

## SCIENTIFIC NOTE

### DETERMINATION OF THE SEX OF CATERPILLARS WITHOUT DISSECTION

LUCIEN LAVENSEAU

Laboratoire de Neuroendocrinologie, E.R.A. CNRS no. 850, Université de Bordeaux I, 351 Cours de la Libération, 33405 Talence Cédex, France

(Accepted 11 June 1982)

SEX determination in larval Lepidoptera is a basic problem for a large range of studies such as hormone and metabolite assays, heterosexual grafts, or analysis of postembryonic developmental and endocrine processes. Methods previously proposed concern only few species. Sometimes, coloured testes are visible through the larval cuticle, such as in *Ephestia kühniella* (Kühn and Henke, 1929), *Ephestia cautella*, *Plodia interpunctella* and *Paramyelois transitella* (Oberlander, personal communication). Rarely, evident morphological details are used. *Orgyia antiqua* for example, exhibits during the 5th larval instar dorsoabdominal bristle tufts differently coloured in the male and the female (Paul, 1937). In the last instar larvae of *Bombyx mori*, obvious Ishiwata's spots can be observed on the ventral side of the abdominal end (Bounhiol, 1938). Some other authors recommend more drastic methods, which are not convenient to differentiate larvae destined for an operation. Fedotov (1939), in Psychidae, examined the gonads by means of an abdominal opening, and Frizzi (1947) in *Bombyx mori* and Allegret (1956) in *Galleria mellonella* stained an heterochromatic body characteristic of female silk gland cells. Finally, other methods appear quite demanding and rather imprecise, such as the weight of the caterpillars or the mean diameter of their head capsules. Those concerning the pupal stage are unsuitable for many experiments. None of these criteria gave us adequate information for the species used in our experiments. Consequently, we searched for a method in which the larvae will not be sacrificed and, if possible, usable for early larval instars. During the preliminary phase of our study, we sacrificed caterpillars and examined their gonads. Those dissections showed a constant correlation between the nature of the gonads and some external signs. Recognition of only such external signs allows determination of larval sex. The devised method is being currently used in our laboratory and has been tried on other wild or bred strains of caterpillars.

For scanning electron microscopy, anesthetized larvae were injected with ethyl alcohol, then dehydrated. The abdominal end, set apart, was carbonated *in vacuo*, then coated with gold-palladium film by cathodic sputtering. The specimens were observed with a Siemens autoscan at 10–30 Kv. Nineteen species from 8 families were examined. *Pachytelia unicolor* (Psychidae), *Ostrinia nubilalis* (Pyralidae), *Galleria mellonella* (Pyralidae), *Paramyelois transitella* (Pyralidae), *Ephestia cautella* (Pyralidae), *Thaumetopoea pityocampa* (Notodontidae), *Lymantria dispar* (Lymantriidae), *Orgyia antiqua* (Lymantriidae), *Sesamia nonagrioides* (Noctuidae), *Heliothis armigera* (Noctuidae), *Earias biplaga* (Noctuidae), *Cirphis unipuncta* (Noctuidae), *Arctia caja* (Arctiidae), *Ilema* sp. (Arctiidae), *Pyrrharctia isabella* (Arctiidae), *Bombyx mori* (Bombycidae), *Antheraea pernyi* (Attacidae), *Philosamia cynthia* (Attacidae), *Pieris brassicae* (Pieridae). Our method is suitable for most caterpillars, except the very small ones, like *Polychrosis botrana* or *Clysia ambiguella* (Tortricidae).

In all studied species, a careful observation either of the 8th or both the 8th and the 9th abdominal segments shows 2 minute cuticular depressions on each segment in the female. Always symmetrical with regard to the anterioresposterior axis of the body, they are present between 2 bristles or 2 groups of bristles. Their location is obvious after the 3rd larval stage in large species (Figs 1, 2). On the other hand, in small species (Figs 3, 4), this little pit latter appears as smooth and shining stria amidst the frosted cuticle. Male abdomen tips appear to be devoid of such anatomical marks. Particularly in small species, the observation is easier if the larvae are alive, epidermis is clean and dry, and the abdomen pressed to provoke swelling. Fixed specimens are still usable, but with more difficulty. Grazing lighting helps for a better observation. The scanning electron microscope enhances the sexual differences (Figs 5–8) and shows the nature of the observed structures (Fig. 9). According to the studies of Bounhiol and Front-Bourgin (1962) in *Bombyx mori*, Leclercq-Smekens (1976) in *Euproctis*, we, interpret these depressions as the visible part of the ectodermal infoldings forming the genital imaginal disks. These disks give rise to the terminal part of the genital duct: *bursa copulatrix*, spermatheca, vagina, and

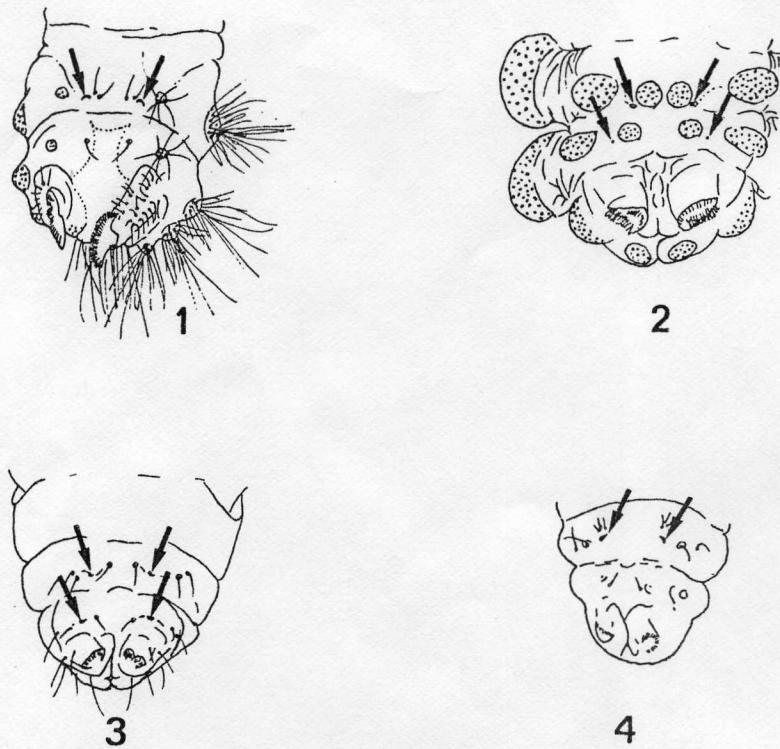


FIG. 1. Optical observation of abdominal end of female *Lymantria dispar* (ventral side). Characteristic pits are indicated by black arrows.  $\times 4$ .

FIG. 2. Same details in female *Pyrrharctia isabella*. Only base of bristles groups are represented.  $\times 4.5$ .

FIG. 3. Same details in female *Paramyelois transitella*.  $\times 13.5$ .

FIG. 4. Same details in female *Pachytelia unicolor*. In such a small larva, cuticle appears more shiny at level of depressions.  $\times 10$ .

accessory glands. Based upon the existence of imaginal disks in all the studied larvae, the proposed method can likely be extended to all Lepidoptera Ditrysia species of adequate size (more than 1-cm long). Its use is less reliable in some cases such as *Solenobia triquetrella* (Amman, 1954; Brunold, 1957) and *Choristoneura murinana* (Wittig, 1960) in which genital imaginal disks appear late in the larval life. It should be noted that the most commonly used optical magnifications are not sufficient to determine the sex of the youngest larvae or of the larvae from very small species.

*Acknowledgements*—I am very grateful to Professors P. Allegret, B. J. R. Philogène, H. Oberlander and A. Maleville for kindly supplying me with different species of Lepidopterous larvae.

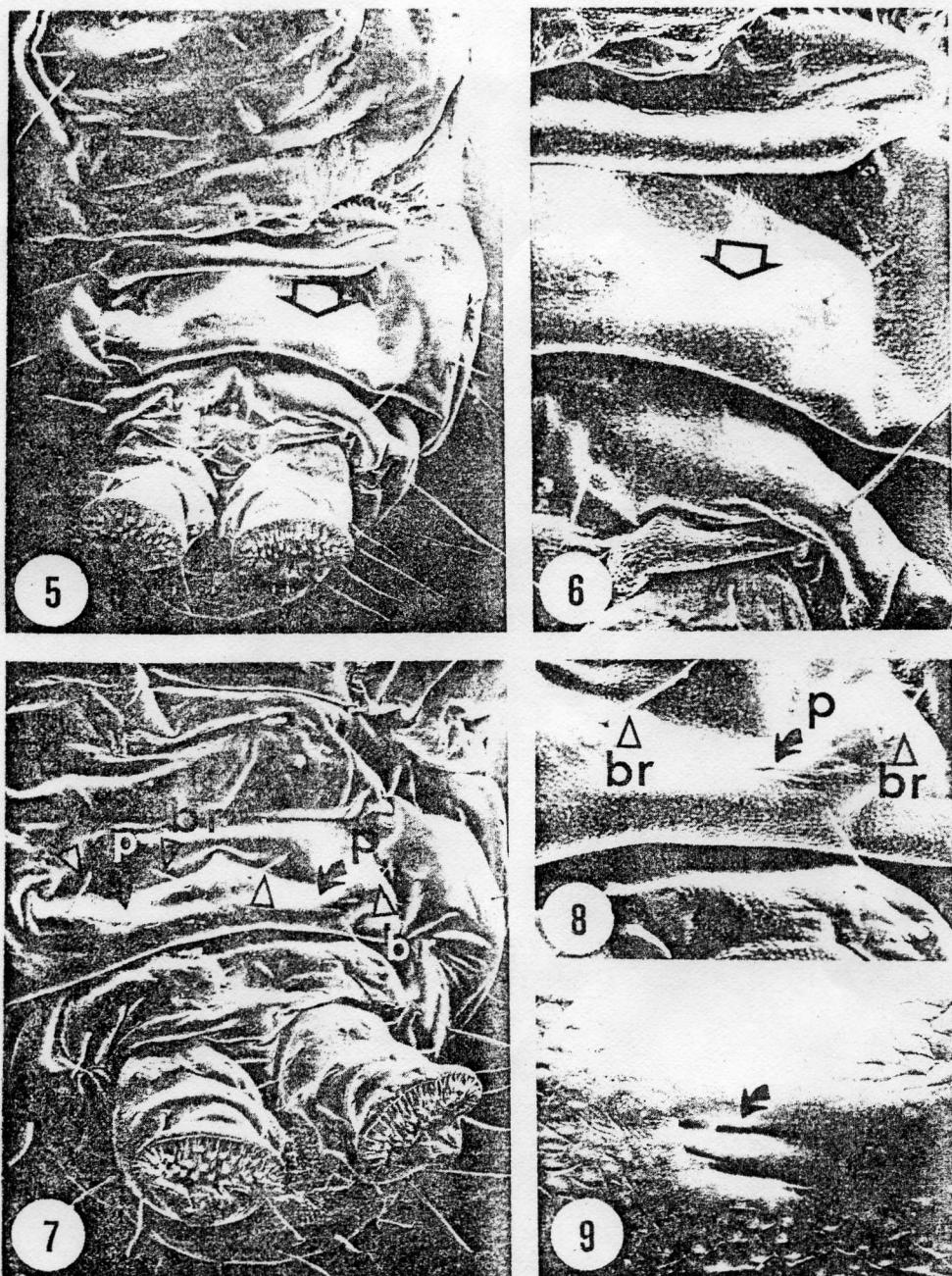


FIG. 5. Scanning electron microscope observation of abdominal end of male *Ostrinia nubilalis* larva (European corn borer). Compare with Fig. 7.  $\times 40$ .

FIG. 6. Detail of previous illustration (zone with light arrow). Compare with Fig. 9.  $\times 65$ .

FIG. 7. Abdominal end of female *Ostrinia nubilalis* larva. Paired pits (p) are clearly visible between typical bristles (br). Female abdomen is broader than that of male.  $\times 40$ .

FIG. 8. Detail of zone surrounding left pit (p), between 2 characteristic bristles (br).  $\times 70$ .

FIG. 9. Detail of left side mark. Cuticle is obviously smooth at this level, contrary to usual rough pavement aspect. In center of smooth zone, pits indicate attachment points of underlying genital imaginal disk.  $\times 400$ .

## **Sexing of Lymantriid Moths**

The difference between male and female larvae is that females have genital (sex-) pores and males do not. To be able to determine if an individual has these pores (and is a female) you will need to use a **microscope** with a **fiber optic light source** (on shelf on eastern wall, room 845a). You will also need some **needles** (preferably those with a wooden handle), **forceps** and some patience. All except patience can be found in DRAWER 060.

Larvae in vials should be taken out of the freezer at least 30 minutes before examination. **DO NOT** open the vial until it has warmed up to room temperature, otherwise you will have the larvae covered with water droplets. After about 30 minutes pick the larvae out of the vial and place it on a paper towel under the microscope. The larvae should have its ventral side up (i.e. lying on its back), and be as straightened out as possible. Focus on the rear end. You will see (check out the handdrawn figure for guidance):

- \* The two hind legs
- \* Hairy bulbs, several pairs, located at the sides
- \* Smaller hairy spots, pairs located between the pairs of hairy bulbs

Now, look at the last two (most posterior) pairs of hairy bulbs and small hairy spots. If you have problems finding the small hairy spot, do some focussing back and forth to see where the hairs come out. If sex pores are present you will find them right between the big hairy bulb and the small hairy spot. The sex pore is a dark spot (sometimes silvery on Gypsy Moth females). In all there are four of them (two pairs). If you see at least three you can be sure it is a female. If you only see e.g. two: keep on looking. It might help to use the needle to squeeze the fluid contents of the larvae towards the rear end, hereby filling out the skin and make the larvae less crumbled up. Also, press/move the part of the skin you are looking at with the needle, since sex pores may sometimes be 'hidden' in small folds.

### Special for White Marked Tussock moths:

WMT are usually all yellow in the area you are looking. Sex pores are therefore comparably easy to spot. Usually they are also *surrounded by a deeper/darker yellow area*, looking like some tissue visible through the skin. Sex pores are usually *located somewhat closer to the small hairy spot than to the hairy bulb*.

### Special for Gypsy moths:

GM are more difficult to sex because they have a brown and heterogeneously colored ventral side. Sex pores may look *silvery/shiny*, and are always *located right between the hairy bulb and the small hairy spot*.

**NOTE:** You cannot give up on a larvae and redo it later; once it starts to dry up you will not be able to identify anything on it. Just keep on going until you either find three pores, or you are convinced that there are none. Sometimes there might be a dark (pigmented) spot in one of the four positions which may confuse you, but if you do not find any more it is probably a male anyway.

### Time:

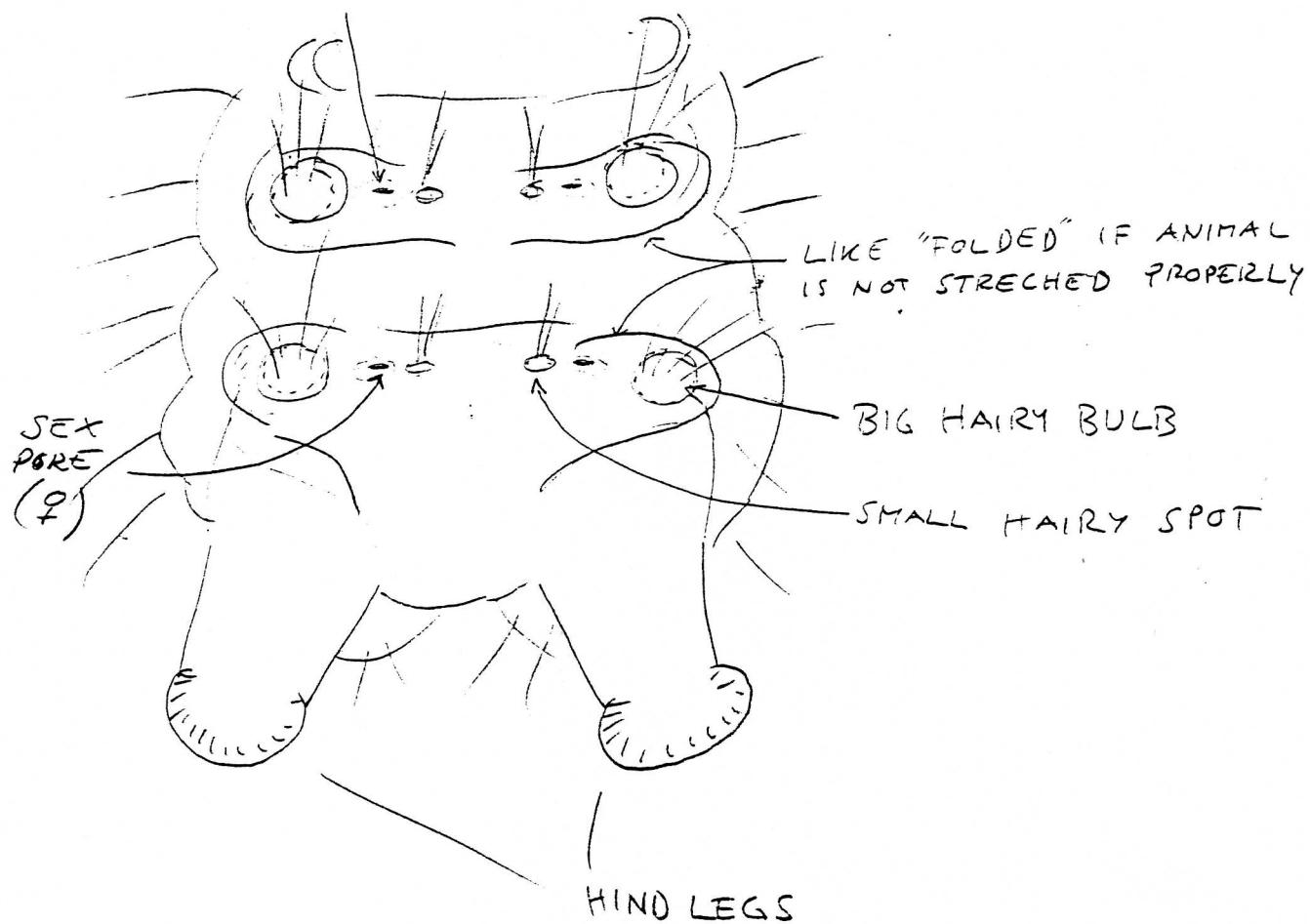
When you get the hang of this you may process about **30-35 WMT/hour** or **10-15 GM/hour**. How time consuming this process is also depend on the size of the larvae.

# White Marked Tussock Moth / Gypsy Moth

REAR END - VENTRAL (UNDER)-SIDE.

SEXPORES ON FEMALES ARE USUALLY RIGHT  
BETWEEN THE BIG HAIRBULB AND THE HAIRY SPOT  
(USUALLY CLOSER TO HAIRY SPOT IN WMT.)

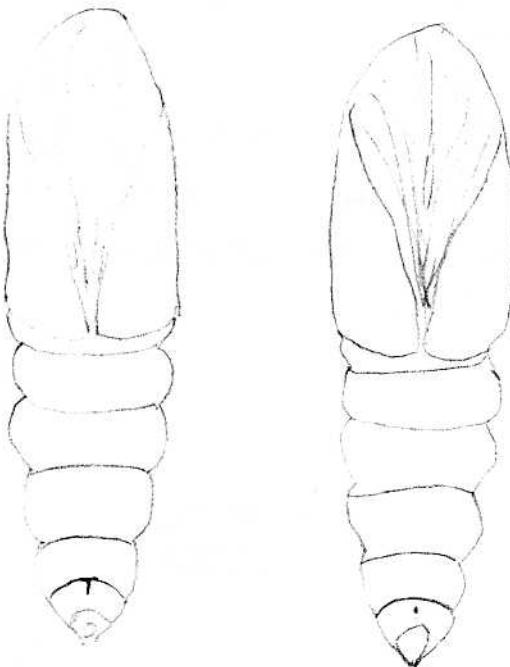
SEXPORE OFTEN SURROUNDED BY  
DARK YELLOW AREA IN WMT



For GYPSET MOTH:

Basically look for the same set pores in females, pores may have a "silvery" look. Pores usually more centered between BIG HAIRBULB and HAIRY SPOT

Sexing GM pupae



♀

(a narrow slit  
in conjugation  
much a segment  
break)

♂

(raised  
dot - below  
the segment  
break)

These are the best pupa

← I'm not sure if this is right, but  
close to the tip, one of them the  
notching/denting feathers can  
be found

These differences are visible  
with the naked eye - once  
you know what to look for

Learn to sex the pupae w/  
a hand lens or dissecting

Dear Gocelyn,  
Here's how.

Maryland Resitter