well as under the $\times 90$ (oil immersion) objective (Figs. 2 & 3).

RESULTS

Results are shown in Table 1. The accuracy of the method is assessed by noting the sex of the butterfly or moth when it emerges, and in the

TABLE 1. Adult emergences and pupae (sexed externally) from several species of Lepidoptera tested for sex chromatin using cells from the larval proleg.

Brood no. & species	No. of larvae tested for "Smith" body	Sex chromatin assessment of larvae	Emergences from tested larvae	Deaths in larval & pupal stages	Overwintering tested pupae sexed externally
15050 <i>P. glaucus</i> ex black female form	9	8 positive 1 negative	6 black females 1 male	2 positive —	— —
15094 <i>P. glaucus</i> ex yellow female form	19	10 positive 9 negative	1 yellow female —	5 positive 4 negative	6 females ¹ 3 males
15219 Joint no. given to several generations of <i>P. dardanus</i> ex female <i>hippocoon</i>	14	1 positive ² 13 negative	— 2 females, 8 males	1 positive 3 negative ³	— —
509z Joint no. given to several generations of <i>E. core amymone</i>	3	1 positive 2 negative	1 female 1 male	— 1 negative	— —
524z Joint no. given to several generations of <i>H. bolina</i> hybrids Sarawak × Sri Lanka	22	11 positive 11 negative	6 females 5 males	5 positive 6 negative	— —
14983 & 14984 <i>L. dispar</i> Japanese race	12	6 positive 6 negative	5 females 3 males	1 positive 3 negative	— —
14985 <i>L. dispar</i> F1 race cross ♀ German × ♂ Japanese	14	12 positive 2 negative	10 females 2 males	2 positive —	— —

¹ Two of these 6 female pupae developed from "Smith" negative larvae. To date, 4 yellow females have emerged from this brood; 2 were "Smith" positive and were "Smith" negative.

² This was the only positive finding in at least 40 insects of both sexes in this brood, tested at various stages of development. Unfortunately the larva died and the cell nuclei were too degenerate to perform a confirmatory test.

³ These 3 insects were killed when moribund and the test was confirmed as "Smith" negative on gut cells.

case of overwintering insects, by scoring the sex of the pupa by its external appearance. Concordance between the larval score and the adult or pupal sex is high.

DISCUSSION

Several points are of interest:

In *P. glaucus*, it had been reported previously (Clarke *et al.*, 1976) that the black females were "Smith" positive and the yellow ones (which are male-like) and the males were negative. It is now clear that frequently yellow females are positive, and there appears to be a polymorphism for the character in the yellow form. All black females have, however, so far been positive.

In *P. dardanus*, in the present material, the *hippocoön* females are consistently negative, though previously a few insects of this and other female forms have been positive, so that here again there is a polymorphism.

This information in both these species could clearly have been obtained without sexing the larva, but prospective testing has the great advantage that it is possible to select and breed from a female of known "Smith" status. Moreover, it obviates the necessity of testing her immediately after death which is obligatory because rapid degeneration of the cell nuclei occurs post-mortem.

The most valuable application of the method, however, will become evident when *L. dispar* is further studied. Here, in race crosses, intersexes may occur (Goldschmidt, 1933), and it will be most informative to relate the sex chromatin status to the phenotype and to the gonadal morphology.

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BOOK REVIEW

BUTTERFLIES. Text by Jo Brewer, photographs by Kjell B. Sandved. 1976. Harry N. Abrams, New York. 176 pp., ill. Price: hardcover, \$18.95; softcover, \$9.95, U.S.

In the last few years a number of fine popular volumes with exquisitely colored plates have been published, but none has had such an innovative and refreshing approach as this book. Since it is broad in scope and supplies the necessary basic information for the study of Lepidoptera in the clear, concise manner for which Brewer is duly noted, the book should stimulate an interest in and appreciation for the insect group from a technical as well as an aesthetic point of view. It is well illustrated with 245 photographs (133 in color) and additional line drawings and scanning electron micrographs.

The organization of the book is quite a departure from traditional treatments. There is a section on the economic impact of butterflies on man ("Historical Notes on Butterflies, Moths and Men"). The section on "Butterflies in Art, Heraldry and Religion" which chronicles the symbolic impact of butterflies on man in everyday life and in legend is especially noteworthy. The remaining sections delve into those areas which man finds so curiously fascinating: metamorphosis, ornamentation of the wings, the compound eye and protective devices. The section on the wings not only examines the physical aspects in terms of wing scales, pigmentation and wing formation, but also the mechanics involved in temperature regulation and flight, all through the enchanted photographic eye of Kjell Sandved. In "Protective Devices," deception, warning coloration and camouflage are discussed. There is also a brief explanation of Batesian and Müllerian mimicry, along with a discussion of larval specificity on certain toxic hostplants and the important role which these plants play in mimetic associations.

In such a volume which includes an array of photographs, there are some organizational problems in fitting the plates with the appropriate text. The last 25 pages illustrate further intricate designs and structural iridescence, so intriguing to the natural observer. While these are interesting, they seem somewhat superfluous. In a few cases the identifications are incorrect or not in keeping with current literature such as *Thecla syncellus* (= *Panhiades bitias*).

The above points by no means diminish the utility and significance of this book for its intended audience. Its true value will be realized indeed by the enthusiasm and appreciation generated for this diverse biological group in both aspiring and professional lepidopterists alike.

JACQUELINE Y. MILLER, *Allyn Museum of Entomology, 3710 Bay Shore Road, Sarasota, Florida 33580.*