U.S. FOREST SERVICE RESEARCH NOTE NE-2

1963

Northeastern Forest Experiment Station

FOREST SERVICE, U.S. DEPT. OF AGRICULTURE, 102 MOTORS AVENUE, UPPER DARBY, PA.

A TECHNIQUE FOR SEXING FULLY DEVELOPED EMBRYOS AND EARLY-INSTAR LARVAE OF THE GYPSY MOTH

Because variation in sex ratio is an important factor in the population dynamics of the gypsy moth (*Porthetria dispar*), it is necessary to have some means of determining the ratio of males to females in a population at the beginning of the larval period as well as in the later stages. For determining the sex of fully developed embryos and early-instar larvae, a simple whole-mount microscope technique has been devised. The procedure is as follows:

Alcohol-preserved larvae are placed in an 88-percent liquefied phenol (carbolic acid) solution for a minimum of 2 hours; then they are transferred to an 88-percent liquefied phenol-rose bengal stain solution. The optimum phenol/stain ratio is 99.5/0.5 by weight.

Live larvae and fully developed embryos (with egg chorion punctured or removed) are placed directly in the 88-percent liquefied phenol-rose bengal stain solution.

The larvae are left in the phenol-stain solution for at least 48 hours; punctured eggs require a minimum of 72 hours in the solution. The specimens are then transferred directly to 1- by 3-inch microscope slides. Three specimens can be placed on each slide.

A cover glass is placed over each specimen, and pressure is applied. Under light pressure the integument of the treated insect fractures readily, allowing the internal organs (including the gonads) to flow out through the anal opening or through a break in the abdominal wall.

Each mount is first scanned under low magnification (X 35) to locate the gonads and then under higher power (X 100) to make positive iden-

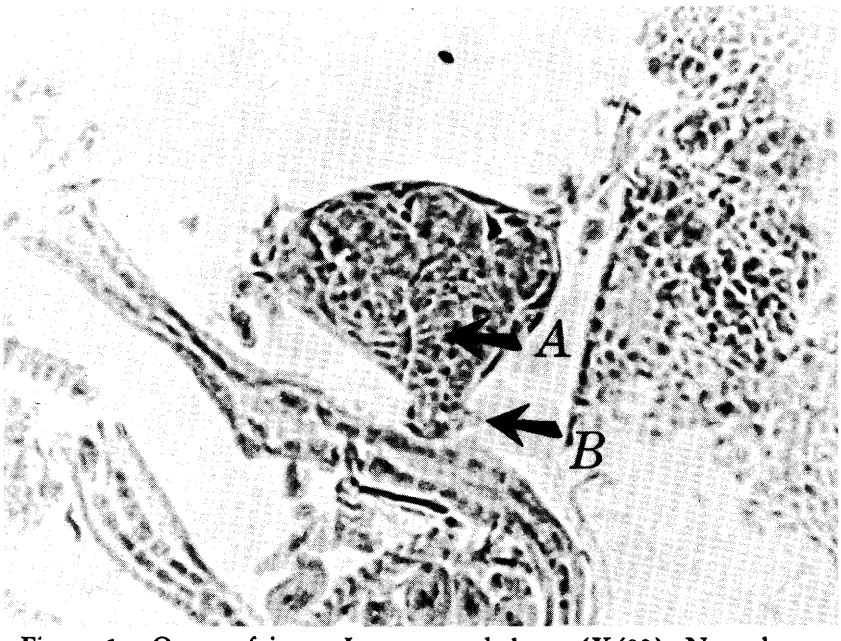

Figure 1.—Ovary of instar I gypsy moth larva (X400). Note the three lobes, the stalk-like outgrowth (A), and the calyx (B).

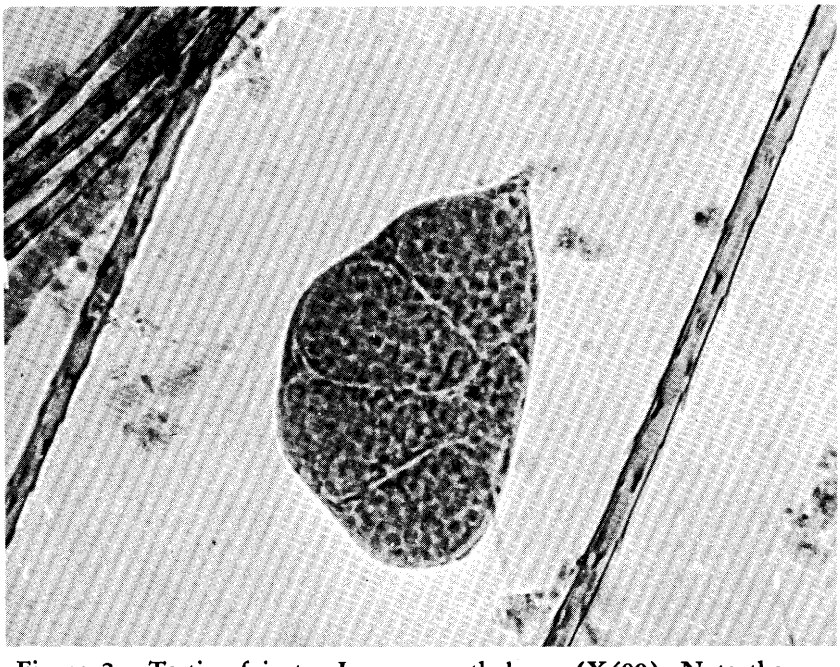

Figure 2.—Testis of instar I gypsy moth larva (X400). Note the four distinct lobes; no stalk-like outgrowth or calyx is present.

tification of the sex. The contrasting characters of the female and male gonads show up clearly.

In the female, the ovaries remain undifferentiated in the embryo and in the hatched first-instar larva. Later in the first instar three definite lobes are formed, which lead to or are connected with a stalk-like outgrowth (fig. 1). Occasionally there appears to be a fourth lobe, but it is apart from the ovarial stalk. The stalk has an enlargement at the distal end, at its junction with the oviduct. This enlargement, or calyx, is normally funnel-shaped and can be seen clearly even in the embryo stage. When pressed between the cover glass and slides, the ovaries present a round to oval-oblong shape.

In the male, the testes consist of four lobes each, all well-defined from the fully developed embryo stage through the later instars (fig. 2). No stalk-like outgrowth or calyx is present. The testes usually appear kidney-shaped.

This technique, which has been used successfully at the Forest Insect Laboratory of the Northeastern Forest Experiment Station at New Haven, Conn., may be applicable to other lepidopterous species.

The mounts can be made in advance; the phenol stain solution will not crystallize. After use, the slides may be washed in running water and used again.

CAUTION: Phenol is highly corrosive to human tissue; so care must be taken in handling it. In case of direct contact with the skin, soap and running water should be applied immediately. The phenol should always be kept in screw-cap glass containers.

— GILBERT LEVESQUE

Biological Aide
Forest Insect Laboratory
Northeastern Forest Experiment Station
Forest Service, U. S. Dept. Agriculture
New Haven, Conn.