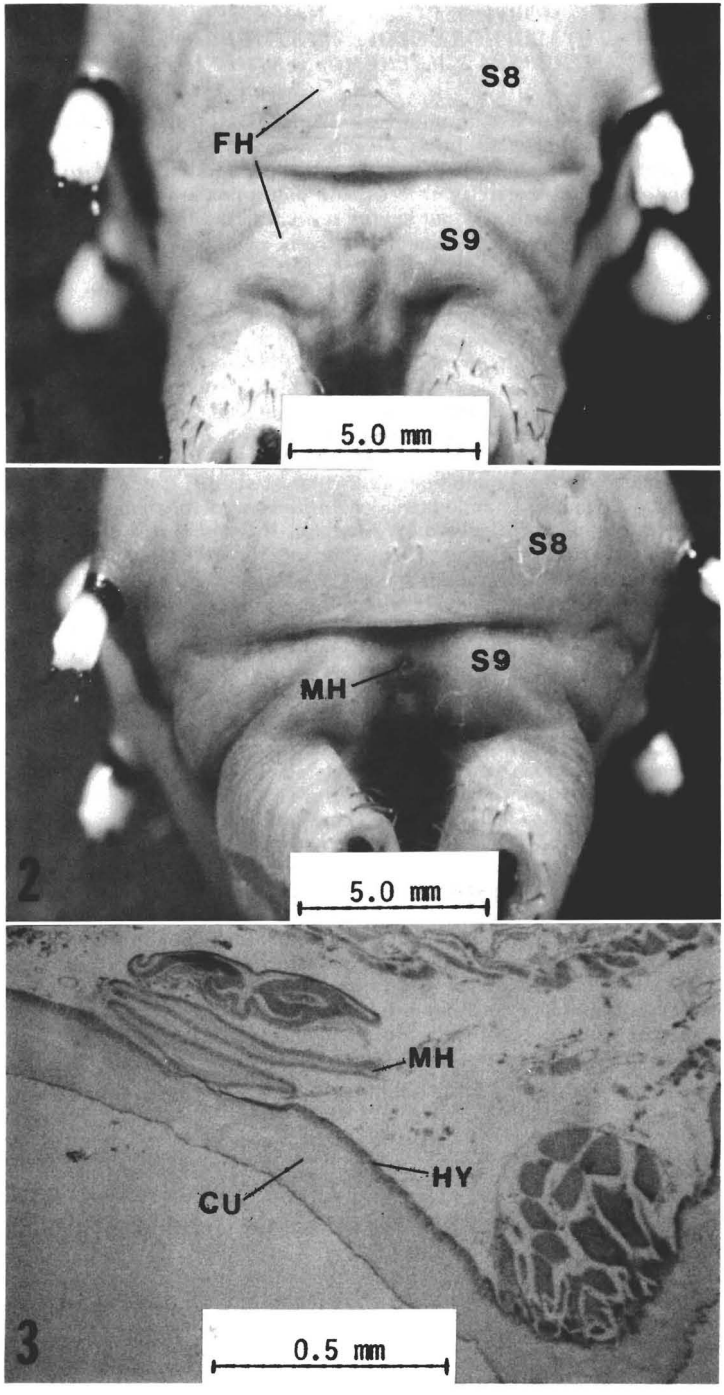
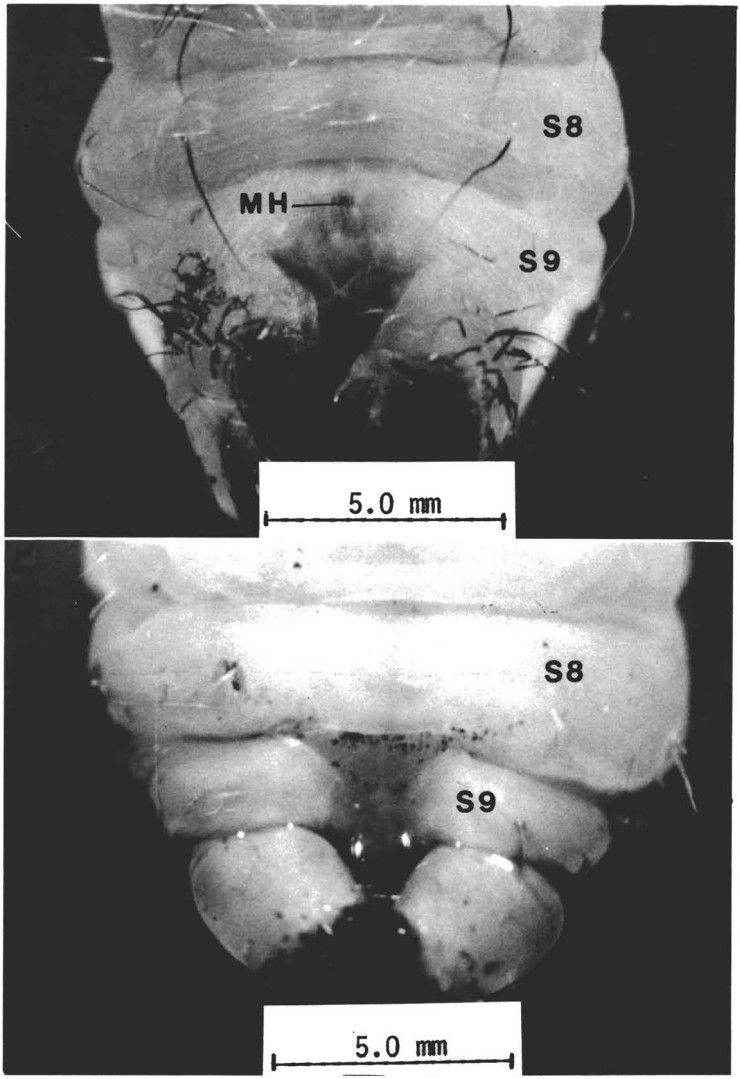
Journal of the Lepidopterists' Society
34(1), 1980, 69–73

SEX-RELATED MORPHOLOGICAL CHARACTERS IN LARVAE OF *HYALOPHORA GLOVERI GLOVERI* AND *ANTHERAEA POLYPHEMUS* (SATURNIIDAE)

Information on sex-related morphological characters that occur in giant silkworm moth larvae has a number of useful applications, including: determination of sex in individual larvae, maintenance of proper sex ratios in breeding stock, rearing of one particular sex for special research purposes, and determination of sex ratios in experimental groups of individuals without having to rear them to pupation or adult emergence. Sex-related morphological characters have been reported for a number of lepidopterous larvae (Stehr & Cook, 1968, Bull. U.S. Nat. Mus. 276: 46–47; Hinks & Byers, 1973, Can. J. Zool. 51: 1235–1241; Kean & Platt, 1973, J. Lepid. Soc. 27: 122–129; Miller et al., 1977, J. Lepid. Soc. 31: 144–146). These characters vary somewhat in form, but they appear to represent either the genital histoblasts, visible externally through the integument; or pits or modifications of the integument associated with the genital histoblasts. In female larvae the genital histoblasts, or modifications of the integument, are found as paired structures on the venter of the 8th and 9th abdominal segments (S8 and S9). In male larvae the genital histoblast, or the modified integument, is found as a single structure on the venter of S9. Examination of 4th- and 5th-instar larvae of four giant silkworm moth species (Miller et al., 1977) demonstrated the occurrence of these characters in male larvae of *Antheraea polyphemus* (Cramer); and in female larvae of *Eupackardia calleta* (Westwood), *Hyalophora cecropia* (Linnaeus), and *Callosamia promethea* (Drury). This paper reports the occurrence of such characters in another giant silkworm moth species, *Hyalophora gloveri gloveri* (Strecker), and provides additional observations on characters previously reported by Miller et al. (1977) for *A. polyphemus*.

The *H. gloveri gloveri* larvae used in this study were from a small breeding-stock colony maintained in sleeve cages on wild black cherry (*Prunus serotina*) in Frederick Co., Maryland. Newly-hatched, 1st-instar larvae were set up in a separate sleeve cage on wild black cherry. They were removed from the sleeves as early 5th-instar individuals and categorized as male or female on the basis of the morphological characters discussed earlier. The larvae were then segregated according to sex into two sleeve cages and reared to pupation to confirm the sex of each individual. A small number of other early 5th-instar individuals from the same original sleeve cage were killed in neutral buffered formalin. These larvae were used, as freshly-killed individuals, to photographically record the appearance of the external morphological characters; and later for histological sectioning and examination for internal histoblasts. In the larvae for histological sectioning, the appropriate abdominal segments (S8 for females; S9 for males) were removed with a scalpel. The fixed abdominal segments were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Seventeen living 5th-instar larvae of *H. gloveri gloveri* were examined for external morphological characters. Seven individuals possessed fairly distinct, white, subsurface spheres between the ventral and subventral setae on S8 and S9 (Fig. 1) and were categorized as females; 10 individuals possessed a single, dark pit on the venter of S9 (Fig. 2) and were categorized as males. These larvae, after being segregated and reared to pupation, produced 7 female pupae and 10 male pupae corresponding to, and confirming, the sex determinations made in the larval stage. Examination of transverse sections prepared from female larvae of *H. gloveri gloveri* did not reveal any structures that could be related to the white, subsurface spheres that had been observed in living specimens. These negative findings are presently without explanation. It is possible that the spheres observed in living larvae were lost in sectioning, particularly if they did not have a firm attachment to the integument. Also, it is possible that the structures that we observed externally were missed in the sectioning process, since we could not prepare thin sections for the entire larval abdominal tissue. Transverse sections from male *H. gloveri gloveri* larvae revealed the presence of an internal structure (Fig. 3) associated with the dark pit observed in living larvae. This structure is similar in form

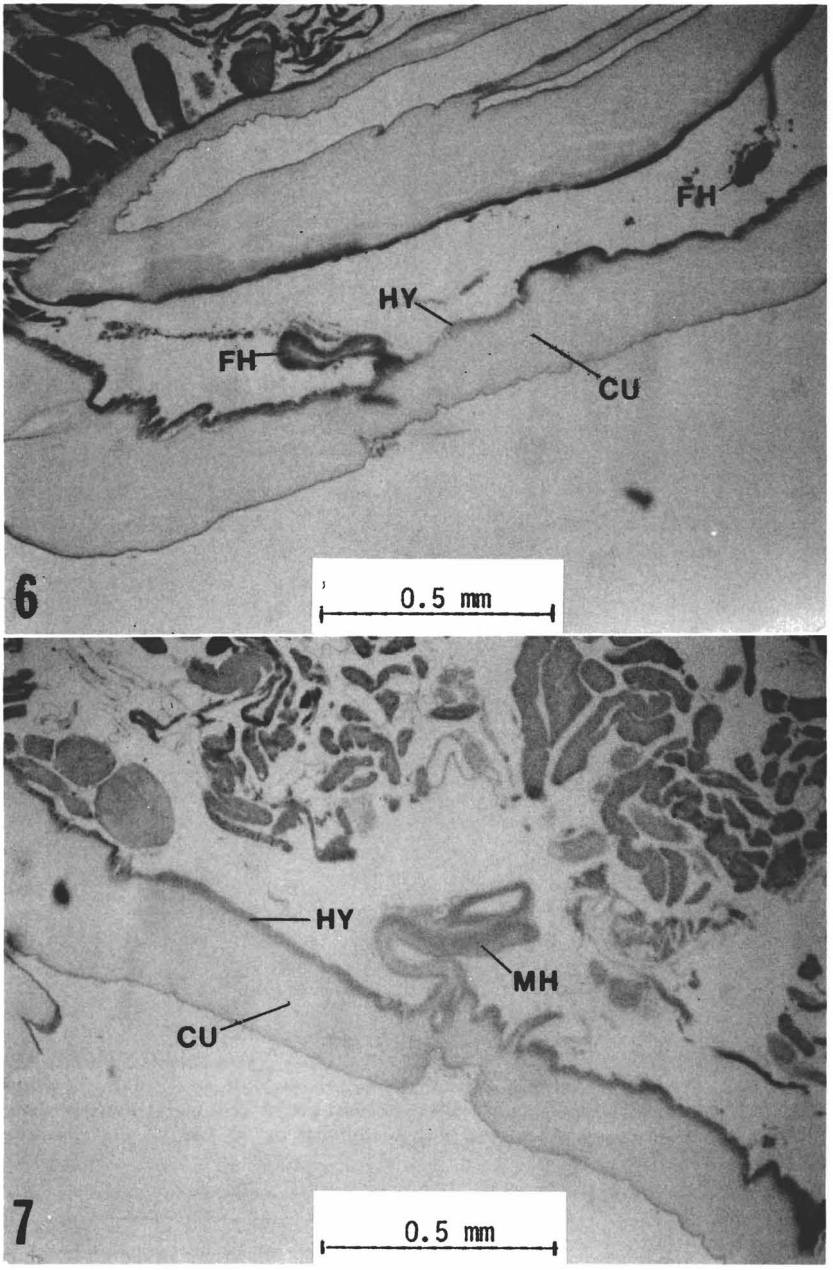

S8
FH
S9
5.0 mm
1
S8
S9
MH
5.0 mm
2
MH
HY
CU
0.5 mm
3



FIGS. 4–5. Ventral views of 5th-instar *A. polyphemus* larvae. 4, male showing location of black pit (MH) associated with histoblast on S9. 5, female showing absence of any external structures associated with histoblasts on S8 and S9, and absence of black pit on S9.

←

FIGS. 1–3. Sex-related morphological characters in 5th-instar larvae of *H. gloveri gloveri*. 1, ventral view of female showing white, subsurface spheres (FH) associated with histoblasts on S8 and S9. 2, ventral view of male showing dark pit (MH) associated with histoblast on S9. 3, transverse section of S9 of male showing single histoblast associated with dark pit observed externally in living larvae. CU, cuticle; FH, female histoblast; HY, hypodermis; MH, male histoblast.



FIGS. 6–7. Transverse sections of venter of S8 and S9 of 5th-instar *A. polyphemus* larvae. 6, female showing paired histoblasts in S8. 7, male showing single histoblast associated with black pit on S9. CU, cuticle; FH, female histoblast; MH, male histoblast; HY, hypodermis.

to the male histoblast (Herold's Organ) described by Hinks & Byers (1973) for noctuid larvae and probably represents the male genital histoblast in *H. gloveri gloveri.*

The *A. polyphemus* larvae used in this study were from a colony maintained in sleeve cages on various maples (*Acer* spp.) in Frederick County, Maryland. During routine maintenance of the colony, we have cursorily examined 4th- and 5th-instar larvae for the presence of external sex-related characters. After several hundred such examinations, we have observed only the male character, in the form of a black pit on the venter of S9, as described earlier by Miller et al. (1977). To improve our ability to reliably determine sex in larvae of this species, we made more detailed examination of a series of individuals. The objective was to locate and describe any external female characters that correspond in form and location to those found in females of other species discussed heretofore. If characters thought to represent female histoblasts were observed, we planned to rear larvae to pupation to verify sex. Larvae were removed from the sleeves as early 5th-instar individuals and examined with the unaided eye for the presence or absence of the black pit characteristic of the male; those without a black pit were examined in greater detail for the presence of female characters using a dissecting microscope (60×). A few 5th-instar larvae from the colony were killed in neutral buffered formalin and used, as freshly-killed individuals, to record photographically the external appearance of S8 and S9; and later for histological sectioning and examination for histoblasts. Histological sectioning was accomplished in the same manner as discussed earlier for *H. gloveri gloveri.* We examined forty-nine 5th-instar larvae of *A. polyphemus.* Nineteen of these had a black pit on the venter of S9 (Fig. 4). In the other 30 individuals we could find no characters that appeared to represent either male or female histoblasts, or modifications of the associated integument, on S8 or S9 (Fig. 5). Although these conditions were described earlier by Miller et al. (1977), they were not illustrated; therefore, we have included them here as figures. Since no female characters were observed, we did not rear any of these 49 individuals to pupation to confirm sex. The information presented by Miller et al. (1977) adequately demonstrated the sex-related nature of the black pit (i.e., pit present = male; pit absent = female) and additional confirmatory data based on our observations was not necessary. In the female *A. polyphemus* larvae, transverse histological sections revealed the presence of paired, pyriform histoblasts (Fig. 6) arising from the ventral hypodermis in the areas between the ventral and subventral setae of S8. There were no apparent modifications of the associated integument, an observation that was also made during examination of living larvae. Transverse sections from male *A. polyphemus* larvae revealed the presence of an internal structure (Fig. 7) associated with the black pit observed in living larvae. This structure, which is elongate and appears to have a firm attachment to the hypodermis, appears to represent the male genital histoblast in *A. polyphemus.* It is similar to the male histoblast (Herold's Organ) described for noctuid larvae by Hinks & Byers (1973).

We have concluded from these observations that the presence of sex-related morphological characters in *H. gloveri gloveri* is confirmed; and that the characters can be reliably used to sex larvae of this species. Although we found both male and female histoblasts in larvae of *A. polyphemus,* those of the female are small and not visible externally; and the associated integument is not modified. Therefore, we have concluded that living larvae of *A. polyphemus* can be sexed only on the basis of the presence or absence of the male character.

THOMAS A. MILLER, *U.S. Army Medical Bioengineering Research & Development Laboratory and* SAMUEL V. MACHOTKA, *U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 27101.* (The opinions contained herein are those of the authors and should not be construed as official or reflecting the views of the Department of the Army.)