

The Taxonomic Value of Pre-alar Scales in the Identification
of *Culex (Culex) pipiens* L. and *Culex (Cx.) torrentium*

Martini (Diptera: Culicidae)

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ABSTRACT: *Culex (Culex) pipiens* L. and *Culex (Cx.) torrentium* Martini are sympatric sibling species ordinarily inseparable at egg, larval or pupal stages. Adult males of both species may however be distinguished on the basis of their differential terminalia, and the females on the basis of the presence or absence of pre-alar patch of scales on the sternopleuron. The latter character is believed to be lacking in *Cx. pipiens*. In this study newly emerged adults of both species, obtained from field egg batches, have been closely examined and compared. The presence of pre-alar scales in *Cx. pipiens* adults is reported, and the unreliability of this character as a diagnostic feature clearly indicated. It is therefore suggested that in distinguishing adult females of *Cx. pipiens* and *Cx. torrentium* eggs should be obtained and the progeny reared to give F₁ males which could be conclusively identified by the structure of the terminalia.

INTRODUCTION

Culex (Culex) pipiens L. is a member of the *Cx. pipiens* complex (Laven 1967; Mattingly 1967). The species occurs mainly in the temperate climates and is rarely found in West Africa (Gutsevich et al. 1971; Horsfall 1955; Knight and Stone 1977). *Culex (Cx.) torrentium* Martini is a member of the *Cx. trifilatus* complex (Mattingly 1967). The species was first described in 1924 from Germany, and is now known to be widespread in the USSR, Central Europe and Asia Minor (Mattingly 1951; Gutsevich et al. 1971). Larvae of both *Cx. pipiens* and *Cx. torrentium* often occur together in the same breeding sites (Service 1968a; Onyeka 1980), though the latter shows a well defined preference in its choice of oviposition sites (Onyeka 1980).

Pre-adult and adult stages very closely resemble each other and separation of the two species can be made reliably only in the adult stage (Service

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1968a; Jupp 1979). The principal taxonomic character used is the difference in shape of the male terminalia of the two species (Mattingly 1951; Service 1968a). One external taxonomic character which has been suggested to identify both adult male and female *Cx. torrentium* is the presence of a pre-alar patch of scales on the upper part of the sternopleuron, a character believed to be lacking in *Cx. pipiens*. Jupp (1979) in a small study in Southern England showed that differentiation of the females of the two species could be made according to the number of pre-alar scales. The presence of two or more scales on one or both sides of the thorax would identify a specimen as *Cx. torrentium*.

In the course of population studies of the two sympatric sibling species of *Culex* in Silwood Park, Ascot, Berkshire, England, some adult mosquitoes bred from egg-rafts deposited by *Cx. pipiens* females were found to possess pre-alar scales. Consequently a detailed taxonomic study of both species was planned, with a view to ascertaining the extent of occurrence of the pre-alar scales and other characters in the adults, and in particular to assess relative values and reliability of using presence or absence of the scales as a basis for distinguishing between the two sibling species.

MATERIALS AND METHODS

COLLECTION AND BREEDING OF MOSQUITOES:

Egg-rafts of *Cx. pipiens* and *Cx. torrentium* were collected from permanent groundwater habitats at Imperial College, Silwood Park, Ascot. The rafts were conveyed to the insectary in separate breeding pans, to which small amounts of yeast had been added as a hatching stimulus (Service 1968b). Emerging larvae were fed on a mixture of brewer's yeast and powdered dog biscuits until pupation (Birtwistle 1971). Representative samples of pupae from each egg-raft were then transferred to a 16 x 16 x 16 cm emergence cage where the adult emerged. All rearing was carried out at $21 \pm 1^\circ\text{C}$, 50% r.h. and 16 h light - 8 h dark photoperiod (McLintock 1960; Miles 1974; Sanburg and Larsen 1973).

PROCESSING OF MOSQUITOES FOR MICROSCOPIC EXAMINATION:

(a) MALE TERMINALIA:

Two male mosquitoes from each egg-raft were removed from the emergence cage with the aid of a glass aspirator and stunned by placing them in a freezing chamber of a refrigerator. The terminalia were dissected and placed in a test tube containing 10% sodium hydroxide. After heating for 5-10 minutes to clear, the specimens were dehydrated with alcohol and xylol and then mounted in Canada balsam. The slides were examined under a binocular microscope at 100x magnification and positively identified as either *Cx. pipiens* or *Cx. torrentium*, based on the following differences: the dorsal arm of the phallosome in *Cx. pipiens* is tubular and does not project up to the level of the spines at the tip of the paraproct, whereas in *Cx. torrentium* the dorsal arm is pointed at the tip and projects to about the level of the spines. Furthermore the basal process of the paraproct in *Cx. torrentium* has a very well developed lateral projection which in *Cx. pipiens* is only vestigial (see Service 1968a).

(b) PRE-ALAR SCALES:

As pre-alar scales easily become detached from mosquitoes (Service 1968a), due cognisance was given in this investigation to the presence or absence of the insertions of these scales which are readily visible in slide preparations.

The following method was adopted for the slide preparations: The mosquito was killed and left for a few days to dry since dry specimens were easier to prepare. The specimen was placed in a solid watch glass and wetted with absolute alcohol to eliminate air bubbles. Ten percent potassium hydroxide was added to the specimen in absolute alcohol and the specimen was left for 1 hour to clear. The mosquito was then transferred to another watch glass containing glacial acetic acid and left for 15 minutes to neutralize the potassium hydroxide. The specimen was then mounted in gum chloral and the slide dried in the oven at about 35°C. The cover slip was ringed round with Euparal and the preparation was then ready for examination under a binocular microscope at 100x.

Slide mounts were prepared of 24 adult mosquitoes (12 males and 12 females) from each of 45 egg-rafts of *Cx. pipiens* and *Cx. torrentium*. Since specimens were mounted on their sides it was not possible to see the scale insertions on both sides of the thorax of a single specimen. Therefore, mounting was done in such a way as to permit dorsal view of the left thorax in 6 males and 6 females and the right thorax in 6 males and 6 females. The presence or absence of pre-alar scale insertion scars was recorded in each case.

RESULTS

Plate 1 and Figure 1 show the pre-alar region of *Cx. pipiens* with one pre-alar scale and a single scale insertion. The proportions of *Cx. pipiens* and *Cx. torrentium* with or without pre-alar scale insertion are presented in Table 1.

Of the 1080 *Cx. pipiens* examined 99 (9.2%) were found to possess pre-alar scale insertions on either side of the thorax. In the same number of *Cx. torrentium* examined, 1045 (96.8%) possess scale insertions but in 35 (3.2) individuals scale insertions were absent. Excluding all mosquitoes without scales, the number of scale insertions found in *Cx. pipiens* varied between 1-4 with a mean of 1.7, whereas in *Cx. torrentium* the number varied between 1-16 with a mean of 6.4 (Fig. 2).

Observation on the occurrence of pre-alar scale insertions in males and females of *Cx. pipiens* and *Cx. torrentium* showed that in *Cx. pipiens* more females possessed scales than males (Table 1). A χ^2 test showed the difference to be significant ($p < 0.05$). The numbers of scales in this species range from 1-4 with a mean of 1.8 in females and 1-3 with a mean of 1.6 in males (Fig. 2). There is no significant difference ($p > 0.05$) between the number of males and females of *Cx. torrentium* with scales. The numbers of scales range from 1-16 in both males and females (Fig. 2), with a mean of 6.3 in males and 6.5 in females.

DISCUSSION

Various taxonomic characters have been employed in the past for distinguishing between adult *Cx. pipiens* and *Cx. torrentium*. For example, Natvig (1948) used differences in the wing venation to separate females of both species. He found that the length of the stem of vein R_1 was between $1/4$ and $1/3$ the length of its fork in *Cx. pipiens* and between $1/5$ and $1/6$ in *Cx. torrentium*. Moreover, the length of the radio-medial cross vein was $2/3$ and $1/2$ its distance from the medio-cubital cross vein in *Cx. pipiens* and *Cx. torrentium*, respectively. Jupp (1979) found this character unsatisfactory. Mattingly (1951) observed that the dark scales on the abdominal sternites were more numerous in *Cx. torrentium* than in *Cx. pipiens*. In addition, Service (1968a) in his studies on the taxonomic characteristics of the two species has noted that the above differences may not be used with confidence to separate the two species. Furthermore, he found no pre-alar scales in *Cx. pipiens* collected from Brownsea Island and Monks Wood which were examined for this character. Also, he reported that in a few *Cx. torrentium* from the same source as the *Cx. pipiens*, pre-alar scales were absent, while in others they were easily detached. Hence, he concluded that absence of pre-alar scales could not always be taken as positive evidence of *Cx. pipiens*. In this respect results of the present investigation are of special interest, as they show that at Silwood Park as much as 9.2% of the *Cx. pipiens* population possess scales. The findings of Jupp (1979) are in agreement with these results although the larger sample taken here indicates that *Cx. pipiens* may occasionally have 3 or 4 pre-alar scales. Mattingly (1980) (personal communication) has also collected two female *Cx. pipiens* on separate occasions from Gloucestershire in Southwest England with pre-alar scales.

Hence, there is evidence of some degree of unreliability in using the presence or absence of pre-alar scales as a diagnostic feature for the separation of males or females of these sibling species of *Culex*. Nevertheless, it may be suggested (see Fig. 2) that when more than four scales are present, the specimen may be safely identified as *Cx. torrentium*; but when the pre-alar scales number between 1-4, identification using this character may not be applied with confidence. As the pre-alar scales are easily detached, it is necessary to look for the insertions of these scales whenever there are doubts. When a male mosquito is involved it is possible to confirm its specific identity by examination of the terminalia. But when a female is involved it is recommended that, whenever possible, eggs be obtained and progeny reared so that F_1 males can be identified by means of phallosome characteristics. The structure of the male terminalia remains the only certain way to differentiate these two species.

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Table 1. Occurrence of pre-alar scale insertions in *Cx. pipiens* and
Cx. torrentium.

Species	No. examined	No. with scale insertions	No. without scale insertions	% with scale insertions
<i>Cx. pipiens</i>	Male 540	39	501	7.2
	Female 540	60	480	11.1
	Total 1080	99	981	9.2
<i>Cx. torrentium</i>	Male 540	520	20	96.3
	Female 540	525	15	97.2
	Total 1080	1045	35	96.8

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PLATE 1



FIGURE 1

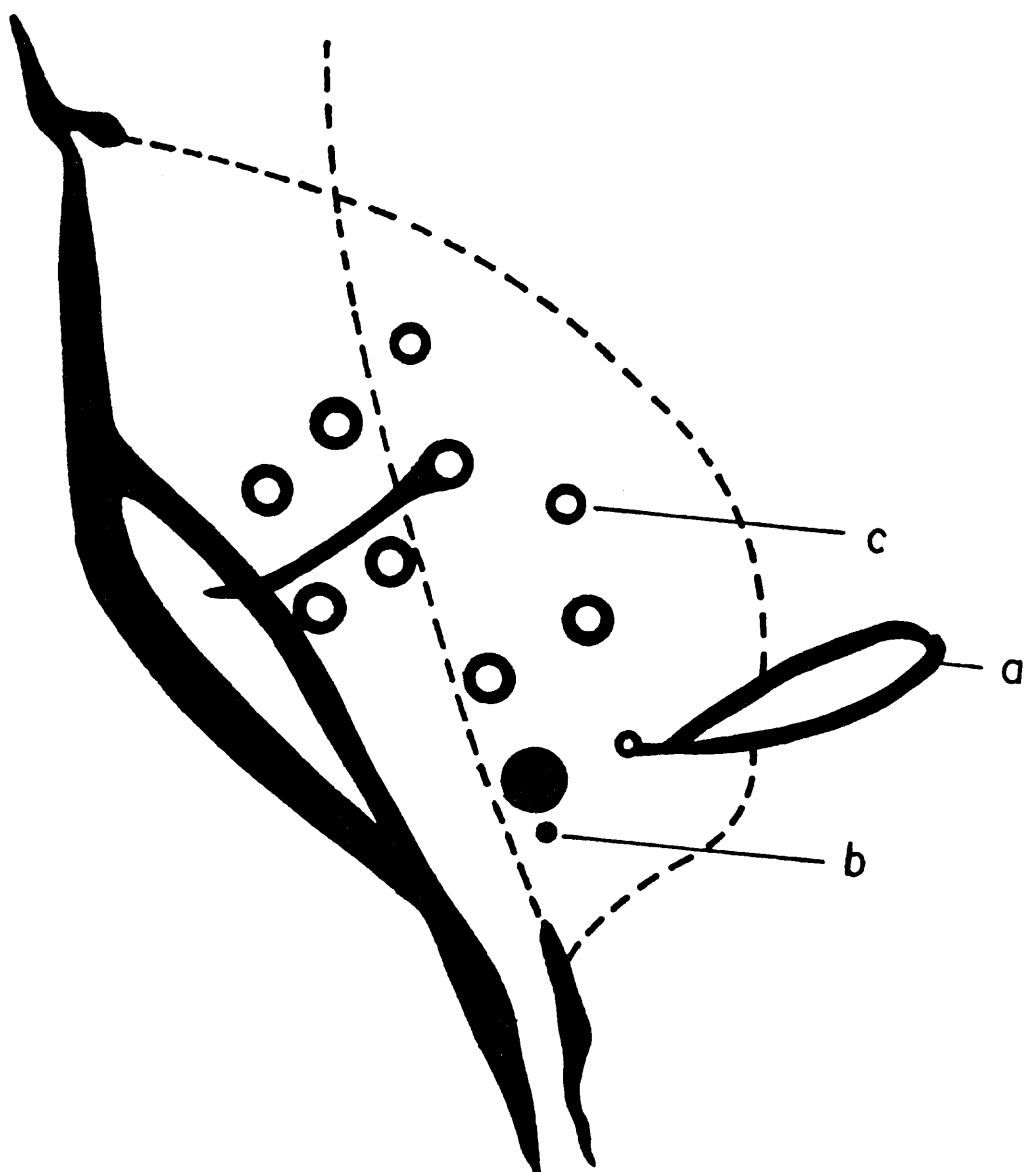


FIGURE 2

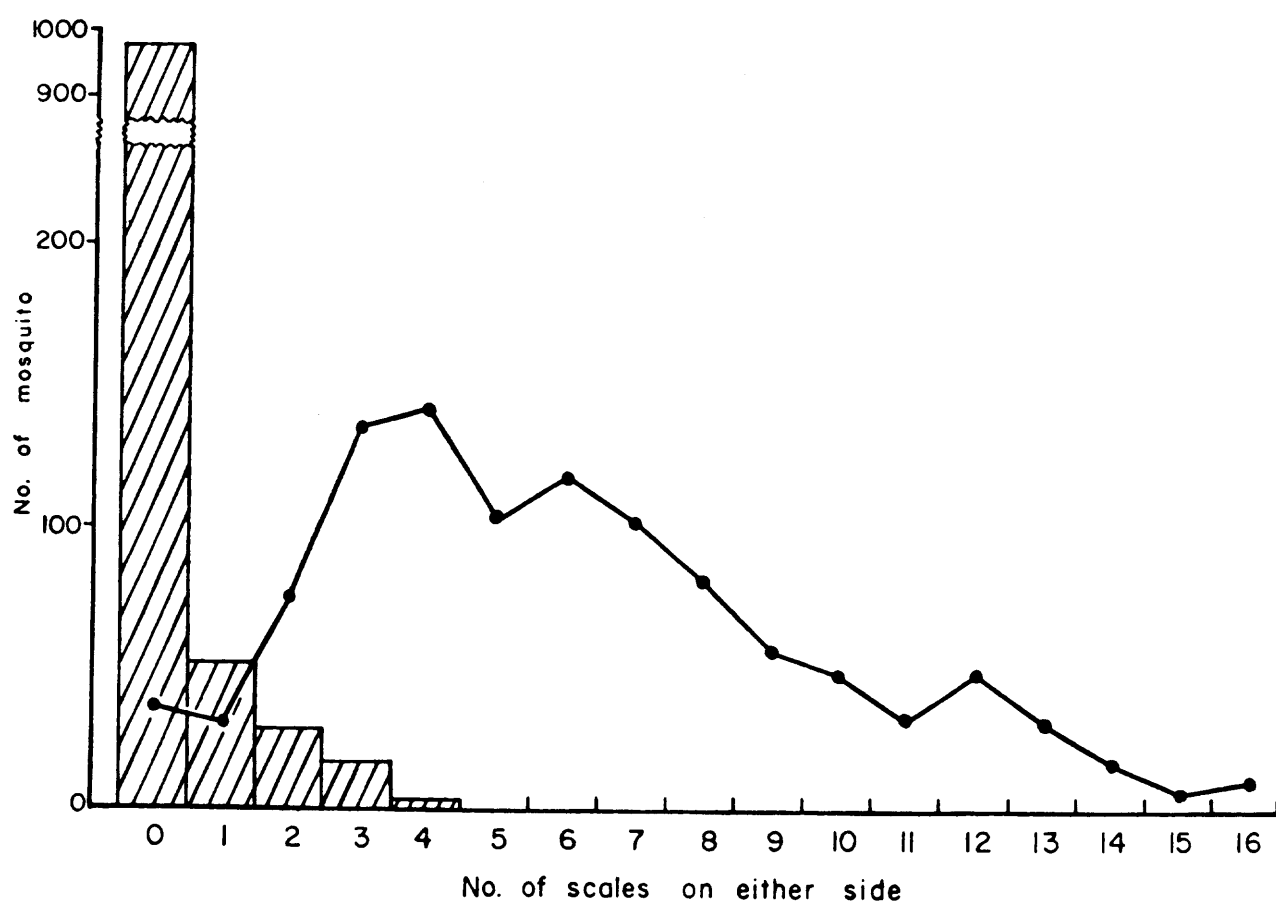


FIGURE 3

