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The chaetotaxy of the second and third instar larvae of Aeoes abserratus (F. & Y.) with particular reference to instar differentiation (Diptera: Culicidae).

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THE CHAETOTAXY OF THE SECOND AND THIRD INSTAR LARVAE OF AEDES ABSERRATUS (F. & Y.) WITH PARTICULAR REFERENCE TO INSTAR DIFFERENTIATION (DIPTERA: CULICIDAE)

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TABLE OF CONTENTS

INTRODUCTION

Mosquitoes comprise one of the most important groups of Diptera because of their role as vectors of disease-producing organisms in man and animals. New knowledge pertaining to this group increases man's ability to protect himself and his livestock from their ravages.

As intensively as the fourth instar larvae of the Culicidae (following the classification of Stone 1959) have been studied taxonomically, little attention has been paid to the first three instars, in particular the second and third instars.

Descriptions of the second and third instars and methods of distinguishing between these instars are important for three primary reasons: first, for instar differentiation within a species; second, for distinguishing between those species in which the well-known characters used in the fourth instar may be less developed in the second and third instars and therefore less reliable; and third, for phylogenetic studies for which descriptions of instars must be available before comparisons can be made and similarities and differences ascertained between species.

Aedes (Ochlerotatus) abserratus (Felt and Young) is a member of the punctor subgroup, a division of Edwards' (1932) Group G of the subgenus Ochlerotatus. This is a nearctic species restricted to southeastern Canada and northeastern

United States. The fourth instar larva has been described by Knight (1951), Carpenter and LaCasse (1955), Barr (1958) and others although knowledge of the chaetotaxy is far from complete. No description of the first and second instars has been published and only an extremely brief note on the third instar (Barr 1958). The purpose of this study is to describe and compare the chaetotaxy of the head, thorax, abdomen (in part), and the terminal segments of the two instars and to investigate characteristics of the chaetotaxy that might prove useful in separating the second from the third instar.

In addition to providing a description of these two hitherto undescribed instars, such a study might serve as a basis for future studies in geographic variation. A description of the chaetotaxy and a knowledge of its variation, both geographic and between the instars, especially when compared with similar studies for other species, may point out inherent variation or stability in certain characters and therefore serve as a guide to the choice of the best possible taxonomic characters and at the same time contribute towards a better understanding of phylogenetic relationships. Finally, it is hoped that methods of instar differentiation found useful in this species can be applied to other species in the family.

LITERATURE REVIEW

Specific Differences

Specific differences between the first instar larvae of the Culicidae have been summarized by Dodge (1966). The fourth instar is well known, having been described for the majority of species. Most of the keys are based on the characters of this instar. In the second and third instars the characters used to differentiate the fourth instar may or may not be satisfactory. According to Dodge (1963), some of these characters, such as hair branching and saddle development, are not fully developed in the second and third instars and therefore should not be used in distinguishing between species. However, he used other characters that remain constant throughout the last three instars to distinguish between species in any of these instars. Unfortunately, in some species of mosquitoes the only taxonomic characters are not fully developed in the second and third instars and become difficult to use, particularly in the second instar, as was demonstrated by Knight (1964) for Aedes sollicitans (Walker) and Aedes taeniorhynchus (Wiedemann). Other characters, such as those used by Linam and Nielsen (1963) for distinguishing Culex tarsalus Coquillett from Culex peux Speiser and Culex erythrothorax Dyar from Culex salinarius Coquillett, have not been investigated for variability in the second and third instars and might well prove unsatisfactory.

Instar Recognition

First Instar

The first instar larva is easily recognized by the presence of an egg burster and the lack of a ventral anal brush (Dodge 1966).

Fourth Instar

There does not appear, at present, to be a universally applicable method for distinguishing the fourth instar from the third or the second. Each group or species has its own criteria. All North American species in at least five genera (Psorophora, Culex, Culiseta, Mansonia, Uranotaenia) and many Aedes species have the anal segment completely ringed in the fourth instar (Carpenter and LaCasse 1955). Hedeen (1959) believed that in all species of mosquitoes in which the anal segment is completely ringed in the fourth instar, the earlier instars possess an incompletely ringed anal segment. Such a condition is true for all species of United States Psorophora (Hedeen 1959), the California Culex (Bohart and Washino 1957), Culiseta morsitans (Theobald) and Mansonia perturbans (Walker) (Barr 1958) and Uranotaenia anhydor Dyar (Belkin and McDonald 1956). In those species of Aedes in which the fourth instar is completely ringed, those of northeastern North America (Smith 1965) and A. infirmatus Dyar and Knab (Breland 1951) are known to be incompletely ringed in the third instar. However, Toxorhynchites rutilus septen-

trionalis (Dyar and Knab) is exceptional in having the anal segment completely ringed in all instars (Breland 1949).

In addition to the previously discussed characteristic, there are other characters that may be used in distinguishing the fourth instar. Smith (1965) identified those species of Aedes in eastern North America which have an incompletely ringed anal segment in the fourth instar by the position of the saddle hair or the shape of the saddle. Toxorhynchites rutilus septentrionalis (Dyar and Knab) in the fourth instar has an obvious increase or decrease in the diameter of certain thoracic hairs and an unusually large increase in the length of one thoracic hair; several thoracic hairs become removed from the sclerotized area they arise from in the third instar (Dodge 1964). In Anopheles punctipennis (Say) transparent areas appear on the abdomen in the fourth instar (Smith 1914). Mitrofanova (1929) reported the appearance of a median suture on the ventral surface of the head in the fourth instar in Anopheles maculipennis Meigan. In the genus Orthopodomyia many species acquire dorsal sclerotized plates on the distal abdominal segments in the fourth instar (Edwards 1932, Marshall 1938). Instar descriptions by Montchadsky (1926-27) for Aedes pulchritarsis (Rondani) and Hearle (1929) for Aedes flavescens Muller showed distinct differences in the ventral anal brush between the third and fourth instar. In addition to these characters, head and siphon lengths and widths (Mitrofanova 1929, Abdel-Malek 1948, Jones 1953, Shaw and Maisey 1961);

numbers of comb scales and pecten teeth (Marshall 1938); and hair branching (Marshall 1938, Breland 1949, Barr 1958) have been used to distinguish the fourth from the earlier instars.

Second and Third Instars

Compared to the amount of published work on the fourth instar, there has been a paucity of papers dealing with the second and third instars. Head and siphon lengths and widths have been shown to be clearly different between the second and third instars (Mitrofanova 1929, Abdel-Malek 1948, Jones 1953, Shaw and Maisey 1961). Hedeen (1959) separated the United States species of Psorophora by the shape of the siphonal apodeme. In addition to the above, Smith (1965) also used the following characters to differentiate between instars: the ratio of adult to larval eye; the ratios between head and collar, head and labial plate, labial plate and foramen; and the length-width ratio of the siphonal felt-chamber. Not one of these characters was applicable for all species.

Characters of the chaetotaxy usually play the major role in distinguishing between the instars. Occurrence of transitory hairs, differences in the numbers of cratal and precratal hairs, differences in the numbers of branches in ontogenetically homologous hairs, differences in the numbers of comb scales and pecten teeth, and a difference in the development of a particular hair are the most obvious characteristics of the chaetotaxy useful in distinguishing between the instars.

Transitory hairs may be defined as hairs appearing for the first time in the second or third instar. This review will be restricted to the third instar. According to Belkin (1952) prothoracic hair 0 is transitory, "appearing, if at all developed, in the second or third instar". This is the only observed reference to a transitory prothoracic hair appearing in the third instar. The meso- and metathoracic segments have one conspicuous transitory hair in roughly the same position on both segments. These meso- and metathoracic transitory hairs have been reported for Anopheles Walkeri Theobald (Hurlbut 1938), Toxorhynchites rutilus septentrionalis (Dyar and Knab) (Dodge 1964) and Uranoteania anhydor (Dyar) (Belkin and McDonald 1956).

The presence of transitory hairs is widespread within the family, occurring in all three subfamilies, and consequently may be of family-wide importance in differentiating between instars. Their presence may be of more common occurrence than reported. Most authors discussing second and third instars have restricted descriptions to the head, the terminal segments, and the lateral abdominal hairs. Therefore, transitory hairs on the thorax and abdomen may have been overlooked.

The ventral anal brush is composed of two parts, the cratal and the precratal hairs, which arise from the cratal (barred) area and the precratal area respectively. There is some difference of opinion as to exactly what constitutes the cratal and precratal areas (see discussion on page 65).

In spite of this, distinct differences occur between the instars of some species in the numbers of hairs in these areas. Belkin and McDonald (1956) for Uranotaenia anhydor Dyar referred only to the ventral brush, not distinguishing between cratal and precratal areas. Under diagnostic characteristics of the larval instars they noted two and four pairs of hairs respectively in the second and third instars. Knight (1964) in a table of differentiating characters for Aedes sollicitans (Walker) and Aedes taeniorhynchus (Wiedemann) listed 6-9 and 1-4 hairs in the second and third instars respectively for the precratal hairs of both species. In the remaining references, the authors did not utilize the described differences to differentiate between the two instars. Abdel-Malek (1949) showed no overlap in the range of the numbers of cratal and precratal hairs between the two instars of Aedes trivittatus (Coquillett). Hurlbut (1938) and Hearle (1929) showed no overlap in the range of the cratal hairs in Anopheles walkeri Theobald or Aedes flavescens (Muller), in the second and third instars. Christophers (1960) stated that "usually" there are four and six hairs in the second and third instars respectively for Aedes aegypti (L.).

Although some authors have observed distinct differences in the number of cratal and precratal hairs, others have reported little or no difference. Maisey (1959) recorded for Aedes atropalpus (Coquillett) almost complete overlap in the ranges of the cratal hairs of the two instars although

the third averaged slightly higher. Montchadsky (1926-27) recorded no difference in the numbers of cratal and precratal hairs in Aedes pulchritarsis (Rondani) and Breland (1949) stated that the ventral brush is similar in both instars for Toxorhynchites rutilus septentrionalis (Dyar and Knab).

Differences in branching of hair 3 of the anal segment was used by Breland (1949) to distinguish between the second and third instars in Toxorhynchites rutilus septentrionalis (Dyar and Knab). Marshall (1938) used the numbers of branches of the inner, middle and outer frontal hairs and the siphon tuft to aid in second and third instar differentiation for 18 species of British mosquitoes in the genera Aedes (11 species), Culiseta (5), Culex (1) and Orthopodomyia (1). In the following references, the author did not specifically use hair branching to distinguish between instars. However, on comparing the descriptions of the two instars, obvious differences (no overlap in range) are often apparent. Bohart and Washino (1957) in describing the second and third instars of the California Culex used the upper and lower head hairs, the pentad formula, siphonal tufts and the inner caudal seta of the anal segment. Only the upper head hairs and pentad hairs 1 and 3 had no overlap and then in only some of the species. Hurlbut (1938) in his study of the chaetotaxy of all four instars of Anopheles walkeri Theobald showed no overlap in one head hair, two prothoracic hairs, two metathoracic hairs, three abdominal hairs and three hairs on the

tenth abdominal segment. Table 1 summarizes the preceding information and gives other references.

In Toxorhynchites rutilus septentrionalis (Dyar and Knab) mesothoracic hair 4 became noticeable reduced in diameter in the third instar (Dodge 1964).

Differences in the number of cratal and precratal hairs and in the number of branches in a particular hair have, so far, been the most common methods of distinguishing between the instars. These two methods, being quantitative in nature (the transitory hairs would be qualitative), are subject to variation. Within each instar, in any one population, there is variability. In addition, branching (or even the number of hairs) may be modified by different environments (Barr 1954, and Belkin and McDonald 1956), therefore presenting the distinct possibility of geographical variation. Extremely important to the evaluation of the description of intra- and interpopulation variation is the amount of material studied and the location(s) from which the material was collected. Most of the existing literature gives inadaquate information on these two points. Commonly, the collection location of the specimens examined is omitted, and often there is no mention of the number of specimens or hairs studied. Also, the range is customarily given but measures of central tendency and the frequency distribution are often lacking. These latter two considerations are important when there is no observed overlap in the second and third instar ranges but assume particular importance when there is an observed overlap.

If only the range is given, any overlap raises a question as to the goodness of the hair as a differentiating mechanism. Measures of central tendency and frequency distribution give more information about the hair and enable the observer to make a more accurate evaluation. Range overlap does not preclude that a hair is entirely useless as a differentiating mechanism.

The problem of evaluating the relative number of comb scales and pecten teeth in the second and third instars as differentiating characters is the same as that for hair branching. The numbers of comb scales and pecten teeth are routinely given in descriptions of the second and third instars. However, in only a few instances have the comb scales and/or the pecten teeth actually been studied from the viewpoint of distinguishing between these instars. Marshall (1938) used both in conjunction with hair branching for the genera Aedes, Culiseta, and Culex, but only the comb scales along with hair branching for the genus Orthopodomyia.

Some studies have demonstrated that the relative numbers of comb scales and pecten teeth were not reliable for instar differentiation. This lack of reliability in both comb scales and pecten teeth has been shown for Aedes sollicitans (Walker) and Aedes taeniorhynchus (Wiedemann)(Knight 1964), and for Uranotaenia anhydor Dyar (Belkin and McDonald 1956), and in pecten teeth for Aedes atropalpus (Coquillett) (Maisey 1959).

The difference in development of a particular hair between the second and third instars was illustrated by Toxorhynchites rutilus septentrionalis (Dyar and Knab); mesothoracic hair 4 became noticeably reduced in diameter in the third instar (Dodge 1964).

METHODS AND PROCEDURES

Collection Locations

Specimens were collected from three locations in western Massachusetts (Table 2). Belchertown Bog in the town of Belchertown is located next to and on the south side of Route 181 and approximately 1.5 miles southeast of the junction of Route 181 and Route 202 in Belchertown. Federal Street Bog in the town of Belchertown can be located by travelling northeast towards Amherst on Route 9 from the junction of Route 9 and Route 202 for approximately 1.3 miles to the junction of Route 9 and Federal Street, then proceeding along Federal Street for approximately .5 miles. Collections were made on the north side of the road. Cushman Bog in the town of Amherst may be reached by walking south on the railroad tracks approximately 450 yards from the junction of Pine Street and the railroad tracks in Cushman. Collections were made on both sides of the tracks.

All three areas were collected in the spring, Belchertown Bog in 1963 and 1964, Federal Street Bog and Cushman Bog only in 1964.

Rearing and Preserving Specimens for Study

Specimens were collected with a long-handled dipper and transported back to the laboratory in a large jar (Fig. 1). At the laboratory the specimens were placed in four-dram vials,

one specimen to a vial. A pipette was used to transfer individual larvae to the vials. Water from the collecting site was used in the vials. Leaf parts or pieces of sphagnum moss from the collecting site were placed in the vial; presumably microorganisms associated with this plant material provided food for the larvae. A cotton plug was placed in the mouth of the vial to prevent dust from collecting on the water surface. Collections were numbered sequentially and each specimen that was studied was identified by the collection number and a subscript, each specimen receiving a different subscript. All subsequent information on the specimen was recorded under this mark or combination of number and subscript. See Fig. 2 for rearing equipment.

The larvae were reared at room temperature which was not controlled. Daily inspection revealed whether moulting had occurred. If so, the larva was killed in hot water and preserved in a vial of 70% alcohol, each vial being affixed with the larva's mark. The cast skin was placed in the vial of alcohol with the larva. At a later date some but not all of the alcohol-preserved material were mounted on microscope slides.

Procedure for Mounting Cast Skins and Larvae on Microscope Slides

Cast skin: 1. Skin was removed from alcohol by a pipette and placed on slide.

2. Excess alcohol was allowed to evaporate off, care being taken not to allow the skin to become dry.

3. A drop of cellosolve was placed on skin and skin positioned in the cellosolve.

4. Most of the cellosolve was drawn off, using paper toweling, again with care being taken to prevent desiccation.

5. Balsam of a runny consistency (diluted with cellosolve) was placed near but not directly on the skin and allowed to slowly envelop the skin and fill the siphon and head capsule. The skin was repositioned if necessary.

6. After 3 to 4 hours at room temperature a second coat of thicker balsam was added so that the skin was completely covered. The slide was placed in a drying oven for 12 to 24 hours. Skins covered with balsam but without a coverslip could not be left in the oven too long or cracking of the balsam occurred in which case the skin also cracked.

7. Upon removal from the oven, a small drop of balsam was placed directly on the skin and a coverslip added. There was little or no movement of the skin when the coverslip was added.

8. The slide was then returned to the oven for further hardening.

Larva: 1. The larva was removed from alcohol with a pipette and placed in the small dish of cellosolve for 10 minutes.

2. The specimen was removed from cellosolve and placed on a slide using a pipette. The 8th through 10th abdominal segments were severed from the body with insect

pins and the two pieces positioned on the slide. Enough cellosolve was left with the specimen to keep it thoroughly moist.

3. Steps 5-8 as given under the procedure for mounting cast skins were then followed.

Identification to Species

Abserratus is one of the easiest of the Aedes to determine to species in the last three larval instars. In the northeastern United States it is the only member of this genus in which anal hairs 3 and 4 are single. Thus the dorsal brush of the anal segment consists of only four hairs.

Criteria for Obtaining Known Instars

All specimens used in this study were reared so that the instar was definitely established. The first instar was easily recognized by the presence of the egg burster and the fourth instar by the completely ringed anal segment. Second-instar material was obtained by placing first instar specimens in individual vials until the first molt. This procedure gave the second instar larva plus the associated cast skin of the first instar. Third instar material was obtained by placing specimens that were not first or fourth instar in individual vials. The next molt resulted in either a third or fourth instar larva with its associated second or third instar cast skin.

Hairs Studied

The complete chaetotaxy of the head and thorax was

described. It was necessary to use slides of the cast skin in order to see the minute hairs. During the preparation of the slides, the abdomen, unlike the head or thorax, often became so twisted that the identification of the minute hairs was impossible. However, the large lateral and subventral hairs were easily identified and therefore the only ones described for this body region. These large hairs are the usual ones referred to in descriptions of the larval abdomen. On the eighth abdominal segment only the pentad hairs were described, the two minute hairs being omitted because of the afore-mentioned twisting. On the siphon the siphonal tuft and the dorsal preapical spine were described, the hairs of the siphon valves being omitted because they could not be adequately observed on the siphon which was mounted laterally. All the hairs of the anal segment were studied.

Measurements

Measurements were made with a binocular compound microscope equipped with a 10X micrometer ocular. Objectives of 10X and 43X provided magnifications of 100X and 430X. At 100X and 430X each division of the micrometer was calibrated to 11.0 and 2.6 microns respectively. Lengths and diameters were recorded in micrometer units and converted to microns before the statistics were calculated.

All hairs were measured from cast skin slides. The darkness of the integument of the larva often made the smaller hairs invisible on whole mounts. The length of the microscopic hairs was measured at 430X, the macroscopic hairs at 100X.

For convenience of description, the hairs were divided into macroscopic and microscopic hairs. The macroscopic hairs are large and usually spiculate hairs in which branching is easily seen under the lower powers of the dissecting microscope. The microscopic hairs are aspiculate and usually shorter and thinner than the macroscopic hairs; their branching is usually clearly seen only with the high power (430X) of the compound microscope. In branched hairs, the longest branch was measured. The length was recorded to the nearest whole micrometer unit. The range and mean length in microns and the number of hairs measured is given for all hairs and, in addition, the standard deviation is given for the macroscopic hairs. Only hairs that were reasonably straight and horizontal to the microscope stage were measured.

A slight difference in length was noted when an individual macroscopic hair was measured at 100X and 430X. This discrepancy is not considered large enough to be of importance when comparing the means of two different hairs, one measured at 100X and the other at 430X (fifteen #9 metathoracic hairs were measured at 100X and 430X and averaged 972 and 968 microns respectively, a difference of only four microns). Measurement at 100X is to be recommended for the macroscopic hairs because it is much faster.

The recorded mean length of the microscopic hairs is approximate, probably being slightly less than the true length for two reasons: 1) these hairs rarely were completely

straight, and some length was lost because of curvature; 2) the exceedingly fine tip of these hairs was often difficult to see and only what could be seen was measured, no allowance being made for possible error. It is assumed that the portion not seen was only a small fraction of the total length. The recorded mean length of the macroscopic hairs is closer to the true mean than is that of the microscopic hairs because these larger hairs showed little curvature and the tips were easily determined. Because of these possible sources of error in the microscopic hairs, the standard deviation was calculated only for the macroscopic hairs.

The diameter of each hair was measured at 430X and recorded to the nearest half unit on the ocular micrometer scale. No attempt was made to estimate the diameter of a hair that was less than one micrometer unit in diameter. The diameter measurement was then converted to microns, so that the diameter statistic was recorded as a whole number or a whole number and a half multiple of 2.6 (the length in microns for one micrometer unit). The resulting product is expressed in fractions of a micron which should not, however, be construed to represent such extreme accuracy. The range in microns and the number of hairs examined are given for each hair. The mean diameter is given for those hairs for which the lower distribution limit is 2.6 microns or more. Hairs that measured less than one unit are expressed as less than 2.6 microns (-2.6). The diameter recorded for branched

macroscopic hairs is that of the widest branch. In microscopic hairs the diameter before branching was recorded. The diameter reading of all hairs was taken at the widest point of the shaft which was invariably along the basal half of the shaft.

When the average length and diameter of different hairs were compared, the means for the most part reflected the situation found on an individual specimen. Therefore, the means were used when comparing hairs. However, variation does exist, and in two hairs with similar averages, the length or diameter of these hairs on one individual might be different from the average. For example, prothoracic hair 3 averaged 76 and 38 microns shorter than hair 2 in the second and third instars respectively. However, in a few individuals these hairs were observed to be very close to equal in length. The ideal method would be to compare these two hairs on each of several specimens. In this case the description might read that hair 3 was usually shorter than but may be equal to hair 2. This latter method for comparing the hairs is very time consuming and the author felt that the increase in accuracy does not warrant this approach. This method was used for the abdominal hairs and consequently their description differs somewhat from that of the other hairs. Because averages are used in all cases not otherwise noted, the word average is omitted and hairs are referred to as being similar to, shorter than, or longer than some other hair or hairs.

Sample Size

When possible, a minimum of thirty observations was made for the branching statistics and eighteen for all other characters. The sample size can be noted in the hair description or the appropriate table. No distinction was made between the hairs on the right and left sides. One or both hairs on a specimen were observed depending on whether they were clearly seen and properly positioned. Thus thirty hairs might represent anywhere from fifteen to thirty specimens. Material was studied from three breeding areas far enough apart to represent fairly independent gene pools. The study of three independent breeding areas provides a better picture of character variation than the study of one breeding area. Each breeding area represented a subsample. Where possible, subsamples of equal size were studied for each character. When this was not possible (a minority of cases), more material from the other areas was studied to make up the deficit and increase the total number of observations to the designated size. No attempt was made to analyze differences between breeding areas. Gross differences, if present, should have been obvious through simple inspection of the data. No such differences were noted.

Position of Hair on Specimen

The position of each hair was determined on specimens preserved in alcohol and examined with the dissecting microscope. The position of the smallest hairs could be seen with some difficulty at 144X, the larger hairs requiring less power.

The only exceptions were meso- and metathoracic hairs 12 which could only be seen on cast skin slides at 430X with the compound microscope. The position of each hair is described verbally and illustrated by drawings.

Hair Spiculation

The typical spiculate condition for each hair is described with obvious variations included in the description. In describing the length of the spicules on a given hair, only the longer ones were used. Density of spicules includes all the spicules present regardless of size.

Hair Branching

The number of branches for the macroscopic hairs was determined on alcohol specimens with the dissecting microscope. The microscopic hairs were examined on cast skin slides and on whole mounts of the larva with the compound microscope at 430X.

The branches of the macroscopic hairs have an equal point of origin near the alveolus and are always on the same plane so that they are spread out like the ribs on a fan (Fig. 12, 13). Branches that arise further up the shaft are rare except in head hair 7 and antennal hair 1. This latter condition is called secondary branching (Fig. 14-16). These branches are not included in the number recorded for a hair. If a hair is two-branched, the branches are equal or one is somewhat longer and thicker than the other. If more branches are present, typically some are longer and thicker than others.

Occasionally all the branches are equal in diameter but never are they all equal in length.

In the microscopic hairs branching typically occurs on the same plane well away from the alveolus, with the branches equal or subequal in length, diameter and point of origin (Fig. 6, 17, 18). All branches, regardless of size, were recorded for the microscopic hairs. In some microscopic hairs the branches arise from more than one plane and may be decidedly unequal in length, diameter and point of origin, giving the hair a conical appearance from an end-on view and a dendritic appearance when viewed laterally (Fig. 7, 19). For these hairs only an estimation of the number of branches per hair was possible. The resolving power of the microscope was not sufficient to differentiate all the branches since these were often numerous and tended to obscure one another. Only an estimated mean and range can be given for these hairs.

For other than conical hairs, the following statistics are given for the number of branches per hair: mode, percentage of branches in the mode (modal value), mean, range, standard deviation, standard error of the mean and coefficient of variability.

Comb Scales and Pecten Teeth

The numbers of both comb scales and pecten teeth were easily counted with the dissecting microscope on alcohol-preserved specimens, and on slides of cast skins and larvae with the compound microscope.

DESCRIPTIONS

Introduction

The system used for numbering the hairs is that of Belkin (1950). Table 19 presents the hairs studied in this paper with the exceptions of Antennal hairs 2-6.

Hairs are described in numerical sequence. Macroscopic hairs are identified by an asterisk before the hair number. Immediately after the hair number references to the figure illustrating the hair position and the tables that apply to the length and branching statistics are given in that order. The figures are primarily to illustrate hair position and typical appearance. Hairs are not drawn to scale and relative sizes are only generally indicated. Data in the text should be consulted when comparing hairs with respect to size and branching. II denotes the second instar and III the third instar in all descriptions and tables. Where there is no difference between the instars, a single description is given applying to both instars.

The characteristics of each hair are described in the following order after the figure and table references:

Position - the position is described.

Length - length statistics are given in parenthesis after the instar in the following order: mean in microns, range in microns, and number of hairs examined. The standard

deviation for the macroscopic hairs is given in tabular form.

Diameter - diameter statistics are given in parenthesis after the instar in the following order: mean in microns, range in microns, and number of hairs examined. Where the same statistics apply to both instars, they are given once after the third instar.

Branching - statistics for the number of branches per hair are given in parenthesis after the instar in the following order: mode, percentage of hairs in the mode (modal value), mean, range, coefficient of variability, and number of hairs examined. The mode, mean, and range are given in tabular form for easy comparison along with the standard deviation and standard error of the mean. When hairs are unbranched, the number examined is given in parenthesis after each instar. Where the same statistics apply to both instars, they are given once after the third instar. For the conical hairs only an estimate of the mean and range is given (also see discussion of conical hairs on p. 23).

Spiculation - description of length and density of the spicules if present.

Sclerotization - description of basal sclerotization if present.

Head Hairs

Belkin (1950) listed sixteen hairs for the head capsule of the fourth instar larva of the Culicidae. All were observed on the second and third instars except #0 and #2. Neither was observed in the fourth instar of A. abserratus. Hair 0 was described but not figured for two species of the subgenus Ochlerotatus in the fourth instar by McDonald (1957 a, b) and illustrated for four species in the fourth instar by Belkin (1962). Belkin and McDonald (1957) listed it as "not seen" in their description of the fourth instar of Aedes (O.) monticola Belkin and McDonald. Hair 2 was said by Belkin (1962) to be "apparently always absent" in the tribe Aedini.

If one examines the figures of the subgenus Ochlerotatus given in Belkin's 1962 work it is apparent that he has deviated from his original description as to the relative positions of head hairs 12 and 13. In his 1951 publication he stated that #12 "may migrate mesad but is always latered of #13." Undoubtedly Belkin is continuously revising his original work as more information comes to light; this apparent descrepancy is probably a product of increased knowledge. However, since there was no mention of this change in the text his 1950 interpretation will be used in this study. It should be noted that if the relative position of these two hairs is constant in the subgenus Ochlerotatus, #12 in this paper would then be #13, and vice versa, according to Belkin's

1962 publication.

Hair 0: Missing

*Hair 1: Fig. 5. Tables 3, 4.

Position- arises from labrum on either side of palatum curving inwards and downwards, the two hairs from each side partially enclosing palatum. Length- II (90, 78-117, 30), III (133, 112-159, 22); II: 0.83 to 1.45 times as long as distance between alveolus of antennal hair 1 and base of antenna, mean 1.04; III: 0.73 to 1.24, mean .97. Diameter- II (6.5, 5.2-7.8, 18), III (9.7, 7.8-10.4, 17) or approximately one fifth greatest diameter of antenna in both instars. Branching- single and spinelike; II and III (64). Spiculation- aspiculate or with a few spicules; spicule length variable, ranging from only notches being visible to equal to width of shaft at base of spicule.

Hair 2: Missing

Hair 3: Fig. 5.

Position- arises from clypeus immediately dorsal to clypeolabral suture and somewhat mesad of lateral corner of labrum. Projects downward and curves towards hair 1, appearing almost pressed to labrum. Extremely difficult to see with dissecting microscope because of lack of pigment. Length- II (41, 31-49, 18), III (63, 52-73, 13). Diameter- II and III (2.6, 18). The length and diameter are only approximations because

the typical position of this hair was never horizontal to the micrometer. Branching- single, II (63), III (64).

Hair 4: Fig. 5. Tables 5, 10.

Position- dorsal; most mesad of dorsal head hairs, mesad of #6 and on or just mesad of a line drawn posteriorly from #1. Length- II (74, 52-104, 19), III (71, 52-101, 20). Diameter- II and III (-2.6, 12). Branching- II (2, 57%; 1.78; 1-3; 35%; 65), III (2, 58%; 2.32; 1-5; 32%; 62).

*Hair 5: Fig. 5. Tables 3,4.

Position- dorsal; most posterior and mesad of macroscopic dorsal head hairs, on or just laterad of a line drawn posteriorly from #1 and somewhat anterior to transverse midline of frontoclypeus. Length- II (258, 231-297, 20), III (340, 297-263, 20) or just shorter than to almost half again as long as antenna, most commonly just longer than antenna, averaging 17% and 5% longer in second and third instars respectively. Diameter- II (4.0, 3.9-5.2, 23), III (5.6, 5.2-7.8, 18). Branching- single II (178), III (175). Spiculation- II, length as in Fig. 20 to so reduced that only notches are visible, density less; III, length as in Fig. 20 or less, density as in Fig. 20 or less.

*Hair 6: Fig. 5. Tables 3,4.

Position- dorsal; anterior and just lateral to #5 and on a line drawn mesally from antennal socket.

Length- II (256, 231-286, 20), III (330, 297-352, 20). Diameter- II (4.2, 3.9-5.2, 23), III (6.5, 5.2-7.8, 19). Branching- single; II (180), III (176). Spiculation- similar to #5.

*Hair 7: Fig. 5. Tables 3,4,5,10.

Position- dorsal; most laterad of macroscopic dorsal head hairs, dorsal to mesal edge of antennal foramen. Length- II (247, 209-297, 20), III (308, 275-341, 20). Diameter- II (4.6, 3.9-5.2, 18), III (6.6, 6.5-7.8, 18). Branching- II (1, 95%; 1.05: 1-2; 21%; 178), III (2, 59%; 1.69; 1-3; 33%; 174); displays proclivity for secondary branching (12 out of 52 and 14 out of 58 for II and III instars respectively). Spiculation- II, length greater than in Fig. 20 to so reduced that only notches are visible, density as in Fig 20 or less; III, length and density same as or greater than in Fig. 20.

Hairs 8-10:

The positions of head hairs 8, 9 and 10 are partially described by their positions relative to the combined larval and adult eyes. No distinction is made between the two eyes because they are not always distinct from each other, especially in the second instar.

Hair 8: Fig. 5. Tables 5, 10.

Position- dorsal; mesad of eye and somewhat mesal to frontoclypeal suture. Length- II (137, 96-185, 20),

III (144, 117-172, 20). Diameter- II (-2.6, 13), III (-2.6,14). Branching- II (2, 64%; 1.86; 1-3; 32%; 99), III (2, 52%; 2.47; 1-4; 25%; 75).

Hair 9: Fig. 5. Tables 5, 10.

Position- dorsal; mesad of eye, somewhat laterad of frontoclypeal suture and posterior to #8. Length- II (138, 104-185, 20), III (154, 112-198, 22). Diameter- II (-2.6, 15), III (-2.6, 14). Branching- II (2, 66%; 1.91; 1-3; 32%; 68), III (2-3, 45%-45%; 2.54; 1-5; 28%; 71).

Hair 10: Fig. 5. Tables 5, 10.

Position- dorsolateral; just dorsal to posterior half of eye and lateral to or just posterior to #9. Length- II (134, 89-182, 26), III (154, 114-185, 21). Diameter- II (-2.6, 17), III (-2.6, 14). Branching- II (2, 84%; 2.00; 1-4; 21%; 72), III (2, 69%; 2.37; 2-4; 25%; 35).

Hair 11: Fig. 5. Tables 5, 10.

Position- ventroloateral; on or just lateral to a line drawn posteriorly from lateral edge of antennal foramen and somewhat anterior to midpoint between antennal foramen and ocular bulge. Length- II (68, 49-83, 24), III (81, 65-104, 20). Diameter- II and III (-2.6, 12). Branching- slightly conical; II (4, 37%; 3.97; 2.7; 26%; 75), III (4, 36%; 4.66; 2-9; 28%; 67).

Hair 12: Fig. 5. Tables 5, 10.

Position- ventrolateral; on or somewhat laterad of a line drawn posteriorly from center of ventral edge of antennal foramen, somewhat posterior to a line drawn laterally from angle formed by ventral edge of oral cavity and anterior end of maxillary suture. Length- II (152, 130-195, 20), III (181, 133-203, 23). Diameter- II and III (-2.6, 12). Branching- II (1, 97%; 1.03; 1-2; 17%; 62), III (1, 86%; 1.14; 1-2; 31%; 64).

Hair 13: Fig. 5. Tables 5, 10.

Position- ventrolateral; somewhat posterior to and mesad of # 12. Length- II (62, 47-99, 20), III (74, 52-104, 24). Diameter- II and III (-2.6, 12). Branching- II (2, 51%; 2.32; 1-5; 36%; 77), III(3-4, 31%-31%; 3.61; 2-7; 30%; 67).

Hair 14: Fig. 5, 24, 25.

Position- ventral; closely associated with angle formed by ventral edge of oral cavity and anterior end of maxillary suture, posterior to ventral articulation of maxilla. Length- II (37, 29-47, 20), III (54, 39-65, 20). Diameter- II (3.2, 2.6-3.9, 18), III (4.0, 3.9-5.2, 16). Branching- single and spine-like; II and III (64).

Four anomalies were noted: on three specimens one of the hairs was forked in a manner similar to Fig. 24; on one of these specimens one branch of the

fork was shorter and thinner than the other; on another specimen there were two hairs on the right side (Fig. 25).

Hair 15: Fig. 5. Tables 5, 10.

Position- ventral; on gular area approximately mid-point between maxillary suture and midventral line, lateral to or just anterior or posterior to #13. Length- II (63, 52-75, 20), III (75, 57-88, 16). Diameter- II (-2.6, 20), III (-2.6-2.6, 18). Branching- II (2, 59%; 2.21; 1-3; 28%; 78), III (3, 43%; 3.26; 2-6; 29%; 53).

Antennal Hairs

Belkin in 1950 numbered, for the first time, the six hairs that regularly occur on the antenna of the fourth instar culicid larva: 1- antennal hair, shaft hair; 2- inner subapical hair, dorsal sabre; 3- outer subapical hair, ventral sabre; 4- terminal antennal hair; 5- hyaline process, papilla; 6- finger process (from Belkin 1950). All of these hairs occurred in both the second and third instar. There was little or no difference between the two instars in the location of each hair. The length and thickness of each hair in relation to the other antennal hairs remained the same in the two instars.

*Hair 1: Fig. 5, 16. Tables 3, 4, 5, 10.

Position- dorsal; alveolus 30% to 50% of antennal length from proximal end of antennal sclerotization. Length- II (126, 99-148, 20), III (167, 156-195, 20); hair does not extend to or barely reaches tip of antenna. Diameter- II (4.4, 2.6-5.2, 20), III (4.6, 2.6-7.8, 20). Diameter of unbranched second instar hairs similar to that of branches of third instar hairs, i.e. the means were similar and only two third instar hairs had diameters over 5.2 microns; these were single hairs. Branching appears to replace diameter increase in third instar except where third instar hairs are single and then there is an increase

in diameter. Branching- II (1, 98%; 1.02; 1-2; 12%; 122), III (2, 60%; 2.32; 1-4; 33%; 1.06). Displays tendency towards secondary branching; secondary branches may occur anywhere along shaft and the apex often breaks up into two or more secondary branches (Fig. 15, 16). In third instar, shafts of branched hair may differ in degree of secondary branching. Spiculation- shaft may be prominently spiculate, especially in second instar, with numerous, easy-to-see spicules, or nearly aspiculate with very small and widely scattered spicules. In some cases even these small and widely scattered spicules are apparently absent. In third instar, shafts of branched hair may differ in amount of spiculation.

From between the central portion to the apex of the shaft small secondary branches may arise often appearing to be no more than greatly enlarged spicules. This condition in conjunction with secondary branching often gives the end of the hair a bushy appearance (Fig. 16).

Hairs 2-6:

These hairs are located at the distal end of the antenna. Their sizes and shapes are illustrated in Figs. 3-4. All are single and as there is no difference, except an increase in size, in any of the hairs between the instars, the two figures illustrate both instars. The following description

of the hairs likewise applies to both instars.

All of these hairs arise from a membranous area at the apex of the antenna. Hairs 2 and 3 arise subapically from the ventral side of the antenna. Hair 2 is the internal member of this pair and the most prominent of the terminal antennal hairs. Hair 3 is shorter and more slender that #2.

Hairs 4-6 are apical in position. Hair 4 is the most dorsal of these apical hairs. Ventral to #4 are hairs 5 and 6. Hair 5 is the internal hair of this pair and is unmistakable because of its terminal transparent portion. Hair 6 is uniquely characterized by a transverse membranous line near its base.

Thoracic Hairs

Prothoracic Hairs

All fourteen hairs listed by Belkin (1950) are present.

Hair 0: Fig. 8.

Position- dorsal; posterior and lateral to submedian prothoracic group. Length- II (61, 44-81, 20), III (60, 49-75, 20). Diameter- II (-2.6, 20), III (2.6, 14). Branching (estimate only) - conical. II: 25 hairs; mean 4.92; range 3-7. III: 10 hairs; mean 7.40; range 6.10;

Submedian prothoracic group

The dorsal submedian prothoracic group consists of three single (#1 is rarely double) macroscopic hairs (1, 2, 3) whose alveoli form an anterio-posterior row angled slightly away from the median line as the row extends posteriorly. The alveolus of #1 is larger and somewhat removed from the alveoli of #2 and #3 which are similar in size and almost touching. There is no sclerotization around the alveoli. Hair 1 is the most prominently spiculate, and the longest and thickest of the three hairs. Hairs 2 and 3 are similar in spiculation and diameter but #3 is often shorter than #2. These three hairs are somewhat stouter than the rest of the macroscopic thoracic hairs.

*Hair 1: Fig. 8. Tables 3, 4, 6, 10.

Length- extends approximately to base of #5 when lying flat; II (564, 451-616, 21), III (763, 660-869, 29). Diameter- II (5.1, 3.9-5.2, 19), III (8.6, 7.8-9.1, 19). Branching- II (1, 100%; 1.00; 1; 0%; 115), III (1, 86%; 1.14; 1-2; 30%; 88). Spiculation- II, length and density as in Fig. 21 or density less; III, as in Fig. 21.

*Hair 2: Fig. 8. Tables 3, 4.

Length- II (480, 396-561, 21), III (607, 473-671, 20). Diameter- II (3.8, 2.6-3.9, 19), III (6.1, 5.2-6.5, 16). Branching- single; II and III (117). Spiculation- length equal to or less than in Fig. 21, density less.

*Hair 3: Fig. 8. Tables 3, 4.

Length- II (404, 352-484, 20), III (569, 495-649, 26). Diameter- II (3.7, 2.6-3.9, 17), III (6.2, 5.2-7.8,22). Branching- single; II (119), III (118). Spiculation- similar to hair 2.

Hair 4: Fig. 8. Tables 6, 10.

Position- dorsal; at midpoint or somewhat lateral to midpoint between submedian prothoracic group and #5. Length- II (222, 174-268, 20), III (259, 211-325, 17). Diameter- II (-2.6, 22), III (2.5, 23). Branching- II (1, 52%; 1.48; 1-2; 34%; 67), III (2, 60%; 1.63; 1.3; 32%; 52).

Hairs 5-7

Hairs 5, 6 and 7 are macroscopic with sclerotized areas

posterior to the alveoli which may or may not connect with each other and which generally increase in size from #5 to #7. Hair 5 has the smallest sclerotized area in both instars; the sclerotized area of #6 is larger than that of #5 in the second instar and equal to or slightly larger than that of #5 in the third instar; the area of #7 is equal to or larger than that of #6 in the second instar and larger than that of #6 in the third instar. Hair 5 is longer than #7 in both instars but is relatively longer in the third instar. In diameter it is similar to #7 in the second instar and considerably thicker in the third instar. Hair 6 is less in length and diameter than #5 and #7 in both instars. However, it is longer and thicker in relation to #7 in the third instar. This increase in the differences in length and diameter between #5 and #7 and the decrease in these differences between #6 and #7 in the third instar may be due to #7 being primarily single in the second instar and double in the third. An increase in the number of shafts may replace a larger gain in length and diameter that is found in the third instar in hairs that are unbranched in both instars. These three hairs are similar in spiculation; the spicules have a tendency to be longer than in the submedian prothoracic group or on #8 and #11.

*Hair 5: Fig. 8-10. Tables 3,4.

Position- dorsolateral; lateral to #4. Length- extends to or beyond membranous base of antenna when lying

flat; II (768, 726-814, 20), III (992, 847-1078, 21). Diameter- II (5.4, 5.2-6.5, 20), III (8.1, 7.8-9.1, 23). Branching- single; II and III (118). Spiculation- length tends to be longer than in Fig. 21, density similar to or less. Sclerotization- small area posterior to alveolus.

*Hair 6: Fig. 8-10. Tables 3, 4.

Position- dorsolateral; ventral to and slightly posterior to #5. Length- II (595, 539-649, 21), III (739, 671-825, 24). Diameter- II (4.5, 3.9-5.2, 19), III (5.9, 5.2-6.5, 19). Branching- single; II (119), III (117). Spiculation- similar to #5. Sclerotization- an area posterior to alveolus.

*Hair 7: Fig. 8-10. Tables 3, 4, 6, 10.

Position- lateral; ventral and slightly posterior to #6. Length- II (670, 550-726, 21), III (787, 704-968, 33). Diameter- II (5.6, 5.2-6.5, 18), III (6.7, 6.5-7.8, 22). Branching- II (1, 98%; 1.02; 1-2; 14%; 142), III (3, 83%; 1.84; 1-3; 21%; 156). Spiculation- similar to #5. Sclerotization- an area posterior to alveolus.

*Hair 8: Fig. 8-10. Table 3,4.

Position- lateral; ventral to #7, dorsal and anterior to prothoracic pleural group. Length- II (370, 275-451, 20), III (640, 539-693, 22). Diameter- II (3.1, 2.6-3.9, 19), III (5.6, 5.2-6.5, 20). Branching- single;

II (120), III (119). Spiculation- this hair is smaller than the other spiculate prothoracic hairs, therefore the spicules are smaller and harder to see, so that the hair appears less spiculate. Discounting size, the spiculation may be compared with Fig. 21. II, length and density as in Fig. 21 or density less; III, as in Fig. 21. Sclerotization- a very narrow ring surrounding alveolus.

Prothoracic Pleural Group

Each thoracic segment has a partially sclerotized tubercle which may be ventrolateral or lateral depending on the segment. Besides a similarity in position, the tubercles have other characteristics in common. The posterior side of the tubercle and the anterior apical portion of the tubercle that holds the hairs are sclerotized; the anterior side is membranous. Four hairs occur in two pairs: an anterior pair in which the external hair is #9 and the internal #10 and a posterior pair in which the external hair is #11 (posterior to #9) and the internal is #12 (posterior to #10). A sclerotized finger-like process of the tubercle projects dorsally between #10 and #12.

The prothoracic tubercle is ventrolateral in position. The posterior pair of hairs contains the largest and smallest hairs which are respectively #11 and #12. The anterior pair, #9 and #10, are inserted opposite #11 and #12 respectively and are similar in size. An apical serrated edge of the tubercle

partially surrounds the alveolus of #11.

Hair 9: Fig. 8.

Length- II (328, 260-382, 22), II (368, 299-419, 16). Diameter- II (2.6, 16), III (2.6, 23). Branching- single, II (71), III (56).

Hair 10: Fig. 8. Tables 6, 10.

Length- II (310, 263-374, 20), III (311, 234-408, 17). Diameter- II (2.6, 19), III (2.6, 20). Branching- II (1, 93%; 1.07; 1-2; 24%; 73), III (1, 58%; 1.15; 1-3; 62%; 59).

*Hair 11: Fig. 8. Tables 3, 4.

Length- often extends to or beyond membranous base of antenna when lying flat; II (689, 649-737, 23), III (876, 803-979, 20). Diameter- II (6.1, 5.2-6.5, 19), III (8.6, 7.8-10.4, 20). Branching- single; II and III (118). Spiculation- as in Fig. 21.

Hair 12: Fig. 8. Tables 6, 10.

Length- II (88, 60-107, 20), III (84, 65-107, 17). Diameter- II (-2.6, 19), III (-2.6-2.6, 22). Branching- II (2, 68%; 1.74; 1-4; 31%; 59), III (3, 41%; 2.74; 1-5; 33%; 41).

Hair 13: Missing

Hair 14: Fig. 8. Tables 6, 10.

Position- ventral; on each side of base of neck close to collar of head capsule. Length- II (82, 70-101, 21), III (104, 65-127, 20). Diameter- II (-2.6, 17), III

(2.6, 22). Branching- usually single and spine-like, curving near the base, with a long thin apex; II (1, 97%; 1.03; 1-2; 15%; 79), III (1, 94%; 1.06; 1-2; 22%; 51).

On one specimen two hairs were present on one side.

Mesothoracic Hairs

There are thirteen hairs in the second instar. Macroscopic hair 8 appears for the first time in the third instar bringing the total to the fourteen of Belkin (1950). Mesothoracic hair 5 has somewhat fewer and shorter spicules than #6 and #7 which are similar in spiculation. Hairs 8 and 10 have the longest and densest spicules on the mesothorax and are similar. Hairs 9 and 11 are similar in spiculation to each other and to #6 and #7.

Mesothoracic Hairs 1-4

Hairs 1-4 are microscopic and form a dorsal transverse row. Hair 1 is just laterad of the midpoint between the dorsal midline and the pleuron and mesad of #5. Hair 2 is laterad of and often slightly anterior to #1, similar in appearance but shorter than #1. Although these two hairs are similar in size, #1 is much less easily seen under the dissecting microscope. Hair 3 is laterad of #2 and is long and single. Hair 4 is laterad of #3 and mesad of #5; it is similar to #3 in diameter but is shorter and multibranched. Hairs 3 and 4 are both dark and easily seen for the first one third to one half of their length; the remaining portion is lightly pigmented, very thin, and difficult to see.

Hair 1: Fig. 8. Tables 7, 10.

Length- II (97, 55-138, 21), III (138, 117-164, 7).

Diameter- II (-2.6, 18), III (-2.6, 19). Branching- II (2, 71%; 1.79; 1-3; 28%; 48), III (2-3, 50%-50%; 2.50; 2-3; 20%; 28).

Hair 2: Fig. 8. Tables 7,10.

Length- II (68, 52-94, 20), III (73, 55-109, 8). Diameter- II and III (-2.6, 18). Branching- II (2, 63%; 1.67; 1-3; 31%; 43), III (2, 53%; 2.36; 1-4; 22%; 36).

Hair 3: Fig. 8.

Length- II (324, 273-382, 20), III (369, 338-393, 9), Diameter- II (-2.6-2.6, 20), III (-2.6-2.6, 16). Branching- single; II (86), III (47).

Hair 4: Fig. 8. Tables 7, 10.

Length- II (205, 182-242, 20), III (202, 166-224, 7). Diameter- II and III (-2.6-2.6, 20). Branching- II (2, 66%; 2.40; 2-5; 26%; 73), III (3, 47%; 3.47; 2-5; 21%; 43).

*Hair 5: Fig. 8-10. Tables 3, 4.

Position- dorsolateral; laterad of #4. Length- extends to or beyond membranous base of antenna when lying flat; II (824, 737-902, 20), III (1101, 968-1177, 20). Diameter- II (5.3, 5.2-6.5, 18), III (7.9, 7.8-9.1, 18). Branching- single; II (119), III (117). Spiculation- length as in Fig. 21, density less. Sclerotization- small area posterior to alveolus.

<u>Hairs 6-7</u>:

Hairs 6 and 7 share a common sclerotized area posterior

to their alveoli which is ventral to #5 on the upper half of the pleuron. Hair 6 is multibranched, dorsal and posterior to the single #7. Hairs 6 and 7 have somewhat more numerous and longer spicules than #5.

*Hair 6: Fig. 8-10. Tables 3, 4, 7, 10.

Length- II (809, 726-902, 20), III (1013, 825-1166, 20). Diameter- II (5.1, 3.9-6.5, 18), III (7.1, 6.5-9.1, 18). Branching- II (3, 82%; 3.00; 2-4; 14%; 119), III (3, 77%; 3.22; 2-4; 14%; 115). Spiculation-length and density less than in Fig. 22.

*Hair 7: Fig. 8-10. Tables 3,4.

Length- II (707, 660-792, 21), III (903, 792-990, 20). Diameter- II (4.6, 3.9-5.2, 18), III (6.3, 5.2-6.5, 18). Branching- single; II and III (117).

Spiculation- similar to #6.

*Hair 8: Fig. 8, 10. Tables 4, 8, 10.

Absent in second instar. Position- lateral; ventral and slightly anterior to #7. Length- III (796, 715-902, 20). Diameter- III (5.4, 5.2-6.5, 18). Branching- III (4, 47%; 4.38; 2-6; 18%; 113). Spiculation- III, as in Fig. 22. Sclerotization- an area posterior to alveolus.

Mesothoracic Pleural Group

The tubercle is lateral on the lower half of the pleuron. It is the most ventral of the sclerotized areas, being ventral to the sclerotized area of hairs 6 and 7 in

the second instar and ventral and posterior to the sclerotized area of hair 8 in the third instar. Hair 10 is the macroscopic multibranched hair and lacks serrations around the alveolus. Posterior to hair 10 is the minute hair 12 which is invisible even with the compound microscope unless in exactly the right position on the slide. A plainly visible round white spot marks the insertion of hair 12. A sclerotized finger-like process of the tubercle projects dorsally between hairs 10 and 12. Lateral to hair 10 is the single macroscopic hair 9. The sclerotized area ventral and posterior to the alveolus of hair 9 forms a short single row of serrations. Posterior to hair 9 and lateral to hair 12 is macroscopic hair 11; ventral and posterior to its alveolus there is a row of serrations that is similar to but separate from and less developed than that of hair 9. The alveoli of the posterior pair of hairs are much closer together than those of the anterior pair. Hair 10 has the longest and densest spicules of the mesothoracic pleural group, hairs 9 and 11 being similar. (For introduction to pleural groups see pg. 40).

*Hair 9: Fig. 8. Tables 3, 4.

Length- extends to and often well beyond membranous base of antenna when lying flat; II (852, 792-902, 21), III (1086, 990-1166, 20). Diameter- II (6.7, 6.5-7.8, 18), III (8.9, 7.8-10.4, 18). Branching- single; II (119), III (118). Spiculations- length

and density less than in Fig. 22.

*Hair 10: Fig. 8. Tables 3, 4, 7, 10.

Length- II (756, 660-858, 20), III (910, 803-1045, 22). Diameter- II (5.3, 5.2-6.5, 19), III (7.4, 6.5-9.1, 18). Branching- II (3, 81%; 3.00; 2-5; 16%; 118), III (4, 45%; 4.48; 3-6; 17%; 119). Spiculation- as in Fig. 22.

*Hair 11: Fig. 8. Tables 3, 4.

Length- II (779, 671-869, 21), III (977, 902-1089, 21). Diameter- II (5.3, 5.2-6.5, 18), III (7.7, 6.5-7.8, 18). Branching- single; II (118), III (116). Spiculation- length and density less than in Fig. 22.

Hair 12: Tables 7, 10.

Length- II (26, 16-34, 3), III (17, 17, 1). Diameter- II and III (-2.6, all hairs observed were obviously less than 2.6 microns in diameter). Branching- II (2, 56%; 2.44; 2-3; 31%; 18), III (2, 55%; 2.45; 1-4; 31%; 20).

Hair 13: Fig. 8-10.

Position- lateral. II: anterior to and just dorsad of or even with dorsal edge of sclerotized area of pleural tubercle; III: same as II, and ventral and anterior to hair 8. Length- II (102, 78-146, 20), III (100, 88-143, 9). Diameter- II (-2.6-2.6, 21), III (-2.6-3.9, 19). Branching (estimate only)- conical. II: 60 hairs; range 3-10; mean 6.5. III: 35 hairs; range 9-20; mean 14.00.

Hair 14: Fig. 8.

Position- ventral; directly posterior to prothoracic hair 14 and mesad of pleural tubercle. Length- II (49, 36-60, 20), III (56, 42-68, 20). Diameter- II (-2.6, 18), III (-2.6-2.6, 16). Branching (estimate only)- conical. II: 46 hairs; range 4-12; mean 7.48. III: 20 hairs; range 8-16; mean 11.40.

Metathoracic Hairs

There are twelve hairs in the second instar. Macroscopic hair 7 appears for the first time in the third instar bringing the total to the thirteen hairs of Belkin (1950). Hair 7 has the longest and densest spicules. Hairs 9 and 10 are similar in spiculation.

Metathoracic Hairs 1-6

Hairs 1-6 are microscopic and form a transverse row; hairs 1-5 are dorsal and hair 6 is dorsolateral. Hair 1 is mesad of and slightly posterior to the pleural tubercle about on a line drawn posteriorly from the submedian prothoracic group. It is the smallest of these dorsal hairs. Hair 2 is laterad of and very slightly anterior to hair 1. Hair 3 is laterad of and slightly anterior to hair 2. Hairs 2 and 3 are similar in appearance except hair 2 is the longer. Hair 4 is laterad of and slightly posterior to hair 3, slightly conical, and much shorter than hairs 2 and 3. Hair 5 is laterad of and slightly anterior to hair 4 and is distinctive, being short and spine-like. Hair 6 is laterad of and slightly posterior to hair 5. It is dorsal to the pleural group in the second instar and dorsal to hair 7 in the third instar. It is long and predominantly single. The anterior and posterior positions of the hairs in relation to the other hairs appear to be more pronounced in the third instar. Hairs 2,

3 and 6 are similar to mesothoracic hairs 3 and 4 in having well-pigmented basal portions and lightly pigmented, very thin and difficult-to-see apical portions.

Hair 1: Fig. 8. Tables 8, 10.

Length- II (40, 18-73, 20), III (45, 29-62, 19). Diameter- II and III (-2.6, 18). Branching- II (2, 50%; 1.71; 1-3; 38%; 58), III (2, 62%, 1.85; 1-3; 33%; 26).

Hair 2: Fig. 8. Tables 8, 10.

Length- II (156, 127-190, 18), III (166, 135-198, 11). Diameter- II and III (-2.6, 18). Branching- II (3, 52%; 2.97; 2-5; 28%, 75), III (3, 53%; 3.44; 2-5; 22%; 34).

Hair 3: Fig. 8. Tables 8, 10.

Length- II (117, 94-140, 20), III (126, 104-148, 13). Diameter- II and III (-2.6, 18). Branching- II (3, 45%; 3.53; 2-7; 28%; 83), III (5, 39%; 5.89; 4-9; 24%; 28).

Hair 4: Fig. 8. Tables 8, 10.

Length- II (59, 42-78, 20), III (68, 52-83, 18). Diameter- II and III (-2.6, 18). Branching- slightly conical. II (3, 53%; 3.15; 2-5; 26%; 66), III (4, 42%; 3.75; 2-5; 21%; 33).

Hair 5: Fig. 8.

Length- II (32, 24-44, 21), III (47, 34-60, 19). Diameter- II and III (-2.6, 18). Branching- single and spine-like; II (98), III (69).

Hair 6: Fig. 8. Tables 8, 10.

Length- II (279, 234-328, 21), III (300, 231-361, 21).

Diameter- II and III (-2.6, 18). Branching- II (1, 97%; 1.01, 1-2; 11%; 76), III (1, 91%; 1.09; 1-2; 26%; 56).

*Hair 7: Fig. 8, 10. Tables 4, 8, 10.

Absent in second instar. Position- lateral; upper half of pleuron, most dorsal of the two sclerotized areas. Length- III (794, 676-920, 20). Diameter- III (5.9, 5.2-7.8, 18). Branching- III (4, 64%; 4.20; 2-7; 16%; 118). Spiculation- III, as in Fig. 22 or slightly less dense. Sclerotization- an area posterior to alveolus.

Hair 8: Fig. 8-10.

Position- lateral. II: anterior to and just dorsad of or even with dorsal edge of sclerotized area of pleural tubercle; III: same as II and ventral and slightly anterior to hair 7. Length- II (51, 39-83, 20), III (62, 55-73, 13). Diameter- II (-2.6, 18), III (-2.6-2.6, 18). Branching (estimate only)- conical. II: 48 hairs; range 2-8; mean 5.65. III: 34 hairs; range 6-16; mean 10.03.

Metathoracic Pleural Group

The tubercle is lateral in both instars but may be slightly more ventral in the third instar; in the second instar it is the only sclerotized area on the segment; in the third instar it is ventral and slightly posterior to the sclerotized area of #7. The external single macroscopic #9

and the internal multibranched macroscopic #10 form the anterior pair of hairs. The external microscopic #11 (posterior to #9) and the minute #12 (posterior to #10) form the posterior pair. A sclerotized finger-like process of the tubercle projects dorsally between #10 and #12. The white spot of #12 is similar to that of mesothoracic #12. The relative position of the alveoli and the serrations around the alveoli of #9 and #11 are similar to those of the mesothoracic group except that the serrations are not quite as well developed.

*Hair 9: Fig. 8. Tables 3, 4.

Length- extends from posterior edge of eye to membranous base of antenna when lying flat; II (961, 880-1034, 21), III (1186, 1100-1287, 22). Diameter- II (6.1, 5.2-6.5, 19), III (8.5, 7.8-9.1, 18). Branching- single; II (116), III (118). Spiculation- length and density less than in Fig. 22.

*Hair 10: Fig. 8. Tables 3, 4, 8, 10.

Length- II (933, 814-1067, 20), III (1052, 968-1155, 20). Diameter- II (6.0, 5.2-6.5, 18), III (7.8, 6.5-9.1, 15). Branching- II (2, 68%; 2.33; 2-4; 22%; 118), III (3, 81%; 3.18; 2-5; 15%; 119). Spiculation- length and density as in Fig. 22 or somewhat reduced.

Hair 11: Fig. 8. Tables 8, 10.

Length- II (256, 209-307, 20), III (275, 231-333, 18). Diameter- II and III (-2.6, 18). Branching- II (1, 94%;

1.10; 1-2; 19%; 87), III (1, 97%; 1.03; 1-2; 18%; 58). Basal portion well pigmented; apical portion long, lightly pigmented, very thin and difficult to see.

Hair 12: Tables 8, 10.

Length- II (25, 18-31, 6), III (36, 31-39, 4). Diameter- II and III (-2.6, 18). Branching- II (2, 81%; 2.19; 2-3; 18%; 21), III (2, 78%; 2.15; 1-4; 31%; 27).

Hair 13: Fig. 8.

Position- ventral; mesad of pleural group, slightly laterad of midpoint between mid-ventral line and pleuron; more lateral than pro- and mesothoracic hairs 14. Length- II (74, 52-96, 21), III (74, 55-88, 13). Diameter- II (-2.6, 18), III (-2.6-2.6, 12). Branching (estimate only)- conical. II: 56 hairs; range 2-7; mean 4.32. III: 21 hairs; range 4-12; mean 8.14.

Abdominal Hairs

Only the macroscopic abdominal hairs were studied. These included #6 on segments I-VI, #7 on segments I-II and #13 on segments III-V. Hairs 6 and 7 are lateral in position and #13 is ventrolateral. Hair 6 is closely associated with #7, being directly dorsal to #7 and usually sharing a common sclerotized area. Study of individual specimens showed that #7 is approximately three quarters to almost equal to the length of #6 on segment I and approximately two thirds to over three quarters the length of #6 on segment II. The diameter of #7 is less than or equal to #6 on both segments. In the following descriptions, the lengths and diameter between segments are compared by the mean statistic and by the comparison of hairs on individual specimens.

*Hair 6: Tables 3, 4.

Position- lateral; midlength of segments. Length- mean decreases from segment I through segment VI (Table 12). Studies of individual specimens- Segment I: when bent anteriorly, extends approximately to the prothoracic pleural tubercle. Segments II and III: length approximately three quarters to equal that of hair on preceding segment. Segment IV: approximately one half to slightly less than length of hair on segment III. Segment V: approximately three quarters

to equal that of hair on segment IV. Segment VI: equal to, longer or shorter than hair on segment V. Diameter (Table 12)- mean of each hair is equal to or less than that of hair on preceding segment. On segments I and II: diameters equal. Segment III: equal to or slightly less than that of hair on segments I and II. Segment IV: equal to or less than that of hair on segment III. Segments V and VI: equal; equal to or less than that of hair on segment IV. Most commonly, diameters equal on segments I-III, on segments IV-VI equal but less than on preceding segments. Branching- single, rarely double (sixty specimens of each instar examined; on segment I, one hair double on second instar, five on third instar; on segment II one hair double on second instar). Spiculation- spicules tend to become reduced in size and number on hairs of succeeding segments. Segments I-III: length often 2X or more width of shaft at base of spicule. Density variable but not as dense as Fig. 22. Segments IV-VI: Length and density similar to segments I-III to so reduced as to give the hair an aspiculate appearance, especially on segments V and VI. Sclerotization- an area posterior to the alveolus on segments I-III; on segment I united with sclerotized area posterior to #7; on segment II united or free; on segment III reduced to an area

smaller than to slightly larger than alveoulus.

*Hair 7: Tables 3, 4.

Position- lateral; ventral to #6. Length- mean length greater on segment I than on segment II (table 11). Study of individual specimens substantiated this. Diameter- mean greater on segment I than on segment II (Table 12); diameter on segments I and II may be equal or slightly less on segment II than on segment I. Branching- single, rarely double (sixty specimens of each instar examined; on segment I, two hairs double on second instar). Spiculation- similar on segments I and II; length and density similar to #6 on segments I-III. Sclerotization- an area posterior to alveolus on both segments (see description under #6.)

*Hair 13 (ventral abdominal hair): Tables 3, 4.

Position- ventrolateral; ventral and mesad of #6, approximately midway between #6 and midventral line. Length- mean length similar on segments III and IV but somewhat less on segment V (Table 11); lengths of the three hairs may be equal or subequal. Diameter- means very similar in second instar, becoming slightly reduced in successive segments in third instar(Table 12); diameters of all three hairs often equal. These hairs differ from #6 and #7 in that they do not show a marked reduction in length and diameter in succeeding

segments. Branching- single on sixty specimens of each instar. Spiculation- similar on all three segments; length and density similar to #6 on segments IV- VI.

Hairs of the Terminal Segments of the Abdomen

The hairs of the segments posterior to abdominal segment VII are discussed under three headings: the eighth abdominal segment, the siphon and the anal segment.

Eighth Abdominal Segment: Fig. 11.

There are seven pairs of hairs on the eighth abdominal segment, the five pentad hairs and the minute hairs 0 and 14. Only the pentad hairs were studied.

The pentad hairs form a lateral, dorsal to ventral crescent-shaped row which is close to and follows the contour of the posterior margin of the segment. These hairs are posterior to the comb scales. Hair 1 is dorsal to or slightly posterior to the tooth of the dorsal comb scale. Hair 2 is posterior to #1. Hair 3 is midway between the siphon tube and the anal segment. Hair 4 is ventral and anterior to #3, at the midpoint between #3 and #5. Hair 5 is ventral to the tooth of the most ventral comb scale.

Hairs 1, 3 and 5 are macroscopic and are the dominant hairs in the pentad group. Hair 3 is the longest with the greatest number of branches, but the branches are similar to those of #1 and #5 in diameter. Hairs 1 and 5 are similar in length, diameter and number of branches although #5 averages slightly higher in these three characteristics. Hairs 2 and 4 are microscopic, similar in diameter and less

conspicuous than hairs 1, 3 and 5. In length hairs 2 and 4 are similar although #4 has a higher mean. In the second instar these two hairs are similar in length to, or longer than, #1 and #5 but are considerably shorter in the third instar. Hair 2 shows no increase in length in the third instar, #4 only a small increase, while #1 and #5 show a definite increase.

Pentad hair 3 has the longest and densest spicules of the spiculate pentad hairs. Compared with other spiculate hairs there is little variation in this hair in the length and density of the spicules between individuals. Pentad hair 1 is somewhat more spiculate than #5; both show variation in spicule length and density in different individuals.

Ventral to and between #1 and #2 there is a lightly sclerotized area which may be much reduced in size in the second instar; it is not visible under the dissecting microscope.

*Hair 1: Fig. 11. Tables 3, 4, 9, 10.

Length- II (216, 161-263, 20), III (302, 241-372, 18).

Diameter- II (2.7, 2.6-3.9, 18), III (3.8, 2.6-5.2, 18).

Branching- II (1, 91%; 1.09; 1-2; 27%; 172), III (2, 65%; 2.22; 1-4; 26%; 172). Spiculation- II: length variable, on some hairs only notches visible, on others some spicules 2X or more width of shaft at base of spicule; density variable but less than in Fig. 23. III: similar to second instar except spicules some-

what longer. Sclerotization- a thin ring around alveolus.

Hair 2: Fig. 11. Tables 9, 10.

Length- II (223, 182-260, 20), III (212, 177-270, 18). Diameter- II (-2.6, 19), III (-2.6-2.6, 19). Branching- II (1, 62%; 1.38; 1-3; 36%; 159), III (2, 81%; 2.12; 1-4; 23%; 109). Sclerotization- a thin ring around alveolus. Basal portion well pigmented; apical portion long, lightly pigmented, very thin and difficult to see.

*Hair 3: Fig. 11. Tables 3, 4, 9, 10.

Length- II: extends to base of last pecten tooth to or slightly beyond base of siphon hair; III: extends to base of antepenultimate tooth to base of last tooth. II (355, 289-403, 20), III (402, 354-478, 18). Diameter- II (3.3, 2.6-3.9, 18), III (4.8, 3.9-5.2, 18). Branching- II (3, 70%; 2.94; 2-5; 22%; 174), III (5, 44%; 4.87; 3-8; 18%; 174). Spiculation- as in Fig. 23. Sclerotization- a ring around alveolus.

Hair 4: Fig. 11. Tables 9, 10.

Length- II (238, 208-283, 20), III (244, 200-296, 18). Diameter- II and III (-2.6-2.6, 18). Branching- II (1, 99%; 1.00; 1-2; 8%; 164), III (2, 51%; 1.51; 1-2; 33%; 105). Sclerotization- a very thin ring around alveolus. Basal portion well pigmented; apical portion long, lightly pigmented, very thin and difficult to see.

*Hair 5: Fig. 11. Tables 3, 4, 9, 10.

Length- II (237, 200-286, 19), III (321, 268-385, 18). Diameter- II (3.8, 2.6-5.2, 18), III (5.3, 5.2-6.5, 18). Branching- II (2, 83%; 2.01; 1-3; 21%; 169), III (3, 58%; 2.57; 1-4; 40%; 168). Spiculation- spicules greatly reduced in length, barely visible or with only notches visible; density variable but less than in Fig. 23. Sclerotization- a ring around alveolus.

Comb Scales: Fig. 11.

The second instar averaged 12.11 scales per specimen for 89 specimens and the third instar 12.26 for 88 specimens. The distribution of the comb scale formulas (see definition of hair formula on page 77 and substitute comb scales for hairs) is essentially the same in both instars (Table 20).

Siphon: Fig. 11.

Belkin (1950) recorded thirteen pairs of hairs for the siphon. Only #1 (siphonal tuft) and #2 (dorsal preapical spine) were studied. The remainder are associated with the siphonal valves.

*Hair 1: Fig. 11. Tables 3, 4, 9, 10.

Position- subventral; alveolus distal to base of distal pecten tooth by approximately length of tooth. Length- approximately one half to three quarters of distance between alveolus and distal end of siphon. II (299, 253-341, 18), III (366, 308-407, 18). Diameter- II (3.2, 2.6-3.9, 18), III (5.6, 3.9-6.5, 18). Branching-

II (2, 87%; 2.07; 1-3; 17%; 174), III (2, 51%; 2.40; 1-4; 23%; 172). Spiculation- length 2X or more width of shaft at base of spicule to so reduced as to give an aspiculate appearance; density variable but less than in Fig. 23.

Hair 2: Fig. 11.

Position- subdorsal; alveolus approximately one tenth or less of the dorsal length of siphon tube from distal end of siphon tube. Length- approximately one half to more than distance between alveolus and end of siphon tube. Approximately one third to one half length of distal pecten tooth. II (31, 26-34, 19), III (41, 34-49, 18). Diameter- II (-2.6-2.6, 18), III (2.6, 18). Branching- single and spine-like; II (62), III (60).

Pecten Teeth: Fig. 11.

The second instar averaged 20.59 teeth per specimen for 87 specimens and the third instar 24.24 for 87 specimens. In distribution of the pecten teeth formulas (see definition of hair formula on page 77 and substitute pecten teeth for hairs), there is extensive overlapping between the instars but a tendency for the third instar to have somewhat higher formulas (Table 20).

Anal Segment: Fig. 11

*Hair 1 (saddle hair): Fig. 11. Tables 3, 4.

Position- lateral; dorsal or slightly posterior to midpoint of cratal area, proximal to insertion of

dorsal anal gill; may be slightly more ventral in third instar. See discussion for position of alveolus in relation to saddle on page 80. Length- approximately three quarters to equal to dorsal length of saddle. II (237, 204-286, 19), III (347, 231-418, 18). Diameter- II (2.6, 18), III (4.0, 2.6-5.2, 18). Branching- single; II (122), III (119). Aspiculate.

Hairs 2 and 3

These two macroscopic but aspiculate hairs comprise the dorsal brush of the anal segment. They arise from a common sclerotized area located in the center of the dorsoapical angle of the anal segment. Hair 2 is just laterad of the midpoint of the dorsoapical angle; hair 3 just ventral and lateral to #2. These hairs are similar although #3 is slightly longer and thicker.

*Hair 2: Fig. 11. Tables 3, 4.

Length- longer than dorsal length of siphon tube. II (1035, 880-1188, 18), III (1409, 1265-1507, 7). Diameter- II (6.5, 5.2-7.8, 19), III (8.7, 7.8-11.7, 18). Branching- single; II (126), III (110).

*Hair 3: Fig. 11. Tables 3, 4.

Length- equal to or less than one fourth longer than #2. II (1082, 968-1210, 18), III (1496, 1397-1628, 7). Diameter- II (6.9, 5.2-7.8, 18). III (8.8, 7.8-10.4, 18). Branching- single, II (133, III (115).

*Hair 4 (ventral anal brush): Fig. 28. Tables 3, 4.

The ventral anal brush is composed of 15 to 21 hairs,

the most distal being 4a, the next proximal 4b, etc., extending medially around the ventroapical angle of the anal segment. The hairs arise alternately from each side of the midventral line and originate from two rather distinct areas.

I. Cratal area (barred area): Fig. 11.

A varying number of distal hairs of the ventral brush have a roughly oval, sclerotized area surrounding the alveolus. Continuous with and extending laterally from each end of the oval sclerotization is a transverse sclerotized bar which connects at its distal end with a sclerotized longitudinal side bar. A succession of transverse bars forms a grid pattern (Fig. 26-27). Because each oval is to one side of the midline, one transverse bar is longer than the other. This longer bar is often thinner than the shorter bar. Thus the grid of one side is made up of alternating thick and thin bars. The side bar becomes expanded at its distal end. The area occupied by the transverse bars and connecting side bars is termed the cratal or barred area. Hairs of this area are collectively called the cratal brush and are the largest hairs in the ventral anal brush.

II. Precratal area: Fig. 11.

Proximal to the cratal hairs are the shorter precratal hairs. The first one to three precratal hairs anterior to the cratal brush may have sclerotization around the alveolus and weak transverse bars which do not connect with the side bars. Maisey (1959) considered these hairs to be included

in the cratal area. Shaw and Maisey (1961) stated that from personal correspondence regarding the cratal area Belkin considered any hair in which the transverse bar does not connect with the side bar to be outside the barred area, and Stone, Breland and Bohart omitted reference to these hairs. The present writer prefers to place them outside the cratal area. Collectively the hairs proximal to the cratal brush are termed the precratal brush.

The length of the hairs of the ventral brush increases proceeding posteriorly, being greatest a few hairs before the end of the brush, the remaining hairs displaying a gradated decrease in length (Fig. 28). The first few proximal hairs are microscopic in both instars but show a definite increase in size in the third instar. Length (of longest hairs)- ranges from approximately the dorsal length of the siphon tube to over two thirds again as long, appearing to be proportionately longer in the second instar; II (1010, 924-1089, 18), III (1179, 1034-1386, 18). The diameter of the hairs increases for the first few proximal hairs, sometimes continuing to increase very slightly proceeding posteriorly. Diameter (of thickest hair)- II (5.9, 5.2-6.5, 18), III (8.6, 7.8-10.4, 19). Branching- cratal hairs: second instar single (110 specimens), third instar with at least some double or triple hairs (in only 1 of 108 specimens were all hairs single); precratal hairs: second instar usually single, rarely double (1 of 61 specimens had one hair double and 2

others appeared to have one hair double); third instar usually with double or triple as well as single hairs. Branching occurs well away from the base but at varying distances, even in the same individual. When double, the shaft forks with the branches being equal or subequal in length and diameter. When triple, the shaft forks and one of the branches forks again a short distance distal to the original fork (Fig. 29). The tips of the branches are equal or subequal in distance from the base of the hair and the branches are equal or subequal in diameter. These hairs are aspiculate.

DISCUSSION OF OBSERVATIONS

General Observations

Hair Position

With the possible exception of anal hair 1, no variation was observed either within or between instars.

Hair Length

The mean length of 26 out of 36 microscopic hairs increased in the third instar; for 8 the mean length of the second instar was very similar to or actually higher than that of the third instar; the remaining two hairs (meso- and metathoracic hairs 12) had sample sizes too small for comparison (Table 19). All macroscopic hairs increased in the mean length in the third instar. It is therefore evident that most but not all hairs increase in length in the third instar.

Hair Diameter

In diameter, the microscopic hairs may be divided into three categories: 1) hairs that increased in the third instar (12 hairs); 2) hairs that did not increase in the third instar (4); and 3) hairs in which the diameters were so small or the difference between the instars was so small that an increase in size would not be measurable with the equipment used in this study (21). The third category can be subdivided into those hairs which appeared the same

in both instars and those which gave the impression of being larger in the third instar (11) (Table 19). All macroscopic hairs increased in diameter in the third instar. Apparently most but not all hairs increase in diameter in the third instar.

Hair Branching

Forty out of 83 hairs were unbranched in both instars, 2 were unbranched in the second and unbranched or branched in the third, 1 was unbranched in the second instar and branched in the third, 24 were branched in both instars although unbranched hairs were recorded in either one or both instars, and in 14 hairs only branched hairs were observed in both instars (Table 19). Among hairs that branch, the trend was towards more branches in the third instar. The mean number of branches increased in the third instar in all hairs with the exception of two (meso- and metathoracic hair 12) in which the means were similar. The mode was the same in both instars or higher in the third instar. Even for hairs in which the third instar was bimodal the second instar mode was lower than the higher third instar mode (head hair 9 and mesothoracic hair 1). The class distribution was the same (see mesothoracic hair 6, Table 7); the number of classes was the same but with the third instar having higher class values (see mesothoracic hair 10, Table 7), or the third instar had a greater number

of classes and the upper class values were higher (see antennal hair 1, Table 5). In only two hairs (head hair 10 and mesothoracic hair 1) was the number of classes larger in the second instar and in these the upper class value was the same for both instars.

Clumping in the modal class, as indicated by the modal value, was similar for both instars for 7 hairs, higher in the second instar for 25 hairs, and higher in the third instar for 4 hairs. Thus clumping was either similar in both instars or greater in one or the other with a general tendency toward clumping in the second instar.

The coefficient of variability was used to determine which instar had the greater relative variability. There was no trend in this characteristic, with 12 and 13 hairs being more variable in the second and third instars respectively and 11 being similar in both instars.

Hair Spiculation: Fig. 20-23.

Spicules are apparently restricted to the macroscopic hairs; they were not visible on microscopic hairs under 430X. All macroscopic hairs except those of the anal segment were spiculate. This aspiculate condition is surprising because in all other ways these anal hairs are similar to the other macroscopic hairs.

The spicules appeared to be minute branches of the shaft. In antennal hair 1 and head hair 7, which had secondary branching, there was a gradation in size between the small secondary branches and the larger spicules. The spicules

arise from what appeared to be a notch in the shaft; when the spicules were exceedingly fine or short only the notch was visible.

There was variation in the length of the spicules on an individual hair with the ratio of longer to shorter spicules usually varying along different parts of the shaft. The density usually varied also along different parts of the shaft. The basal portion of the shaft starting at the alveolus and extending distally was always aspiculate for a variable distance. In addition to variation on an individual hair, the spiculation pattern showed variation on the same hair between the right and left side of one individual and between different individuals. In spite of this variation, a particular hair usually had a typical spiculate condition. There was of course variation between different hairs. A gradation existed in the typical spiculation from the extremely reduced number and length of spicules characteristic of hair 6 of abdominal segments V and VI to the long and numerous spicules of mesothoracic hairs 8 and 10. The shortest spicules were so small as to be almost invisible under 430X and the longest 13 times or more the width of the shaft at the base of the spicule. Only in head hairs 5, 6 and 7, prothoracic hairs 1 and 8, and pentad hair 1 was the spiculation more prominent in the third instar.

Basal Sclerotization

The alveolus of certain hairs touched or was surrounded by a sclerotized area of the otherwise unsclerotized

integument. Most but not all of the macroscopic hairs had this condition. The exceptions included the submedian prothoracic group; hair 6 of abdominal segments IV through VI and the saddle hair when not on or bordering the saddle. In addition, the ventral abdominal hairs had very weak or no sclerotized areas. No microscopic hairs, except those of the pleural tubercles and pentad hairs 2 and 4, possessed sclerotization around the alveoli. Little or no difference was noted in the sclerotization between the instars except in prothoracic hair 6 which appeared to have less in the third instar.

Thoracic Chaetotaxy

Comparison of the Thoracic Segments

The total numbers of hairs for the pro-, meso-, and metathoracic segments are 14, 14 and 13 respectively. There was a decrease in the number of macroscopic hairs with a corresponding increase in microscopic hairs on successive segments. The hairs having dense, extremely long spicules were the transitory hairs on the meso- and metathorax, hair 10 of the meso- and metathoracic pleural groups and hair 9 of the metathoracic pleural group.

In their chaetotaxies, the three thoracic segments had certain features in common. Pleural groups were present in similar positions, dorsolateral and lateral macroscopic hairs were present, and the venters each had a single microscopic hair in the same position. However, the prothorax

differed in other ways from the meso- and metathorax which were similar to each other. The prothorax had dorsal macroscopic hairs and lacked a lateral conical hair and a transitory hair. The hairs of the pleural group differed from those of the meso- and metathorax (see comparison of pleural hairs on page 74). On the other hand, each of the meso- and metathoracic segments lacked dorsal macroscopic hairs and possessed a lateral conical hair in the same relative position, a transitory hair in the same relative position, and a ventral conical hair in a similar although not identical position; the hairs of the pleural groups were similar on these two segments.

Serial Homologies

Ontogenetic homologies of the thoracic hairs (i.e. between instars) may now be unquestionably determined in many cases (Belkin 1960, 1962). This is not true of the serial homologies for the three thoracic segments, which are based on such criteria as hair position and branching characteristics. These criteria leave much to be desired when it is understood that hairs on the different segments may come under different selective pressures and could therefore have evolved differently. One would therefore not expect serially homologous hairs to be always similar. Also, hairs not homologous serially but under similar selective pressures could evolve in a like manner. In spite of these inherent difficulties, similarities between hairs must be used as evidence if serial homology is to be ascertained.

In comparing the characteristics of the hairs of the three thoracic segments, it appears questionable whether some of the hairs are correctly homologized serially in Belkin's system of numbering. Belkin has recognized this possibility repeatedly but has suggested that corrections be made only after the early instars of all the mosquito genera have been studied (Belkin and McDonald 1956). The observations on this species should be a step in this direction.

One of the problems with using one species to determine serial homologies is that if there are weak or no apparent similarities the investigator is forced to make conclusions based on insufficient evidence. The present discussion will therefore be limited to the certain obviously similar hairs on the last two thoracic segments.

Hairs 8 and 7 on the meso- and metathoracic segments respectively were transitory in nature, occupied the same position relative to the pleural tubercle, were macroscopic in size, and were similar in branching statistics and spiculation. Hairs 13 and 8 on the meso- and metathoracic segments respectively occupied the same position relative to the pleural tubercle and were conical in shape. Hairs 14 and 13 on the meso- and metathoracic segments respectively were similar in position but not to the extent of the aforementioned hairs and were conical in shape. The similarity between the two hairs of each of these three pairs and the

obvious similarity of the four pairs of hairs of the meso- and metathoracic pleural groups (see next section) suggest that the hairs of these seven pairs are homologous.

Thoracic Pleural Groups

The thoracic pleural groups are associated with the imaginal buds of the adult legs (Root 1932; Christophers 1960). These pleural groups occur throughout the family; they are similar in position throughout the family and on all three thoracic segments. Because of this consistency in occurrence and position they serve as useful land marks for describing the position of certain hairs on the segment.

The three pleural groups, although similar in position, showed a gradual straight line gradation from the ventro-lateral prothoracic group to the lateral metathoracic group (Fig. 9, 10). There was no change in position in the third instar other than that the metathoracic group may have been slightly more ventral. The prothoracic tubercle was the smallest; those of the meso- and metathorax were similar to each other in size with that of the mesothorax being slightly larger. Serrations were associated with the alveolus of #11 on all three segments and were also associated with #9 on the meso- and metathorax. Hairs 10 and 12 lacked serrations. A finger-like process projected dorsally between hairs 10 and 12 on all three segments. Serrations and the finger-like process were more prominent in the third instar.

The chaetotaxal composition of the pleural groups was different for each segment. The prothoracic group consisted of

a single macroscopic hair (#11), two long microscopic hairs (#9 and #10) and a much shorter microscopic hair (#12). On the mesothorax there were three macroscopic hairs, of which one was multibranched (#10) and two were single (#9 and #10), plus a minute hair (#12). On the metathorax there were two macroscopic hairs, one multibranched (#10) and the other single (#9), plus a long microscopic hair (#11) and a minute hair (#12). Comparing these hairs serially, hair 9 was a long, single microscopic hair on the prothorax and a single macroscopic hair on the meso- and metathorax. Hair 10 was a long, usually single or double, microscopic hair on the prothoracic segment and a multibranched macroscopic hair on the meso- and metathorax. Hair 11 was a single macroscopic hair on the pro- and mesothorax and a long predominantly single microscopic hair on the metathorax. Hair 12 was the most constant, being short on the prothorax and minute on the meso- and metathorax. As has been pointed out on page 72, the prothorax differed in its chaetotaxy from the meso- and metathorax which were similar to each other. The prothorax had one hair (#11) similar to its homologue on the mesothorax but none that resembled the corresponding metathoracic hairs. On the other hand, the meso- and metathorax had three hairs (#9, #10 and #12) similar on the two segments.

Association with the imaginal buds of the adult legs, similarity in position, shape and configurations of the basal tubercle, and possession of four similarly placed hairs

strongly suggest that the three pleural groups are serially homologous. Serial homology of the hairs seems to be established, in spite of the variation in size and branching between the segments, because the hairs maintain a constant relative position to each other on all three segments (see description of prothoracic pleural group).

Possible Differentiating Characters

Certain characteristics studied, because of their demonstrated ability to differentiate between the second and third instars in A. abserratus or because they may be of value in other species, deserve special comment.

Transitory Hairs: Fig. 9, 10.

Transitory hairs appearing in the third instar were observed on the meso- and metathorax and according to Belkin (1950) are #8 and #7 respectively. These two hairs were never present in the second instar and always present in the third instar (126 and 101 specimens examined respectively). Because of their large size and the complete lack of variation in their presence or absence they serve as the best method of distinguishing between the two instars.

Hair Branching

The desired condition in hair branching for positive identification is one of no overlap in the distribution of the number of branches per hair between the instars. This condition was very nearly achieved by the hairs of the cratal

area. In this structure all the hairs were single in the 110 second instar specimens examined, and in all but 1 out of 108 third instar specimens at least some of the hairs were double or triple.

In spite of some overlap, hair branching may be useful in differentiating between instars if the range in the number of branches per hair for a given hair on the majority of third instar specimens is outside the range of the same hair on the majority of second instar specimens. Using antennal hair 1 as an illustration, 93% of the second instar hairs were unbranched while 89% of the third instar hairs had 2-4 branches (Table 5). As a consequence of the difference in hair branching between the instars, the majority of third instar formulas[1] fell outside the range of the second instar (Table 5). By using the distribution of the hair formulas for antennal hair 1 to differentiate between instars, one can say that a formula of 2-3 to 3-4 denotes a third instar; a formula then of 1-1 to 2-2 could be either instar. It is possible then to positively identify only a portion of the third instar (11% of the total number of individuals observed). It could also be said that a 1-1 formula is usual for the second instar and all other formulas are unusual; in the third instar a 1-1

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[1]The hair formula refers to the number of branches of a particular hair on each side of an individual. For example, if antennal hair 1 is single on one side and double on the other the formula would be 1-2. No distinction is made between the right and left side.

formula could be called unusual and higher formulas more usual. Therefore, in using the formula for one hair to identify a specimen, there is a good chance of the specimen having an unusual formula and thus being misidentified. However, if two or three hairs with the above-described qualities are used, the chances are much less that all the hairs will have unusual formulas which would result in misidentification. In other words, a combination of hairs, each with some degree of differentiating ability, is better than any one of the hairs used alone.

In addition to antennal hair 1, prothoracic hair 7 and pentad hair 1 showed a very limited degree of overlap in their formula distributions (Table 13) as did mesothoracic hair 10 and pentad hair 3. By combining these former three hairs, it was possible to achieve a high level of differentiation between the instars.

The formulas for antennal hair 1, prothoracic hair 7 and pentad hair 1 were recorded for 93 known second instar specimens and 88 known third instar specimens. Two by two tables were employed to pair antennal hair 1 with prothoracic hair 7 (Table 14), antennal hair 1 with pentad hair 1 (Table 15), and prothoracic hair 7 with pentad hair 1 (Table 16). Each square in a table was identified by a number. Each specimen was recorded in one square of each table according to the formulas of the three hairs (a specimen with formulas 1-1, 1-1 and 1-2 for antennal hair 1, prothoracic hair 7 and pentad

hair 1 respectively was recorded in square 1 in table 14, in square 4 in tables 15 and 16). The number of second instar specimens which fell in a particular square is in the upper left hand corner, the number of third instar specimens is in the lower right hand corner. Each square was then assigned to a particular instar, assignment depending on whether the square had more second instar or more third instar specimens in it (Table 14 has squares 1, 2 and 4 assigned to the second instar and 3 and 5-9 to the third instar). The 2X2 table is thus divided into second and third instar portions, the two being separated by a red line.

Next the 181 known instar specimens were reclassified as second or third instar, or as unidentifiable, according to two methods:

1) If a specimen fell into the same instar area in all three tables it was classified as that instar. If it fell in two out of three it was classified as unidentifiable. By this method, 1 specimen was misidentified, 12 were unidentifiable and 168 or 93% were correctly identified.

2) If a specimen fell in two out of three instar areas it was classified in that instar. By this method, 4 specimens were misidentified and 177 or 98% were correctly identified.

Comparing the first method with the second, the first minimized the misidentifications but also reduced the number of specimens that could be reliably identified. For taxonomic purposes in which quality would be at a premium, the first

method would be the more desirable.

If one is willing to assume that the sample formula distribution of each hair represents the true distribution for the study area, a high degree of differentiation is possible with relatively little misidentification. It is not possible to say positively how large a sample is needed to reflect the true distribution but 93 second instar specimens and 88 third instar specimens can be considered large samples although more specimens would be desirable. The author feels that the samples were large enough to show that the major difference in the formula distribution between the instars was clumping of the formulas in squares 1 and 9 in the second and third instars respectively. Larger samples would give a clearer picture of the strength of the remaining squares.

Position of the Alveolus of the Saddle Hair in Relation to the Saddle

The position of the alveolus of the saddle hair was variable but can be placed into rather definite categories in both instars: Second instar: 1) on membrane ventral to saddle (Fig. 30), 2) bordered by both membrane and saddle (Fig. 31), 3) on saddle dorsal to ventral edge by less than diameter of alveolus (Fig. 32), 4) on saddle dorsal to ventral edge by diameter of alveolus or more (Fig. 33); third instar: categories 2 through 4 above. The position of the alveolus in relation to the saddle was typically different for the two instars. The second instar usually (287 out of

303 observations) fell in categories 1, 2 or 3. This gave the alveolus the appearance of being on or near the ventral edge of the saddle and posterior to the midpoint of the ventral edge of the saddle (Fig. 34). The third instar usually (100 out of 109 observations) fell in category 4 giving the alveolus the appearance of being in the posterior ventral corner of the saddle subequal in distance from the ventral and posterior edges of the saddle (Fig. 35). Because there was a characteristically different position for the alveolus for each of the two instars, the position of the alveolus is of some value in differentiating between the instars. However, because of overlap, this character is considered to be of limited value.

Numbers of Cratal Hairs, Precratal Hairs and Hairs in the Ventral Anal Brush

There was a progressive weakening of the sclerotization of the transverse bars and of the two side bars extending anteriorly (see descriptions of cratal and precratal areas on page 64). There were often one to three hairs that, because of this condition, were difficult to place in either the cratal or precratal brushes. Sometimes only one of the two transverse bars connected with a side bar in these in-between cases. In counting the transverse bars (and thus the number of hairs in the cratal brush), only one side (that most clearly seen) was used, and only those transverse bars that clearly connected with the side bar were recorded as

part of the cratal area.

The numbers of cratal and precratal hairs and the total number of hairs in the ventral brush were studied by two methods: 1) the means and ranges of a number of specimens of second and third instars were compared, 2) the cast skin of the second instar was compared with the third instar larva of the same individual. In the first method an individual was used only once, in either the second or third instar but not in both.

Cratal Hairs

First method: mean 3.9 hairs higher for third instar (Table 17). Range distribution of second and third instars with overlap at higher and lower ends respectively (Table 18).

Second method: mean difference 2.8 hairs, range of difference 1-5 hairs. Of 37 third instar specimens examined, all had at least one more hair than the second instar.

Precratal Hairs

First method: mean 3.3 hairs higher for second instar (Table 17). Range distribution of second and third instars with overlap at lower and higher ends respectively (Table 18).

Second method: mean difference 2.4 hairs, range of difference 1-5 hairs. Thirty out of 33 specimens of the second instar had at least one more hair than the third, the remainder an equal number in both instars.

Total Number of Hairs in the Ventral Brush

First method: means similar, third instar slightly

higher (Table 17). Mode same, modal values similar. Range distributions similar (Table 18).

Second method: mean difference 0.6 hairs, range of difference 1-2 hairs. Sixteen individuals had same number in both instars, ten had one more hair in third instar, only two had two more hairs in third instar, four had one more hair in second instar.

Analysis of the results obtained in the two methods used indicates that the number of cratal hairs increases at the expense of the precratal hairs, not by the addition of new hairs.

In the first method the ratio of precratal hairs to cratal hairs in the second instar (.72) decreased in the third instar (.29) while the means and range distributions of the total number of hairs were very similar in both instars.

In the second method the total number of hairs was either the same in both instars or there was a difference of one or two hairs, either the second or third instar having the extra hair(s). There was always an increase in the cratal hairs in the third instar while at the same time there was usually a decrease in the precratal hairs. Where there was a higher total number of hairs in the second instar, there was still an increase in the number of cratal hairs in the third instar. In nine out of twelve specimens where there was an increase in the total number of hairs in the third instar, there was a greater increase in the number of cratal hairs than in the total number of hairs. In the remaining

three specimens there was an equal increase in the total number of hairs and in the number of cratal hairs.

The total number of hairs in the ventral brush is clearly useless as a differentiating mechanism because of the similarity in number between the two instars. However, since the numbers of cratal and precratal hairs are quite different in the two instars, two possibilities are suggested for distinguishing between the instars, neither of which is entirely satisfactory: 1) the use of the difference in the range of the numbers of cratal or precratal hairs between the instars. Inspection of the range distribution (Table 13) shows that, for both hairs, a majority of the specimens of each instar were outside the range of the other instar. 2) the use of the difference in the ratio distribution of precratal to cratal hairs between instars. The mean and range of the ratio of the precratal to cratal hairs for the second instar is 0.72 and 0.50 to 1.11 respectively and 0.29 and 0.12 to 0.64 for the third instar. There is a large difference between the means but 21 out of 59 of the second instar specimens fell in the zone of overlap as did 3 out of 51 in the third instar. This method correctly segregated 88% of the total number of specimens examined.

The potential of these two possibilities in distinguishing between the instars is limited because of overlap and the often occurring difficulty of assigning certain hairs to either the cratal or precratal brush.

Comb Scales and Pecten Teeth

The average number of comb scales per specimen is practically identical for both instars. There is an increase in the average number of pecten teeth in the third instar. Because of the high degree of overlap between the instars in the formula distributions of the comb scales and pecten teeth, neither of these has any value in differentiation of the instars.

CONCLUSIONS

This study describes and compares the chaetotaxy of the head, thorax, abdomen (in part), and the terminal segments of the second and third instars of Aedes abserratus. Certain characters are shown to be of limited or no value in distinguishing between the second and third instars, while others serve as excellent criteria.

Hair position is constant between instars. Hair length and diameter may increase or remain approximately the same.

Hairs may be unbranched in both instars, unbranched in the second and branched in the third, or branched in both instars. The trend is towards more branching in the third instar in the latter condition. The degree of clumping is usually, but not always, higher in the second instar and may be similar in both instars or higher in the third. The relative variability may be higher in either instar or similar in both.

Spicules visible under 430 magnification are restricted to the larger hairs although the hairs of the anal segment which include the largest hairs areaspiculate. Spicules are subject to variation in length and density along a single shaft, between branches of a multibranched hair, between the same hair on each side of an individual, between the same hair on different individuals and between different

hairs. Nevertheless, most hairs have a typical spiculate condition. With the exception of a few hairs, the spiculation is similar between the instars.

Basal sclerotization is characteristic of the larger hairs but is not always associated with them. The smaller hairs are without basal sclerotization except on the pleural tubercles and two of the hairs of the eighth abdominal segment. There is little or no change in the amount of sclerotization between the instars.

The chaetotaxy of the prothorax is somewhat different from that occurring on the meso- and metathorax which are similar in chaetotaxy. The pleural groups on all three segments are undoubtedly homologous. Three mesothoracic hairs have very close counterparts on the metathorax, suggesting serial homologies which are not recognized in Belkin's 1951 numbering system.

The differences in the numbers of cratal and precratal hairs, the total number of hairs in the ventral anal brush, and the number of comb scales and pecten teeth are of no value for instar differentiation. The position of the alveolus of the saddle hair in relation to the saddle is of only limited value.

Three characters of the chaetotaxy were shown to be of use in separating the second from the third instar in the following order of reliability: 1) the presence or absence of transitory hairs on the meso- and metathorax, 2) an almost complete difference in the number of branches per

hair in the ventral anal brush, and 3) a minimum of overlap in the branching of certain hairs which permitted the use of a combination of two or more of these hairs. The presence or absence of the transitory hairs is an absolute method for distinguishing between the two instars. Transitory thoracic hairs, because of their appearance in all three subfamilies, may be found in most or all species of mosquitoes and be a family-wide method for distinguishing between the second and third instars. The second characteristic is satisfactory but secondary to the transitory hairs in reliability for two reasons: a) one exception was observed, and b) the presence or absence of a hair is less variable than the amount of branching. The third characteristic, because of potential variation and the amount of observed overlap, is the least reliable of the three characteristics.

SUMMARY

Aedes abserratus (Felt and Young) has not been described in the first and second instars. The third instar has been described only briefly. The vast majority of mosquito species are likewise essentially undescribed in the second and third instars. The absence of descriptions has resulted in a lack of criteria for distinguishing between the second and third instars both within and between species. In addition, second and third instar descriptions could contribute considerably to phylogenetic studies in the family Culicidae.

This study describes and compares the chaetotaxy of the head, thorax, abdomen (in part), and the terminal segments of the second and third instars. The characteristics of the chaetotaxy are examined for possible value in differentiating between these two instars.

Larvae in the first three instars were collected from three breeding areas within a thirteen-mile radius of Amherst and individually reared so that only specimens of known instar were studied.

The following characters are described for each hair: position, length, diameter, number of branches per hair, spiculation, basal sclerotization, and characters typical for one hair but not found on most others. Certain statistics are a part of the descriptions of the length, diameter

and number of branches. The three pleural groups, position of the alveolus of the saddle hair in relation to the saddle, the ventral anal brush, and the relative numbers of comb scales and pecten teeth are described for both instars. The three thoracic segments are compared and observations made on possible serial homologies.

It is found that hair position and basal sclerotization change little or not at all between instars. A few hairs appear to have slightly longer and denser spicules in the third instar. Length and diameter increase for some hairs but remain approximately the same for others in the third instar. Hairs may be unbranched in both instars, unbranched in the second and branched in the third, or branched in both instars. The mean number of branches increased in all but two homologous hairs on the meso- and metathorax.

Obvious similarities were observed for the following pairs of hairs on the meso- and metathorax respectively (following Belkin's 1950 system of numbering hairs): hairs 8 and 7, 13 and 8, and 14 and 13. The hairs of the pleural tubercles are considered homologous on the three segments.

The invariable absence or presence of transitory hairs (meso- and metathoracic hairs 8 and 7 respectively) in the second and third instars respectively is the most reliable method of identifying these instars. Hairs of the ventral brush provide a second excellent method, being single in the second instar and with at least some being branched in the

third instar in all but 1 of 218 specimens. A method of using two or more hairs having only a small amount of overlap in the range distribution of the number of branches per hair between the second and third instars is described. This method is not as reliable or efficient as the previous two but gives satisfactory results. Five hairs are sufficiently different between the instars to be used in this method. No other characters studied could be used to differentiate between the instars.

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Fig. 1

Fig. 2

Fig. 1.-Collecting equipment. Fig. 2.-Rearing equipment.

Fig. 3-7.-Head, antennae and microscopic hair branching. Fig. 3.-Left antenna, dorsal view. Fig. 4.-Right antenna, ventral view. Fig. 5.-Head capsule; left half dorsal view, right half ventral view. Fig. 6.-Typical branching of a microscopic hair. Fig. 7.-Conical microscopic hair.

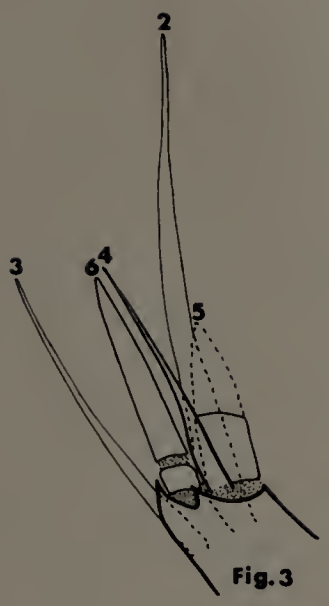

Fig. 3

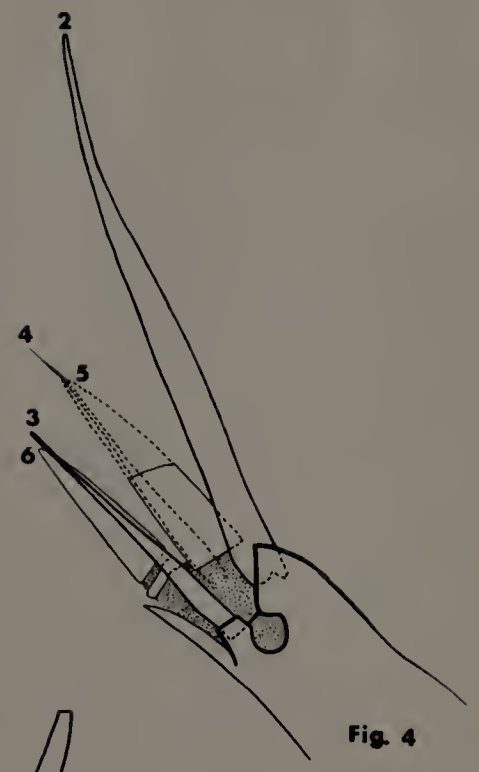

Fig. 4

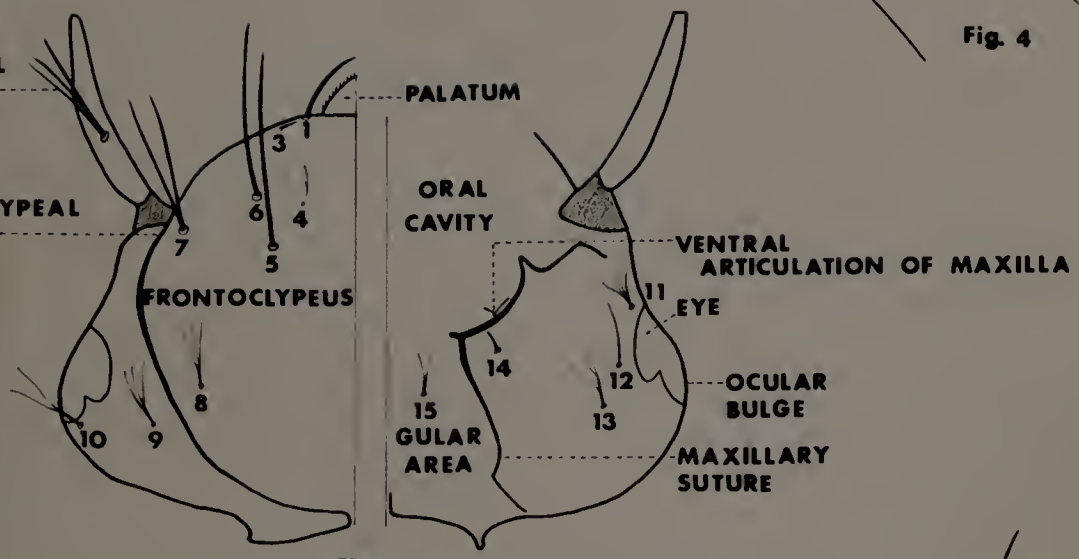

Fig. 5

Fig. 6

Fig. 7

Fig. 8-11.-Thorax and terminalia. Fig. 8.-Thorax; left half dorsal view, right half ventral view. Fig. 9-10.-Thorax, lateral view; 9, second instar; 10, third instar. Note absence of transitory hairs in second instar. Fig. 11.-Terminalia. P, prothorax; MS, mesothorax; MT, metathorax.

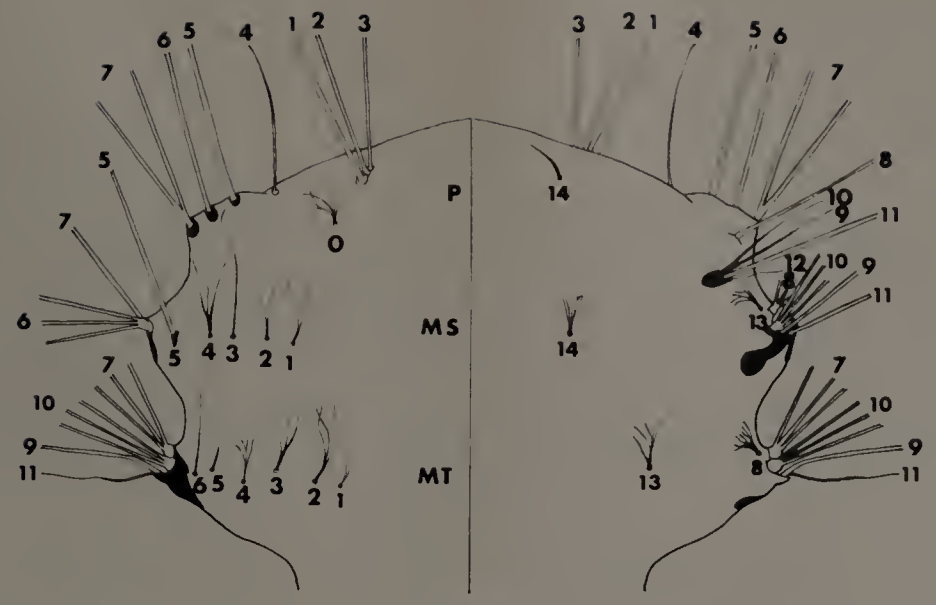

Fig. 8

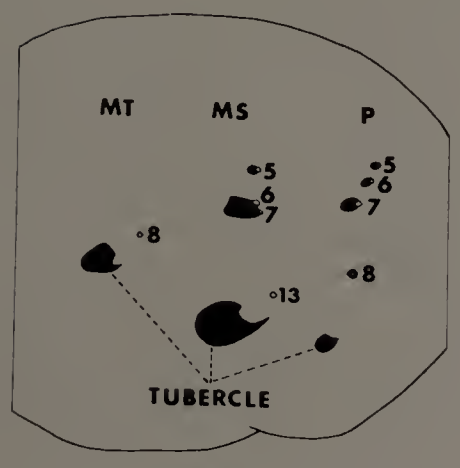

Fig. 9

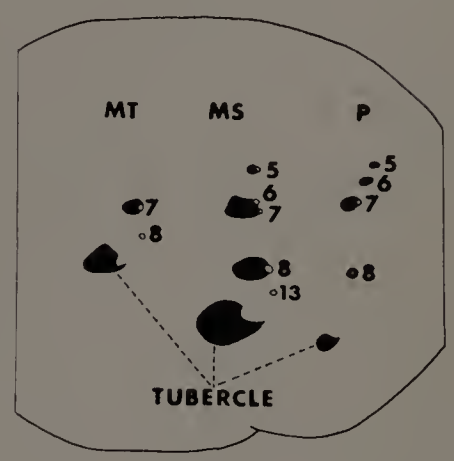

Fig. 10

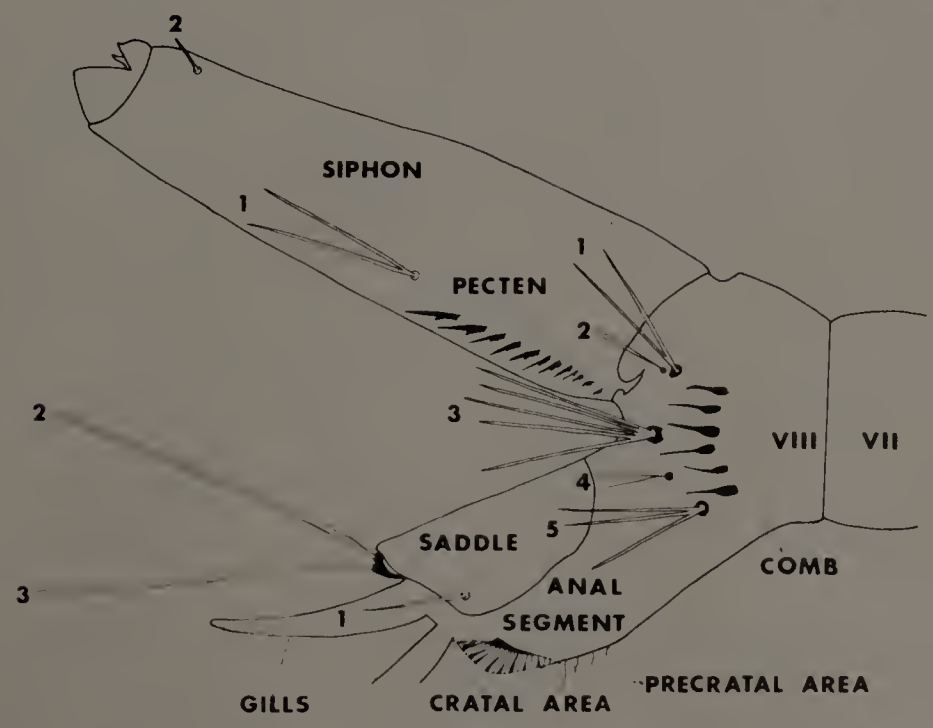

Fig. 11

Fig. 12

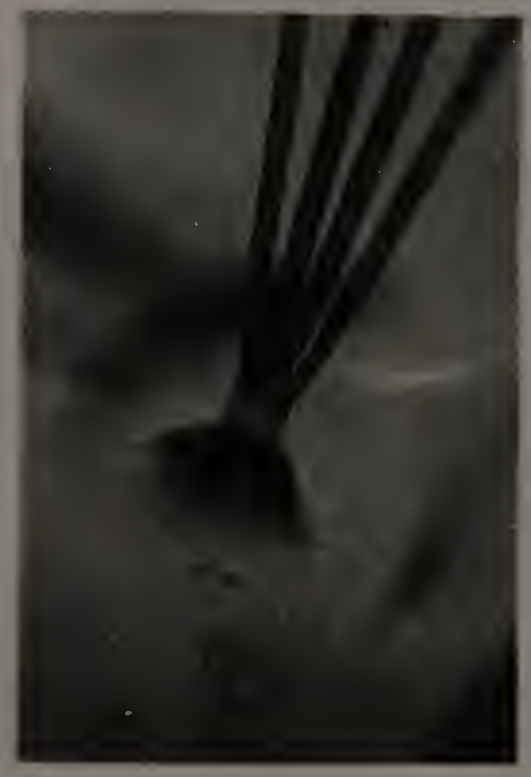

Fig. 13

Fig. 14

Fig. 15

Fig. 12-13.-Branching of macroscopic hairs. Fig. 14-15.-Secondary branching of macroscopic hairs.

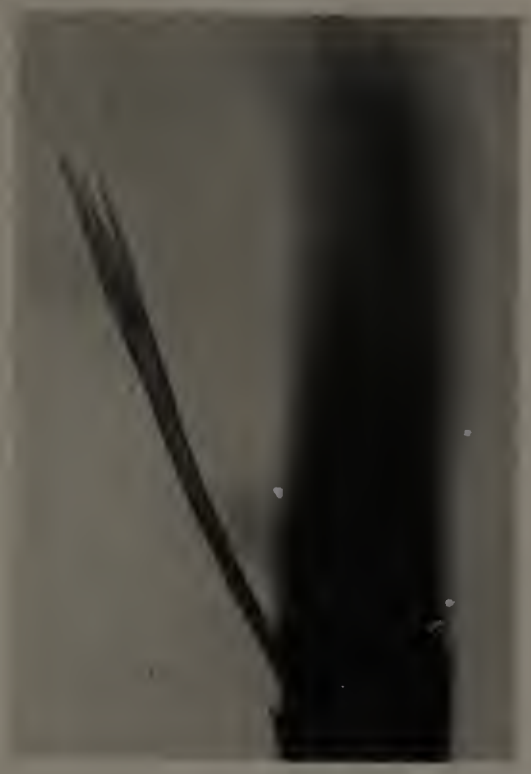

Fig. 16

Fig. 17

Fig. 18

Fig. 19

Fig. 16.-Secondary branching of antennal hair 1.
Fig. 17-18.-Typical branching of microscopic hairs.
Fig. 19.- A microscopic conical hair.

Fig. 20

Fig. 21

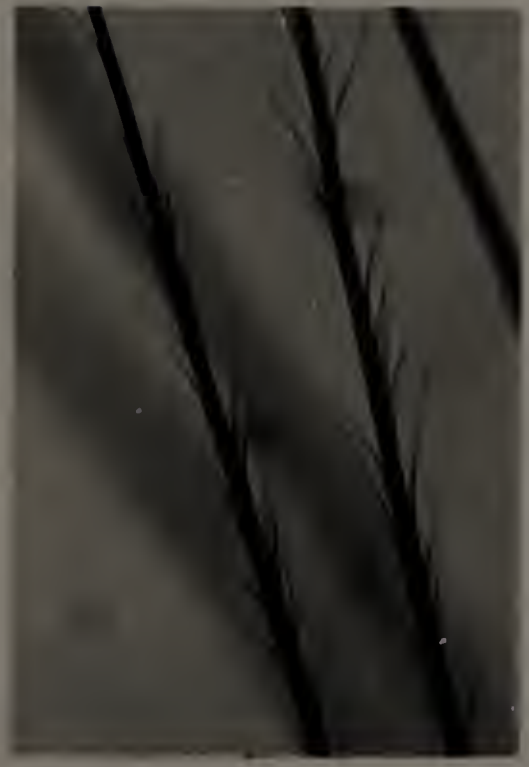

Fig. 22

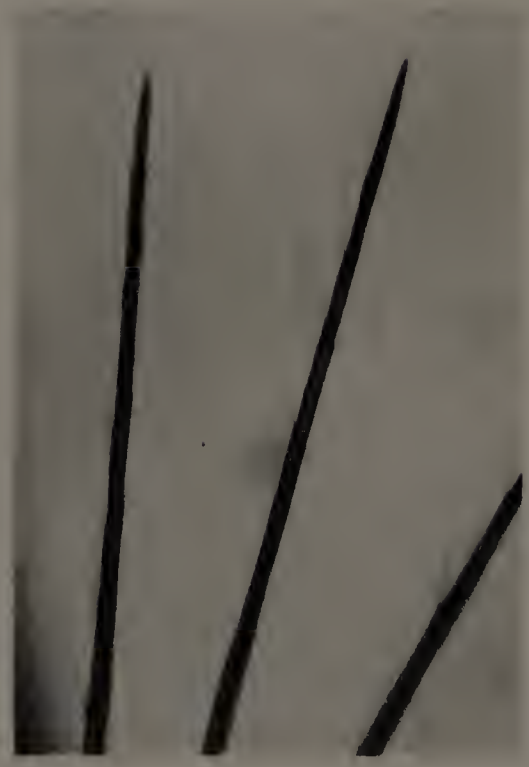

Fig. 23

Fig. 20-23.-Typical spiculation of macroscopic hairs.

Fig. 24

Fig. 25

Fig. 26

Fig. 27

Fig. 24.-An anomaly of head hair 14. Fig. 25.-Head hair 14, two on one side. Fig. 26.-Lateral view of cratal area. Fig. 27.-A ventrolateral view of cratal area.

Fig. 28

Fig. 29

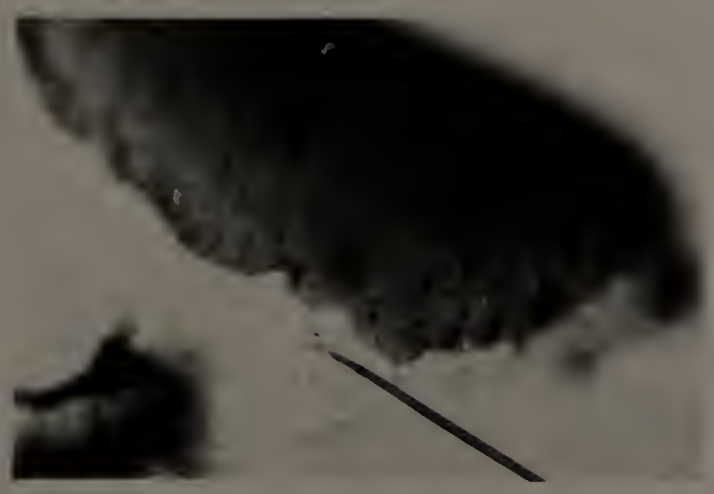

Fig. 30

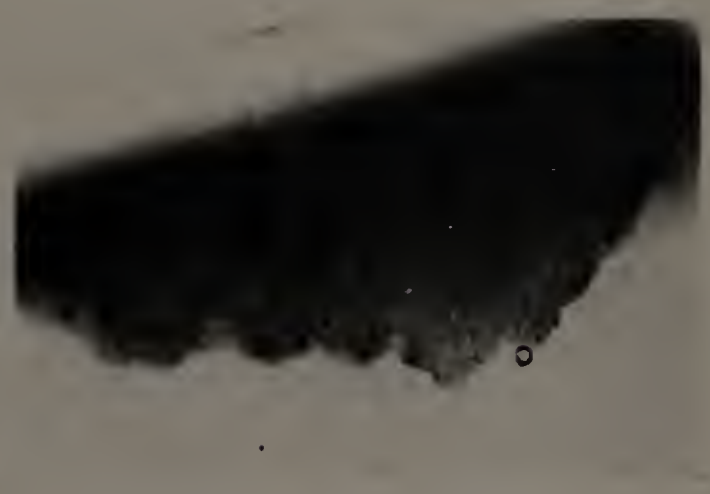

Fig. 31

Fig. 28.-Ventral anal brush; anterior end to the left. Fig. 29.-Hair branching of the ventral anal brush in the third instar. Fig. 30-31.-Position of saddle hair alveolus in relation to ventral edge of saddle; 30, alveolus on membrane ventral to saddle; 31, alveolus bordered by both membrane and saddle.

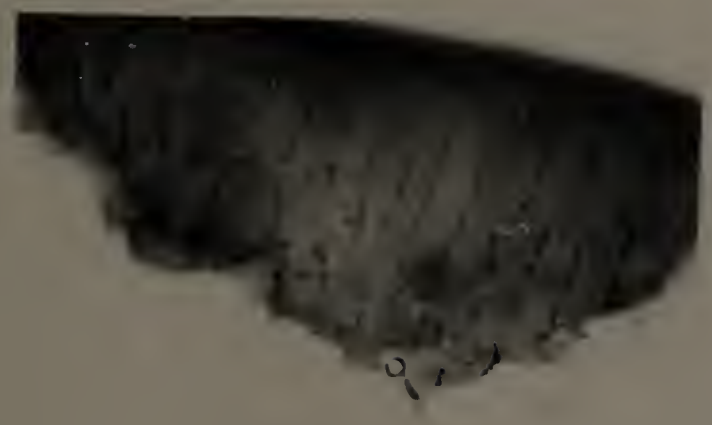

Fig. 32

Fig. 33

Fig. 34

Fig. 35

Fig. 32-35.-Position of saddle hair alveolus in relation to ventral edge of saddle; 32, alveolus dorsal to ventral edge by less than width of alveolus; 33, alveolus dorsal to ventral edge by width of alveolus or more; 34, typical position of alveolus in second instar; 35, typical position of alveolus in third instar.

Table #1- Hairs of Various Mosquito Species That show No Overlap in Range of Hair Branching between Second and Third Instar.

Author	Species	Hair
Abdel-Malek (1949)	Aedes trivittatus	Antennal tuft, ante-antennal hair, pentad hairs A and C, upper lateral abdominal hairs, upper caudal hairs, precratal tuft.
Bohart and Washino(1957)	California Culex (10 species)	Upper and lower head hairs, pentad formula, siphonal tuft, inner caudal seta. (1)
Breland(1949)	Toxorhynchites septentrionalis	Lower tuft of the dorsal brush.
Breland(1951)	Aedes infirmatus	Upper caudal hair of dorsal brush.
Hearle(1929)	Aedes flavescens	Antennal tuft, air tube tuft, anal hair 2, hairs from barred area, hairs basal of barred area.
Hurlbut(1938)	Anopheles walkeri	Head hair 3; prothoracic hairs 7&8; metathoracic hairs 5&8; abdominal hair 6 on seg. I-II; ab. hair 5 on seg. V; outer dorsal, inner dorsal, ventral brush of anal segment.
MacFie (1916-1917)	Aedes aegypti	dorsal pair of the dorsal hairs of the anal segment.
Marshall (1938)	English species of the genera Aedes, Culiseta, Culex, and Orthopodomyia	Outer, middle, inner frontal hairs; siphonal tuft. (1)
Montchadsky (1926-27)	Aedes pulchritarsus	Antennal tuft, head hair 7, siphonal tuft.

(1) These hairs are used in the descriptions of the instars and may or may not show overlap depending on the species.

Table #2- Collection Localities

Belchertown				
*Belchertown Bog:	Latitude	42°	15'	59"
	Longitude	72°	22'	55"
*Federal Street Bog:	Latitude	42°	18'	33"
	Longitude	72°	25'	33"
Amherst				
**Cushman Bog:	Latitude	42°	24'	38"
	Longitude	72°	30'	25"

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*Belchertown, Massachusetts quadrangle
U. S. Geological Survey Map, 1949
Scale: 1: 24, 000.

**Mt. Toby, Massachusetts quadrangle
U. S. Geological Survey Map, 1955
Scale: 1: 24, 000.

Table #3- Standard Deviations of the Length for the Macroscopic Hairs of the Second Instar.

Head		Thorax		Abdomen		Terminal Segments	
Hair	s.d.	Hair	s.d.	Hair	s.d.	Hair	s.d.
1	10.7	P1	48.6	6I	51.8	PH1	26.2
5	17.3	P2	48.9	II	38.4	PH3	30.1
6	17.1	P3	36.7	III	35.7	PH5	21.0
7	25.1	P5	23.2	IV	47.1	SH1	27.1
A1	12.0	P6	33.1	V	38.6	AH1	23.1
		P7	36.1	VI	45.7	AH2	86.2
		P8	53.3	7I	44.6	AH3	69.4
		P11	26.0	II	40.8	AH4	41.4
		MS5	43.3	13III	46.8		
		MS6	48.1	IV	31.1		
		MS7	36.0	V	46.1		
		MS9	35.1				
		MS10	50.7				
		MS11	54.8				
		MT9	41.1				
		MT10	67.9				

A, antennal hair; P, prothoracic hair; MS, mesothoracic hair; MT, metathoracic hair; PH, pentad hair; SH, siphon hair; AH, anal hair; I, first abdominal segment; II, second abdominal segment; etc.

Table #4- Standard Deviations of the Length for the Macroscopic Hairs of the Third Instar.

Head		Thorax		Abdomen		Terminal Segments	
Hair	s.d.	Hair	s.d.	Hair	s.d.	Hair	s.d.
1	14.1	P1	16.6	6I	61.7	PH1	33.5
5	19.1	P2	56.5	II	44.6	PH3	31.6
6	16.3	P3	38.7	III	45.2	PH5	35.3
7	20.8	P5	64.4	IV	58.6	SH1	27.4
A1	11.6	P6	40.6	V	34.5	AH1	50.0
		P7	54.6	VI	23.9	AH2	81.7
		P8	44.5	7I	44.2	AH3	73.0
		P11	48.3	II	38.6	AH4	93.9
		MS5	64.4	13III	36.7		
		MS6	76.7	IV	58.6		
		MS7	62.3	V	47.6		
		MS8	52.0				
		MS9	44.6				
		MS10	58.3				
		MS11	66.0				
		MT7	67.7				
		MT9	62.1				
		MT10	64.1				

Table #5- Frequency Distribution of the Number of Branches per Hair for the Branching Head Hairs and Antennal Hair 1.

Hair	Instar	Number of Branches per Hair									Total # of Hairs
		1	2	3	4	5	6	7	8	9	
4	II	21	37	7	0	0					65
	III	5	36	18	2	1					62
7	II	169	9	0							178
	III	63	102	9							174
8	II	25	63	11	0						99
	III	2	39	31	3						75
9	II	13	45	9	0	0					68
	III	3	32	32	3	1					71
10	II	6	61	4	1						72
	III	0	24	9	2						35
11	II		7	16	28	21	2	1	0	0	75
	III		2	8	24	19	7	6	0	1	67
12	II	60	2								62
	III	55	9								64
13	II	10	39	22	5	1	0	0			77
	III	0	11	21	21	12	1	1			67
15	II	8	46	24	0	0	0				78
	III	0	11	23	14	4	1				53
AH 1	II	188	15	0	0						203
	III	21	134	39	5						199

Table #6- Frequency Distribution of the Number of Branches per Hair for the Branching Prothoracic Hairs.

Hair	Instar	Number of Branches per Hair									Total # of Hairs
		1	2	3	4	5	6	7	8	9	
1	II	115	0								115
	III	76	12								88
4	II	35	32	0							67
	III	20	31	1							52
7	II	139	3	0							142
	III	26	129	1							156
10	II	68	5	0							73
	III	34	14	2							59
12	II	23	52	1	1	0	0	0			77
	III	2	12	14	5	1					34
14	II	77	2								79
	III	48	3								51

Table #7- Frequency Distribution of the Number of Branches per Hair for the Branching Mesothoracic Hairs.

Hair	Instar	Number of Branches per Hair 1	2	3	4	5	6	Total # of Hairs
1	II	12	34	2				48
	III	0	14	14				28
2	II	15	27	1	0			43
	III	3	19	12	2			36
4	II		48	22	2	1		73
	III		3	20	17	3		43
6	II		11	97	11	0		119
	III		1	89	24	1		115
8	II							
	III		1	11	53	40	8	113
10	II		12	96	8	2	0	118
	III		0	9	53	48	9	119
12	II	0	10	8	0			18
	III	1	11	6	2			20

Table #8- Frequency Distribution of the Number of Branches per Hair for the Branching Metathoracic Hairs.

Hair	Instar	Number of Branches per Hair									Total # of Hairs
		1	2	3	4	5	6	7	8	9	
1	II	23	29	6							58
	III	7	16	3							26
2	II		20	39	14	2					75
	III		2	18	11	3					34
3	II		8	37	29	6	1	2	0	0	83
	III		0	0	3	11	6	4	2	2	28
4	II		13	35	13	5					66
	III		1	12	14	6					33
6	II	75	2								77
	III	51	5								56
7	II										
	III		1	9	76	30	1	1			118
10	II		81	35	2	0					118
	III		2	96	18	3					119
11	II	83	4								87
	III	56	2								58
12	II	0	17	4	0						21
	III	2	21	2	2						27

Table #9- Frequency Distribution of the Number of Branches per Hair for the Branching Hairs of the Terminal Segments.

Hair	Instar	Number of Branches per Hair								Total # of Hairs
		1	2	3	4	5	6	7	8	
PH 1	II	156	16	0						172
	III	12	111	48						172
PH 2	II	99	59	1						159
	III	5	88	14	2					109
PH 3	II		31	121	18	3				173
	III			7	53	76	32	5	1	174
PH 4	II	163	1							164
	III	51	54							105
PH 5	II	16	140	13	0					169
	III	1	76	85	6					168
SH 1	II	5	151	18	0					174
	III	3	100	66	3					172

Table #10- Statistics for Those Hairs That Display Branching.

Hair	Mode		Range		Mean		SD		SE		# Hairs Examined	
	II	III	II	III	II	III	II	III	II	III	II	III
H4	2	2	1-3	1-5	1.78	2.32	0.63	0.74	0.08	0.09	65	62
H7	1	2	1-2	1-3	1.05	1.69	0.22	0.57	0.01	0.09	178	174
H8	2	2	1-3	1-4	1.86	2.47	0.59	0.62	0.06	0.07	99	75
H9	2	2-3	1-3	1-5	1.91	2.54	0.62	0.71	0.08	0.08	68	71
H10	2	2	1-4	2-4	2.00	2.37	0.44	0.60	0.05	0.10	72	35
H11	4	4	2-7	2-9	3.97	4.66	1.05	1.31	0.12	0.16	75	67
H12	1	1	1-2	1-2	1.03	1.14	0.18	0.35	0.02	0.04	62	64
H13	2	3-4	1-5	2-7	2.32	3.61	0.83	1.10	0.10	0.13	77	67
H15	2	3	1-3	2-6	2.21	3.26	0.61	0.94	0.07	0.13	78	53
A1	1	2	1-2	1-4	1.02	2.32	0.13	0.76	0.01	0.07	122	106
P1	1	1	1	1-2	1.00	1.14	0.00	0.35	0.00	0.04	115	88
P4	1	2	1-2	1-3	1.48	1.63	0.50	0.53	0.06	0.07	67	52
P7	1	2	1-2	1-3	1.02	1.84	0.14	0.39	0.01	0.03	142	156
P10	1	1	1-2	1-3	1.07	1.15	0.25	0.72	0.03	0.09	73	59
P12	2	3	1-4	1-5	1.74	2.74	0.55	0.90	0.07	0.14	59	41
P14	1	1	1-2	1-2	1.03	1.06	0.16	0.24	0.02	0.03	79	51
MS1	2	2-3	1-3	2-3	1.79	2.50	0.50	0.51	0.07	0.10	48	28
MS2	2	2	1-3	1-4	1.67	2.36	0.52	0.52	0.08	0.09	43	36
MS4	2	3	2-5	2-5	2.40	3.47	0.62	0.74	0.07	0.11	73	43
MS6	3	3	2-4	2-4	3.00	3.22	0.43	0.46	0.04	0.04	119	115
MS8		4		2-6		4.38		0.79		0.07		113
MS10	3	4	2-5	3-6	3.00	4.48	0.49	0.75	0.05	0.07	118	119
MS12	2	2	2-3	1-4	2.44	2.45	0.77	0.76	0.18	0.17	18	20
MT1	2	2	1-3	1-3	1.71	1.85	0.65	0.61	0.09	0.12	58	26
MT2	3	3	2-5	2-5	2.97	3.44	0.75	0.75	0.09	0.13	75	34
MT3	3	5	2-7	4-9	3.53	5.89	0.98	1.40	0.11	0.27	83	28
MT4	3	4	2-5	2-5	3.15	3.75	0.83	0.79	0.10	0.14	66	33
MT6	1	1	1-2	1-2	1.01	1.09	0.12	0.29	0.01	0.04	76	56
MT7		4		2-7		4.20		0.66		0.06		118
MT10	2	3	2-4	2-5	2.33	3.18	0.51	0.49	0.05	0.05	118	119
MT11	1	1	1-2	1-2	1.10	1.03	0.21	0.18	0.12	0.02	87	58
MT12	2	2	2-3	1-4	2.19	2.15	0.40	0.66	0.09	0.13	21	27
PH1	1	2	1-2	1-4	1.09	2.22	0.29	0.57	0.02	0.04	172	172
PH2	1	2	1-3	1-4	1.38	2.12	0.50	0.49	0.04	0.05	159	109
PH3	3	5	2-5	3-8	2.94	4.87	0.63	0.90	0.05	0.07	174	174
PH4	1	2	1-2	1-2	1.00	1.51	0.08	0.50	0.01	0.05	164	105
PH5	2	3	1-3	1-4	2.01	2.57	0.42	1.04	0.03	0.08	169	168
SH1	2	2	1-3	1-4	2.07	2.40	0.36	0.56	0.03	0.04	174	172

Table #11- Range and Mean for Length in Microns for the Macroscopic Abdominal Hairs.

		Hair #6			Hair #7			Hair #13		
		Range	$\bar{x}$	#	Range	$\bar{x}$	#	Range	$\bar{x}$	#
Seg. 1	II	682-891	767	21	506-682	623	20			
	III	836-1067	952	23	693-825	764	20			
Seg. 2	II	583-770	683	22	418-550	496	20			
	III	748-913	844	21	539-671	593	21			
Seg. 3	II	484-616	559	22				374-572	459	21
	III	715-902	799	26				649-770	706	20
Seg. 4	II	297-462	380	25				396-528	459	20
	III	550-792	618	23				627-858	700	19
Seg. 5	II	231-385	320	21				341-528	431	17
	III	495-616	548	24				539-726	638	22
Seg. 6	II	209-374	287	22						
	III	451-539	488	20						

: Number of hairs examined.

Table #12- Range and Mean for Diameter in Microns for the Macroscopic Abdominal Hairs.

		Hair #6			Hair #7			Hair #13		
		Range	$\bar{x}$	#	Range	$\bar{x}$	#	Range	$\bar{x}$	#
Seg. 1	II	5.2	5.2	18	3.9-5.2	5.0	18			
	III	7.8	7.8	19	5.2-6.5	6.1	18			
Seg. 2	II	5.2	5.2	19	3.9-5.2	4.1	18			
	III	6.5-7.8	7.5	18	5.2-6.5	5.8	18			
Seg. 3	II	3.9-5.2	4.7	20				2.6-3.9	3.6	18
	III	5.2-7.8	7.5	18				5.2-6.5	5.7	18
Seg. 4	II	2.6-3.9	3.3	18				2.6-3.9	3.5	19
	III	5.2-6.5	5.6	18				5.2-7.8	5.4	18
Seg. 5	II	2.6-3.9	3.1	18				2.6-3.9	3.5	18
	III	3.9-6.5	5.2	18				5.2-6.5	5.3	18
Seg. 6	II	2.6-3.9	2.8	18						
	III	3.9-5.2	5.1	17						

Table #13- Formula Distribution for Antennal Hair 1, Prothoracic Hair 7 and Pentad Hair 1.

Formula	Antennal Hair 1		Prothoracic Hair 7		Pentad Hair 1	
	II	III	II	III	II	III
1-1	90	1	68	7	72	0
1-2	8	4	1	12	9	10
1-3	0	1	1	57	0	2
2-2	3	21		1	3	35
2-3		11				27
2-4		4				2
3-3		3				8
3-4		1				
4-4		1				
Total	101	48	70	77	84	84

Table #14- 2X2 Table for Antennal Hair 1 and Prothoracic Hair 7.

Prothoracic Hair 7	Antennal Hair 1: 1-1	Antennal Hair 1: 1-2[1]	Antennal Hair 1: 2-2[2]
1-1	79 1 1	8 2 2	3 3 5
1-2[1]	2 4 0	0 5 3	0 6 10
2-2[2]	1 7 2	0 8 10	0 9 55

[1]At least one hair is single, the other has two or more branches.

[2]Each hair has at least two branches.

Table #15- 2X2 Table for Antennal Hair 1 and Pentad Hair 1.

		Antennal Hair 1		
		1-1	1-2	2-2
Pentad Hair 1	1-1	72 1 0	7 2 0	2 3 1
	1-2	8 4 0	1 5 2	0 6 7
	2-2	2 7 3	0 8 12	1 9 63

Table #16- 2X2 Table for Prothoracic Hair 7 and Pentad Hair 1

Prothoracic Hair 7

Pentad Hair 1	1-1	1-2	2-2
1-1	79 1 0	2 2 0	0 3 1
1-2	8 4 3	0 5 3	1 6 3
2-2	3 7 5	0 8 10	0 9 63

Table #17- Range and Mean of the Cratal Hairs, Precratal Hairs and the Total Number of Hairs in the Ventral Anal Brush.

	Range		Mean		Number of Specimens	
	II	III	II	III	II	III
Cratal Hairs	8-12	11-17	10.3	14.2	97	83
Precratal Hairs	6-10	2-8	7.4	4.1	61	83
Total Number of Hairs in Ventral Brush	15-20	16-21	17.9	18.3	59	81

Table #18- Frequency Distribution of the Number of Hairs in the Precratal Brush, Cratal Brush and for the Total Number of Hairs in the Anal Brush in the Second and Third Instars.

Precratal Hairs

Instar	Number of Hairs									Total # of Specimens
	2	3	4	5	6	7	8	9	10	
II					10	21	25	4	1	61
III	10	18	23	22	7	2	1			83

Cratal Hairs

	Number of Hairs										
	8	9	10	11	12	13	14	15	16	17	
II	1	12	48	26	10						97
III				3	8	14	23	19	13	3	83

Total Number of Hairs

	Number of Hairs							
	15	16	17	18	19	20	21	
II	1	4	13	27	12	2		59
III		1	9	43	22	4	2	81

Table #19- The hairs studied in This Paper (with the Exception of Antennal Hairs 2-6) and Some of Their Characteristics.

1. Microscopic hair.
2. Macroscopic hair.
3. Hair unbranched in second and third instar.
4. Hair branched in second and third instar.
5. Hair unbranched in second instar and branched in third.
6. Hair unbranched in second instar and unbranched or branched in third.
7. Microscopic hair for which mean length higher in third instar.
8. Microscopic hair for which mean length of second instar equal to or larger than that of third instar.
9. Microscopic hair that showed an increase in diameter in third instar.
10. Microscopic hair that showed no increase in diameter in third instar.
11. Microscopic hair in which diameter was so small or the difference between instars so small that an increase in size was not measurable.
12. Microscopic hair which appeared to increase in diameter in third instar not withstanding 11 above.

	1	2	3	4	5	6	7	8	9	10	11	12
Head hairs												
1		x	x									
3	x		x			x			x			
4	x			x[1]			x			x		
5		x	x									
6		x	x									
7		x		x[1]								
8	x			x[1]		x				x		
9	x			x[1]		x				x		
10	x			x[2]		x				x		
11	x			x		x				x		
12	x			x[1]		x				x		
13	x			x[2]		x				x		
14	x		x			x		x				
15	x			x[2]		x		x				
Antennal Hair 1												
Prothoracic Hairs		x		x[1]								
0	x			x				x	x			
1		x				x						
2		x	x									

Table #19 (Cont.)

	1	2	3	4	5	6	7	8	9	10	11	12
Prothoracic Hairs												
3		x	x									
4	x			x[1]			x		x			
5		x	x									
6		x	x									
7		x		x[1]								
8		x	x									
9	x		x				x			x		
10	x			x[1]				x		x		
11		x	x									
12	x			x[1]				x	x			
14	x		x				x		x			
Mesothoracic Hairs												
1	x			x[2]			x				x	x
2	x			x[1]			x				x	x
3	x		x				x				x	x
4	x			x				x			x	x
5		x	x									
6		x		x								
7		x	x									
8		x		x[3]								
9		x	x									
10		x		x								
11		x	x									
12	x			x[4]							x	
13	x			x				x	x			
14	x			x			x		x			
Metathoracic Hairs												
1	x			x[1]			x				x	x
2	x			x			x				x	x
3	x			x			x				x	x
4	x			x			x				x	x
5	x		x				x				x	x
6	x			x[1]			x				x	x
7		x		x[3]								
8	x			x			x		x			
9		x	x									

Table #19 (Cont.)

	1	2	3	4	5	6	7	8	9	10	11	12
Metathoracic Hairs												
10		x		x								
11	x			x[1]			x				x	x
12	x			x[4]							x	
13	x			x				x	x			
Abdominal Hairs[5]												
Pentad Hairs												
1		x		x[1]								
2	x			x[1]				x	x			
3		x		x								
4	x					x	x			x	x	
5		x		x[1]								
Siphon Hairs												
1		x		x[1]								
2	x	x	x				x		x			
Anal Hairs												
1		x	x									
2		x	x									
3		x	x									
4		x			x							

[1]Hairs may be unbranched in both instars

[2]Hairs may be unbranched in second instar only.

[3]Transitory hairs which are absent in second instar.

[4]Hairs may be unbranched in third instar only.

[5]All abdominal hairs are macroscopic and single.

Table #20- Formula Distribution of the Comb Scales and Pecten Teeth.

Comb Scales			Pecten Teeth		
Formula	II	III	Formula	II	III
4-6	1	0	7-10	1	0
5-5	8	3	8-8	2	0
5-6	10	11	8-9	5	1
5-7	2	4	8-10	2	0
6-6	40	40	8-11	4	0
6-7	19	20	8-12	1	0
6-8	0	3	9-9	3	0
7-7	8	7	9-10	8	4
7-8	1	0	9-11	9	1
			9-12	1	1
Total	89	88	9-13	1	0
			10-10	9	0
			10-11	18	3
			10-12	5	6
			10-13	0	2
			10-14	0	1
			11-11	3	6
			11-12	6	9
			11-13	1	7
			11-14	0	1
			12-12	3	5
			12-13	2	12
			12-14	1	8
			12-15	0	1
			13-13	2	3
			13-14	0	11
			13-15	0	1
			14-15	0	2
			15-15	0	1
			16-16	0	1
			Total	87	87

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