EXTERNAL MORPHOLOGY OF ABDOMINAL SETAE FROM MALE AND FEMALE HYLESIA METABUS ADULTS (LEPIDOPTERA: SATURNIIDAE) AND THEIR FUNCTION

Authors: Rodriguez, Jessicca, Hernández, Jose Vicente, Fornés, Lizette, Lundberg, Ulf, Arocha Piñango, Carmen-Luisa, et al.

Source: Florida Entomologist, 87(1) : 30-36

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0030:EMOASF]2.0.CO;2

EXTERNAL MORPHOLOGY OF ABDOMINAL SETAE FROM MALE AND FEMALE *HYLESIA METABUS* ADULTS (LEPIDOPTERA: SATURNIIDAE) AND THEIR FUNCTION

Jessicca Rodriguez[1], Jose Vicente Hernández[2], Lizette Fornés[2], Ulf Lundberg[3], Carmen-Luisa Arocha Piñango[3] and Frances Osborn[1]

[1]Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumaná, Venezuela

[2]Laboratorio de Comportamiento, Dept. Biología de Organismos, Universidad Simón Bolívar, Caracas, Venezuela

[3]Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

Abstract

Hylesia metabus is a species of moth, distributed principally in northeastern Venezuela. Female moths use their abdominal setae to cover their egg masses. Contact with these setae can cause a severe dermatitis in humans. Setae from males do not produce these symptoms. The external morphology of the abdominal setae in male and female moths was described and the effect of the setae on ant behavior was studied. We classified the setae into four types, S1, S2, S3 and S4. In females, two of these types were found in the dorsal region; S1 and S2 show a porous structure and measure 2000 µm and 155 µm in length, respectively. In the ventral part of the abdomen we observed three setae types, S1, S3 which are 190 µm long and have small barbs along their length directed towards the apex, and S4 which have numerous barbs at the base, but further up flatten out, with barbs along both sides, before tapering off at the apex. S4 also were found in the lateral zones of the abdomen and were the predominant type of setae covering the egg masses. Only S1 setae were found in males. Egg masses not covered by setae were examined and transported by *Pheidole* ants, whereas covered eggs were largely avoided. The morphology of the S3 and S4 setae types suggests that these may be related to the urticating properties reported for the moth. Ant avoidance of setae covered eggs suggests that these protect the eggs from at least some predators.

Key Words: Urticating moth, egg protection, ants

Resumen

Hylesia metabus es una especie de Lepidóptero, distribuida principalmente en el noreste de Venezuela. Las hembras adultas cubren sus huevos con sus setas abdominales. El contacto con estas setas causa una severa dermatitis en los humanos. Las setas provenientes de los machos no producen tales sintomatologías. Se describió la morfología externa de las setas abdominales en mariposas machos y hembras y se estudio el efecto de las setas sobre el comportamiento de las hormigas. Las setas se clasificaron en cuatro tipos, S1, S2, S3 y S4. En las hembras, dos de estos tipos se encontraron en la región dorsal; S1 y S2, las cuales presentan una estructura porosa y con una longitud de 2000 µm y 155 µm respectivamente. En la parte ventral del abdomen se observaron tres tipos de setas, S1, S3, que miden 190 µm de largo y tienen pequeñas espinas a lo largo de la seta, dirigidas hacia el ápice y S4, que presentan espinas en la base, para luego aplanarse, con espinas a los lados, antes de volver a afilarse hacía el ápice. Las S4 se encuentran también en las zonas laterales del abdomen y son el tipo de seta predominante cubriendo los huevos. En los machos, el único tipo de seta presente son las S1. Grupos de huevos separados de la postura y desprovistos de setas fueron examinados y cargados por hormigas del género *Pheidole*, mientras que éstas evitaron contacto con las posturas cubiertas de setas. La morfología de las S3 y S4, sugiere que éstas podrían estar relacionadas con el efecto urticante de las mismas. La evasión por parte de las hormigas de los huevos cubiertos por las setas indica que éstas protegen los huevos de por lo menos algunos depredadores.

Translation provided by author

Urticating hairs or setae are found in species belonging to 13 families and four superfamilies in the order Lepidoptera. In most cases larvae have urticating setae, and it is only in a few species that they are found on adults, for example in the genera *Acyphas* and *Euproctis* in the Lymantridae and in the genus *Hylesia* in the Saturniidae.

The genus *Hylesia* is a Neotropical moth distributed in the Americas from Mexico to Argentina (Lamy et al. 1984). *Hylesia metabus*

(Cramer) 1775, (common name "Palometa Peluda"), is distributed principally in mangrove swamps in northeastern Venezuela (Fornés & Hernández 2001). The abdomens of the adult females are exceedingly hairy and the females use these hairs to cover their egg masses. It has been speculated that the hairs protect the eggs from predators and parasites, although this has not been demonstrated. Although *Hylesia metabus* moths normally inhabit mangrove swamps, swarming adult moths are attracted by lights of nearby towns and arrive in the thousands, releasing the urticating hairs into the air. Exposure to hairs from female moths leads to severe urticarial and papilovesicular dermatitis. Hairs from male moths are not urticating.

Studies of the external morphology of *H. metabus* adult females have been undertaken, (Lamy & Lemaire 1983; Olivares & Vásquez 1984) and different types of setae have been described. Lamy & Lemaire (1983) described what they called "flechettes" assuming that these were the only setae to have urticating properties. Later, Vásquez (1990) reported three different types of setae present on female abdomens, and considered that two of these types were urticating. Nonetheless, the only setae illustrated are similar to those previously described by Lamy & Lemaire (1983). No detailed description of the external morphology of the different setae found on female abdomens and egg nests is known to us. Neither has anyone described the non urticating setae from male abdomens. Here we describe the setae from male and female abdomens and from the egg nests of *Hylesia metabus* moths. In addition we studied the effect of the setae covering the egg masses on ant behavior in order to ascertain if these protect the eggs from foraging by the ants.

Materials and Methods

Preparation of Samples for Scanning Electron Microscopy (SEM)

Male and female *Hylesia metabus* pupae, and the hairballs used by the females to cover their egg masses were collected from mangrove swamps close to the town of Yaguaraparo, Cajigal District, Sucre State, Venezuela. The pupae were maintained until the eclosion of the adults in the Biological Control Laboratory, at the Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumaná, Venezuela. The larvae were maintained at 24 ± 2°C, relative humidity; 60 ± 5% and a photoperiod of 12:12 (L:D). For SEM, abdomens from moths, and egg nests, were cut in pieces of approximately 1 mm^3, placed in glass vials and dehydrated in an oven at 40°C for 48h in the presence of silica gel. The abdomens were divided into three sections: dorsal, ventral, lateral, with each section being further divided into anterior (towards the thorax), middle, and posterior (towards the ovipositor). Some of the abdomens were scraped to remove the hairs before being divided into equal sized sections as described above. The samples were coated with gold/palladium and observed in a Philips SEM 505 and a JEOL T-300 scanning electron microscope (Stobbart & Shaw 1964).

Behavioral Experiments

The experiments with ants were carried out at the Universidad Simón Bolívar, Caracas, Venezuela, following the methodology used by Osborn & Jaffé (1998) with some modifications. We used *Pheidole* sp. for the purposes of this study, as it is commonly found in the vegetation and thus probably regularly encounters lepidopteran eggs and larvae. Two colonies of *Pheidole* sp. were collected in the surroundings of the university and maintained in the laboratory in plastic bowls of 1-m diameter and 50-cm depth, at a constant temperature of 25°C and a relative humidity of 70-80%. A metal tripod 40 cm high was put in each bowl on which we placed a glass platform of 20 cm^3 as the foraging area. Two plastic lids of 5-cm diameter and 1-cm depth were placed on the glass platforms, and in these we put pieces of soft netting, one soaked in water and the other soaked in a 1:1 ratio of water and honey. In addition, every two days insects collected with entomological nets were placed on the foraging area.

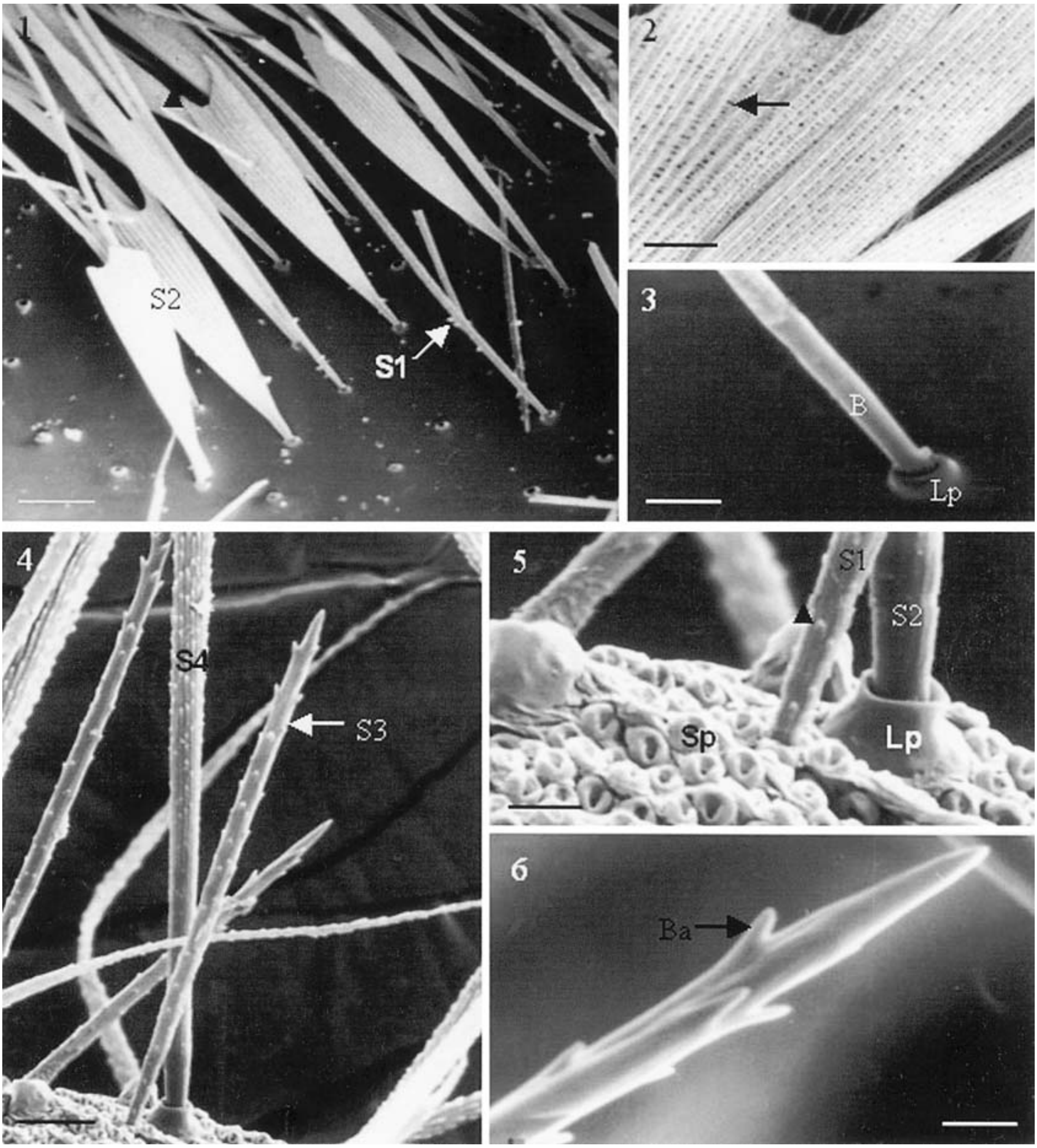
For the bioassays, we placed an egg nest, with or without setae, on the foraging area of one of the ant colonies. Eggs without setae were prepared by removing the setae with forceps and washing the eggs with distilled water. The behavior of the ants was then observed during 15 mins. The egg nests, with or without setae, were tested separately, one after the other (the first to be tested was chosen randomly), with each pair of assays being considered an experiment. Nine replicates were performed, alternating between colonies so that each colony participated in only one experiment on any given day.

The results of the replicates were analyzed by a Wilcoxen-Mann-Whitney test, comparing each response by the ants (exploration, touching, walking over and transport of the eggs) towards setaeless, and setae-covered eggs separately (Siegel & Castellan 1988).

Results

External Morphology of the Setae

The hairs (or setae) of adult moths are distributed in lateral bands along the abdomen. In the dorsal (anterior, middle and posterior) sections of female abdomens we observed two types of setae which we shall refer to as types S1 and S2 (Fig. 1).



Figs. 1-6. 1) General view of the dorsal region of the female abdomen showing setae types S1 and S2. Note the blunt apex of the S1 setae. Bar = 38 µm. 2) Detail of abdominal setae types S1 and S2. Note the porous nature of the setae (arrow). Bar = 32 µm. 3) Detail of the base of a S1 type setae. B: base of setae. Lp: Large pocket Bar = 13 µm. 4) General view of an S3 type setae (S3). Bar = 20 µm. 5) Detail of the base of an S3 type setae. Note the bluntness of the barbs. Sp: small pocket. (▲) Bar = 7 µm. 6) Detail of the apex of an S3 type setae. Note the sharpness of the barbs (Ba). Bar = 5 µm.

S1 type setae were approximately 2 mm long, with a diameter of 5 to 5.3 µm at the base and variable widths of between 4.6 and 24 µm along the rest of their length. These setae showed a very porous lattice type structure and the apices of the setae were blunt (Figs. 2 and 3, Table 1). S2 type setae were between 182 and 220 µm long and were similar to S1 setae in that they also showed a very porous lattice type structure. They were cylindrical at the base, with a diameter of 5.5 to 6 µm, but then fanned out in the shape of a shield, with the apex in the form of a W (Fig. 1, Table 1).

TABLE 1. MEASUREMENTS OF THE DIFFERENT TYPES OF ABDOMINAL SETAE FROM MALE AND FEMALE *H. METABUS* MOTHS.

	Width at base (µm)		Maximum width (µm)		Length (mm)		Width of pocket opening (µm)	
Setae type	Av.	Std. Dev.	Av.	Std. Dev.	Av.	Std. Dev.	Av.	Std. Dev.
S1	5.1	0.1	11.0	6.3	29.1	2.0	7.3	0.2
S2	5.7	0.1	46.0	2.7	12.8	0.2	7.7	0.4
S3	2.9	0.2	8.0	0.7	15.0	0.19	4.0	0.6
S4	5.5	1.2	58.6	3.8	32.0	1.0	7.0	0.4

N = 20.

In the ventral sections of female abdomens we identified three types of setae; S1 type setae (already described) were abundant in the ventral anterior part of the abdomen. S3 type setae were predominant in the middle ventral area, they were approximately 190 µm long, with a diameter of 2.5 to 3.1 µm at the base (Figs. 4 and 5, Table 1). They were smooth, without any holes or pores (Fig. 4). Along their length were small barbs, directed towards the pointed distal end. At the base of the setae these had rounded points which became sharper towards the apex (Figs. 5 and 6). S4 type setae were abundant in the ventral posterior part of the abdomen; they were approximately 1 mm long and showed a more complex morphology (Figs. 7-10); the base of the setae had a diameter of 4.2 to 7.5 µm and the surface was made up of numerous barbs (Fig. 8, Table 1), further up, the setae flattened out to a width of approximately 60 µm, with triangular barbs along both sides (Fig. 9, Table 1). Towards the apex, the setae thinned out into a thin cylindrical tube where, in some photographs, secretion drops were observed (Fig. 10, Sd). S4 setae were also found in the lateral sections of the female abdomens, where they were the only type of setae observed.

The S1, S2 and S4 setae were inserted in pockets with diameters of 7 to 7.7 µm (Figs. 1, 3, 5, 8, 11, Table 1), and S3 setae in pockets with diameters of 3.2 to 4.6 µm (Figs. 5 and 11, Table 1). These pockets varied in density depending on their position on the abdomen. In the dorsal parts of the abdomen where types S1 and S2 were located, the pockets had a density of approximately 1000/mm^2 (Fig. 5) The pockets on the lateral parts of the abdomen, where the S4 type setae were located had a density of 3500/mm^2 (Fig. 8) In the ventral zone of the abdomen, the large pockets, holding S1 and S4 setae, had a density of 2000/mm^2, and in the ventral middle zone, where the S3 setae were located, the small pockets were very abundant; approximately 50000 / mm^2, and tightly packed, so that the cuticle could not be seen (Fig. 5). In the anterior ventral part of the abdomen only large pockets could be observed. In Figure 11, the abrupt change between the middle and anterior parts of the abdomen can be seen.

In the egg nests the dominant type of setae observed was S4, although S1 and S3 setae were also present (not shown) In males only S1 type setae were found.

Behavioral Experiments

Definition of Behavioral Responses of Ants. Preliminary experiments with eggs allowed us to define the following behavioral responses of the *Pheidole* ants towards the larvae: (1) Exploration: ants touch the eggs with their antenna for a period of >1 sec., (2) Touch: ants touch the eggs with their mandibles for a period of >1 sec., (3) Walk over: ants walk over the eggs, (4) Transport: ants transport the eggs towards their nests.

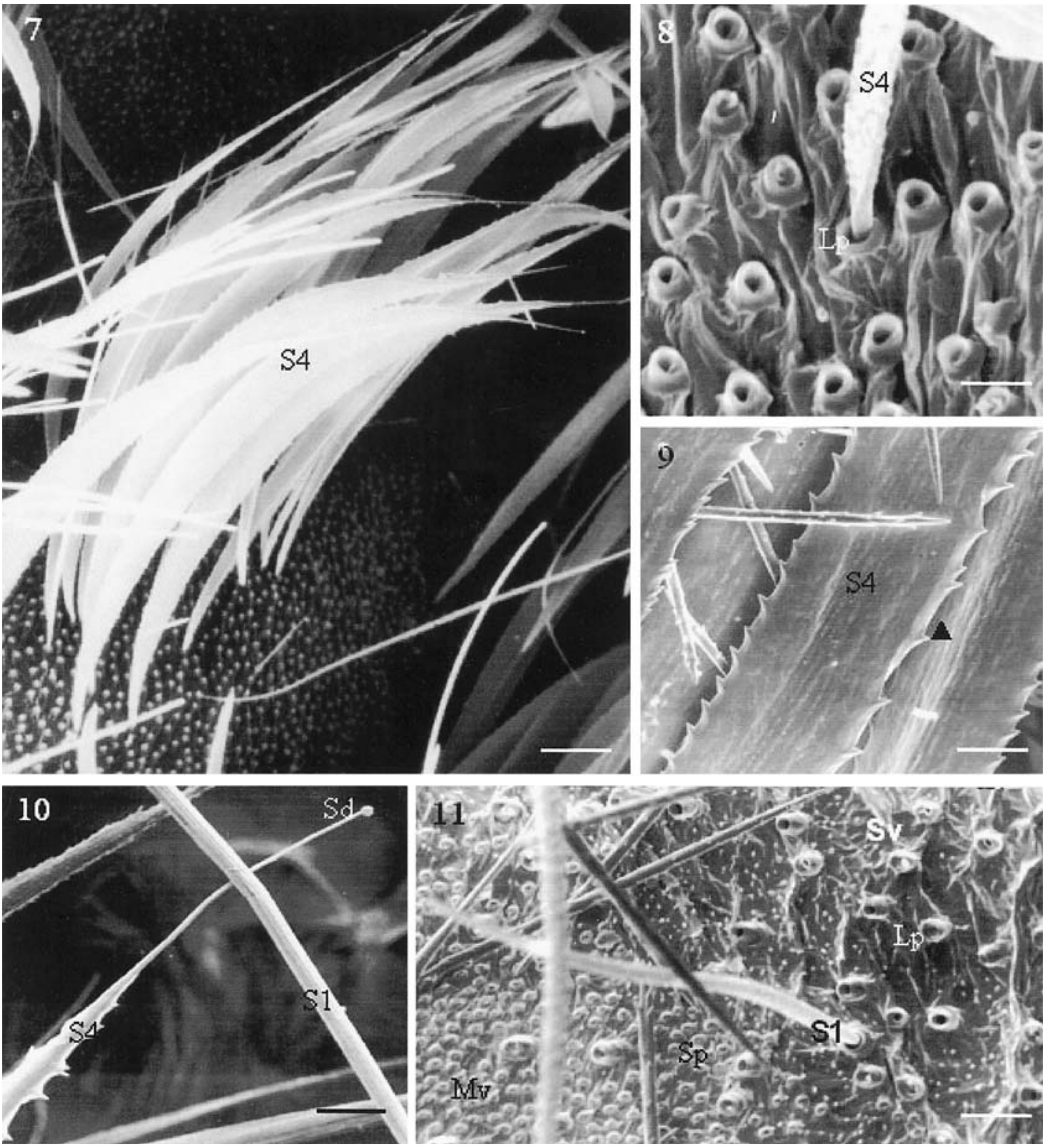
Behavioral Responses of the Ants to Eggs with and without Setae

Table 2 shows the results of the experiments with the ants. It can be observed that there is a significant difference between treatments for the exploration and carrying behaviors with a total of 19 ants exploring uncovered egg masses compared with 0 ants exploring covered egg masses. A total of 16 ants carried uncovered egg masses to their nest compared with 0 ants carrying covered egg masses. In fact, the ants seemed to largely ignore the covered egg masses, and avoided contact with them.

DISCUSSION

This study represents a first detailed description of the morphology of the setae of adult male and female *Hylesia metabus* moths. Based on morphological characteristics, we have classified the setae into four distinct types that we have called S1, S2, S3, and S4, respectively.

Due to their porous nature and lack of sharp points or barbs, S1 setae are unlikely to be urticating. Furthermore S1 was the only type of setae found in male moths which do not have urticating properties. S2 type setae, observed from the dorsal zone of female abdomens, and structurally similar to S1 are also unlikely to be urticating.



Figs. 7-11. 7) General view of the lateral region of the female abdomen showing setae type S4. Note the curved nature of the setae. Bar = 76 µm. 8) Detail of the base of an S4 type setae. Lp: large pocket. Bar = 10 µm. 9) Detail of the middle section of an S4 type setae. Note the lateral barbs (▲). Bar = 23 µm. 10) Detail of the apex of an S4 type setae. Note the secretion drop (Sd). Bar = 210 µm. 11) Detail of the change in pocket sizes between the anterior ventral and middle ventral regions of the female abdomen. Sv: anterior ventral region, Mv: middle ventral region, Sp: small pocket. Bar = 20 µm.

The S3 and S4 types of setae are found only in females and may be related to the urticating properties. The urticating setae found on larvae belonging to the genus *Thaumetopoea* (Notodontidae) show a similar length (150-250 µm) and share some structural similarities with the S3 setae of the *Hylesia* moths, i.e., small, sharp barbs pointing towards the distal end (Lamy & Novak 1988). Urticating setae similar to the S3 type have been described from several species of *Hylesia* moths (Lamy & Lemaire 1983). These authors reported that in *H. metabus* the setae were 100 to 225 µm in length. Further descriptions of these setae report a density of 50000 setae/mm^2, as

TABLE 2. RESPONSES OF *PHEIDOLE* SP. ANTS TO SETAE-LESS AND SETAE-COVERED EGG MASSES OF *H. METABUS*.

Ant behavior	Eggs without setae	Eggs with setae	Value of Z	Probability of difference between treatments
Exploration	18	0	-2.842	0.014*
Walk over	22	8	-0.287	0.796
Touch with mandibles	10	0	-1.835	0.258
Carry	16	0	-2.85	0.014*

The numbers refer to the total number of ants that behaved in a certain way to the egg nests for all nine replicates.
*Significant difference between treatments.
N = 9.

found in this study (Lamy et al. 1984; Pelissou & Lamy 1988).

Setae similar to the S4 type have not, as far as we know, been previously described. Nonetheless, their morphology (sharp barbs at the base, and triangular barbs further up) suggests that they may have urticating properties. The secretion drops observed at the apex of one of these setae also suggests that they contain a liquid substance. Biochemical studies have demonstrated the presence of a kallikrein-like substance (associated with increased vascular permeability and the production of pain) in the urticating hairs of female *H. metabus* moths (Lundberg et al. 2002). Furthermore, the setae are associated with the ovipositor in female moths and are the most abundant type of setae in the egg nests which are known to be very urticating (personal experience of three of the authors).

Although several species of larvae having urticating setae with different morphologies have been described (e.g., Perlman et al. 1976; Press et al. 1977), the information on setae from adult moths is scarce. Apart from *H. metabus*, the only other species of moth reported as having more than one type of urticating setae is *Anaphe venata* (Notodontidae: Thaumetopoinae). Nevertheless, although the setae on *A. venata* are different in size and posture, both types of setae show the same structure: they are sharp at both ends and square when cut transversally, with barbs on all four sides. (Lamy 1984; Lamy et al. 1984). In this species, the female moths release the urticating setae by means of abdominal contractions, placing them over the eggs, in a similar way to that of *H. metabus* (Lamy 1984; Lamy et al. 1984).

Behavioral experiments with ants suggest that they are not repelled by the abdominal hairs covering the egg nests (no alarm behavior was recorded), but rather ignore or avoid them. The eggs of some species of insects, e.g., *Gastrophysa cyanea* (Coleoptera: Chrysomelidae) contain oleic acid which repel several species of ants (Howard et al. 1982). The hairs covering *H. metabus* eggs may simply hide the eggs from the ants. This suggests that the urticating characteristics of the hairs may be directed towards avian or mammal predators.

In conclusion, we have characterized four distinct classes of setae present in the abdominal wall of male and female adults of *Hylesia metabus*. We have chosen to call these different types of setae S1, S2, S3, and S4. In the egg nests, the S4 type of setae is the predominant type. This study thus represents the first description of four morphologically different types of setae from one species of moth, two of which are probably urticating.

Setae covered eggs seem to be protected from predation by ants, although the setae seem to act as a physical barrier rather than a chemical one.

ACKNOWLEDGMENTS

The authors thank Srs. Andrés Villegas and Norberto Pino for assistance in the collection of the *H. metabus* moths and egg nests, and the Centro de Ingenieria de Superficies-Universidad Simón Bolívar and Glen Ródriguez for technical assistance. This study was supported in part by the Consejo de Investigación of the Universidad de Oriente, Project No. CI-5-1901-0820198 and by FONACIT (Fondo de Investigación Regional) to FO, and the Universidad Simón Bolívar to JVH.

REFERENCES CITED

FORNÉS, L., AND J. V. HERNÁNDEZ. 2001. Reseña histórica e incidencia en la salud pública de *Hylesia metabus* (Cramer). (Lepidoptera: Saturniidae) en Venezuela. Entomotropica 16(2): 137-141.

HOWARD, D. F., M. S. BLUM, T. H. JONES, AND D. W. PHILLIPS. 1982. Defensive adaptions of eggs and adults of *Gastrohysa cyanea* (Coleoptera: Chrysomelidae). J. Chem. Ecol. 8: 453-462.

LAMY, M. 1984. La processionnaire du colatier: *Anaphae venata* Butler (Lepidoptere: Thaumetopoeidae): papillon urticant d´Afrique. The cola processionary moth: *Anaphae venata* Butler (Lepidoptere: Thaumetopoeidae): an urticating moth from Africa [Morphology, cause of human diseases]. Insect Science and its Application 5(2): 83-86.

LAMY, M., AND C. LEMAIRE. 1983. Contribution á la systématique des *Hylesia*: etude au microscope électronique á balayage des "flechettes" urticantes. Systematics of the *Hylesia* spp. study with scanning electronic microscopy. Bulletin de la Societé Entomologique de France 88(3/4): 176-192.

LAMY, M., AND F. NOVAK. 1988. Mise en place et différenciation de láppareil urticant de la Chenille processionnaire du chêne (*Thaumetopoea processionea* L.) (Lépidoptéres, Thaumetopoeidae) au cours de son développment larnaire. Annales des Sciences Naturelles, Zoologie, Paris. 13 Série 9(1): 55-65.

LAMY, M., M. H. PASTUREAUD, F. NOVAK, AND G. DUCOMBS. 1984. Papillons urticants dÁfrique et dÁmérique du sud (*g. Anaphae* et *g. Hylesia*): Contribution du microscope électronique a balayage a l´etude de leur appareil urticant et a leur mode d´e action. Urticating moths of Africa (genus *Anaphae*) and of South America (genus *Hylesia*). Bulletin de la Societé Zoologique de France 109(2): 163-177.

LUNDBERG, U., F. R. OSBORN, Z. CARVAJAL, A. GIL, B. GUERRERO, AND C. L. AROCHA-PIÑANGO. 2002. Isolation and partial characterization of a proteasen with kallikrein-like activity from the egg nests of *Hylesia metabus* (Cramer 1775) (Lepidoptera: Saturniidae), preliminary communiation. Revista Científica 12(2)97: 102.

OLIVARES, M. A., AND L. N. VÁSQUEZ. 1984. Morfología externa de escamas de *Hylesia* sp. a nivel de microscopía electrónica. IX Congreso Venezolano de Entomología, Abstract No. 69, Tachira, Venezuela. 1984.

OSBORN, F., AND K. JAFFÉ. 1998. Chemical ecology of the defense of two butterfly larvae against ants. J. Chem. Ecol. 24(7):1173-1186.

PELISSOU, V., AND L. LAMY. 1988. Le Papillon Cendre: *Hylesia metabus* (Cramer (= *H. urticans*) Floch et abonnenc) (Lepidopteres Saturniidae) Papillon urticant de guyane Francaise: Etude Cytologique de Son Appareil Urticant. Insect Science and its Application 9(2)185-189.

PERLMAN, F., E. PRESS, J. A. GOOGINS, A. MALLEY, AND H. POAREA. 1976. Tussockosis: Reactions to Douglas Fir Tussock moth. Annals of Allergy 36:302-307.

PRESS, E., J. A. GOOGINS, H. POAREO, K. JONES, F. PERLMAN, AND J. EVERETTE. 1977. Health to timber and forestry workers from the Douglas Fir Tussock moth. Archives of Environmental Health 32: 206-210.

SIEGEL, S., AND J. CASTELLAN. 1988. Nonparametric statistics for the behavioural sciences. McGraw Hill. New York, London. 399 pp.

STOBBART, R. H., AND J. SHAW. 1964. Salt and water balance: excretion, pp. 189-235. *In* R. Rockstein (Ed.), The Physiology of Insects, Vol. 3. Academic Press, New York, London. 350 pp.

VÁSQUEZ, L. N. 1990. Estudio bioecológico y tacticas de control de la Palometa *Hylesia metabus* Crammer. en el oriente de Venezuela. Saber 3(1): 14-20.