GENERAL NOTES

DETERMINATION OF SEX IN FOUR SPECIES OF GIANT SILKWORM MOTH LARVAE (SATURNIIDAE)

We consider the ability to determine the sex of giant silkworm moth larvae to be of applied value from two standpoints. First, we could rear only (or primarily) individuals of the sex required for special research purposes. Second, we could insure that small colonies ($\leqslant 12$ individuals), maintained from year to year as breeding stock, contain sufficient numbers of each sex. Except for information reported for the domestic silkmoth, *Bombyx mori* Linnaeus (Tazima, 1964, The genetics of the silkworm, Prentice-Hall: Englewood Cliffs, New Jersey), we have found very little information concerning sex determinations in lepidopterous larvae. A recent paper by Hinks & Byers (1973, Can. J. Zool. 51: 1235–1241) reported structures that appear to be reliable indicators of sex in certain noctuid larvae. In female noctuid larvae these characters consist of four pits or other modifications of the integument associated with the developing female genitalia and occur between the ventral and subventral setae on the 8th and 9th abdominal segments; in male noctuid larvae they consist of a pit or other modification of the integument associated with the developing male genitalia (Herold's Organ) and occur on the venter of the 9th abdominal segment. Similar, but less detailed, information has been reported for the genus *Malacosoma* (Stehr & Cook, 1968, Bull. U.S. Nat. Mus., 276: pp 46–47).

During 1975, while maintaining large research colonies of several giant silkworm moth species, we decided to examine some of our colonized larvae to determine whether they exhibited similar sex-related characters. We selected random samples of four giant silkworm moth species (Table 1) and categorized individuals as male or female on the basis of the characters reported by Hinks & Byers. The larvae were then segregated according to sex and reared to pupation or adulthood to confirm the sex of each individual.

Larvae of *Antheraea polyphemus* (Cramer) were examined in both the 4th and 5th instars. We found only the structure related to the developing male genitalia, visible to the naked eye in both instars as a single black pit on the venter of the 9th abdominal segment; female characters were not observed even at 60× magnification. Individuals exhibiting this black pit were categorized as males; those without it were categorized as females.

Larvae of *Eupackardia calleta* (Westwood) were also examined in both the 4th

TABLE 1. Larval sex determinations for four giant silkworm moth species.

Species	Number Larvae Examined	Number Categorized Male or Female	Actual Number Male or Female[1]	Probability of Misclassification[2]
A. polyphemus	27	10 ♂	10 ♂	0 (0–0.27)
		17 ♀	15 ♀ 2 ♂	0.12 (0.02–0.34)
E. calleta	33	16 ♂	16 ♂	0 (0–0.18)
		17 ♀	17 ♀	0 (0–0.17)
H. cecropia	28	15 ♂	14 ♂ 1 ♀	0.07 (0.0003–0.3)
		13 ♀	13 ♀	0 (0–0.23)
C. promethea	43	21 ♂	20 ♂ 1 ♀	0.05 (0.0002–0.21)
		22 ♀	22 ♀	0 (0–0.13)

[1] Sex determined by examination of pupae or adults.
[2] Estimates of probability of misclassification and 95% confidence interval.

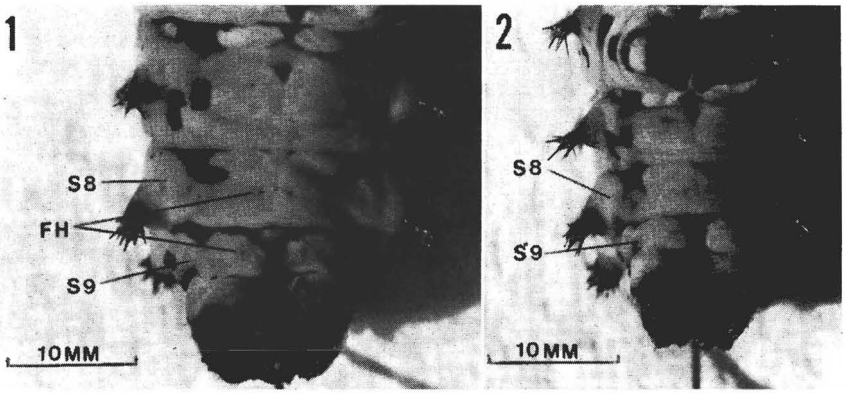

Fig. 1–2. Ventral views of 5th-instar *E. calleta* larvae. 1, female larva showing location of the pits associated with the developing genitalia (FH) on the 8th and 9th (S8 and S9) abdominal segments. 2, male larva showing the absence of these pits on S8 and S9.

and 5th instars. We found only the developing female genitalia, visible to the naked eye in both instars as four prominent dark pits on the ventral side of the 8th and 9th abdominal segments (Fig. 1). We did not observe any male character (Fig. 2), even at 60× magnification. Individuals with the four dark pits were categorized as females; all others were categorized as males.

Of the *Hyalophora cecropia* (Linnaeus) specimens, we examined 3rd-, 4th-, and 5th-instar larvae. We found only the developing female genitalia in the 4th- and 5th-instar larvae, visible to the naked eye as four white subsurface spheres on the ventral side of the 8th and 9th abdominal segments. Microscopic examination of the integument over these white spheres did not reveal any pits or other modifications of the surface. Individuals with these white spheres were categorized as females; all others as males.

Larvae of *Callosamia promethea* (Drury) were examined only in the 4th instar. Using the naked eye, we were unable to find any evidence of developing male or female genitalia. However, microscopic examination (60×) revealed the presence of developing female genitalia in the form of two obscure, irregular, subsurface, dark green to black bodies on the ventral side of the 8th abdominal segment. There was a slight modification of the integument over these structures. Male characters could not be found even at 60× magnification. Individuals with the dark subsurface structures were categorized as females; all others as males.

Our observations (Table 1) demonstrate that the characters associated with the developing genitalia can be used to determine the sex of larvae of these four giant silkworm moth species. When selecting larvae, the probability that an individual will be misclassified depends on how distinct the characters are, the sex in which the characters occur, and the sex being sought. In *A. polyphemus* only the male character was observed. Thus, if one is seeking to obtain only male larvae of this species, the chance of selecting a group free of females is very good, particularly if any questionable individuals are excluded. Conversely, if one is seeking to obtain only female larvae of *A. polyphemus*, the chances are not as good because males with indistinct genital characters might be included with the females. Our findings bear this out for *A. polyphemus* and the other species we studied, although for *E. calleta*, *H. cecropia*, and *C. promethea* the situation is reversed.

In addition to the colonized larvae, we examined five wild larvae of *H. cecropia*

that were collected in the 4th instar and examined only at that stage of their development. We categorized all these larvae as males, but later examination of the pupae showed four males and a female. These results agree with our findings for colonized *H. cecropia* larvae (Table 1).

The findings from this study should be of interest, and perhaps of applied value, to lepidopterists, dealers, and researchers who colonize or study giant silkworm moths.

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A METHOD FOR HANDLING EGGS AND FIRST INSTAR LARVAE OF *CALLOSAMIA PROMETHEA* (SATURNIIDAE)

In an earlier paper (Miller & Cooper, 1976, J. Lepid. Soc. 30: 95–104) we reported the use of portable outdoor cages to effect the mating of various giant silkworm moths, including *Callosamia promethea* (Drury). Since that time we have conducted studies to evaluate methods for the collection of eggs and the transfer of newly-hatched larvae to food plants.

We routinely collect eggs from giant silkworm moths by placing fertile females in paper bags where they can oviposit on the inner surfaces. For larvae reared outdoors, we turn the paper bags inside out and place them in sleeve cages already attached to branches. For larvae reared indoors, we cut the bags into small pieces of paper containing the eggs masses, and these are variously attached to food plant cuttings. These methods are not novel and have long been used, with variations, by lepidopterists who colonize giant silkworm moths (Crotch, 1956, A silkmoth rearer's handbook, The Amateur Entomologist's Society, London; Taschenberg & Roelofs, 1970, Ann. Ent. Soc. Amer. 63: 107–111; Waldbauer & Sternburg, 1973, Biol. Bull. 145: 627–641; Dirig, 1975, Growing moths, N.Y. State College of Agriculture & Life Sciences). For large-scale indoor colonization of giant silkworm moths we found that the time required to cut around the egg masses and then attach them to the food plants was unacceptable. Therefore, we developed a modified procedure for collecting the eggs and transferring the larvae to food plants. This paper reports our results with *C. promethea*.

We used 12 *C. promethea* females, each placed in a brown paper bag (lunch size) on the first night after mating. The following morning the female moths were removed and the bags, containing the eggs, were folded to their original flattened configuration and held for 8 days. On the 9th day 3 fresh wild cherry (*Prunus*) cuttings, each 15–20 cm long and containing 4–5 large leaves, were inserted into each bag. The tops of the bags were folded over about 1.5 cm and a small hole was made at the crease to allow the stems to protrude about 5.0 cm. The bags were inverted and the stems were placed in water containers. Observations of hatching and migration of larvae to the food plants were made by carefully opening the creased end of the bags and looking inside.

The eggs hatched on the 10th day and the larvae crawled about on the inner surfaces of the bags; a few transferred to the wild cherry leaves, but none of these were observed feeding. By the end of the 11th day most of the larvae had transferred to the food plants and were feeding. Observations were continued through the 13th day after oviposition, but no additional larvae transferred to the food plants after day 11. On the 14th day the bags were removed and cut open to record rele-