

CONTACT CHEMORECEPTORS OF FEMALE TABANIDS (DIPTERA)

CONTACT CHEMORECEPTION IN HAEMATOPHAGOUS TABANIDS: A STUDY OF THE  
DISTRIBUTION AND STRUCTURE OF TARSAL AND LABELLAR TASTE RECEPTORS  
AND THEIR SENSITIVITY TO SUGARS AND OTHER CHEMICALS.

By

SURESH BEHARI LALL  
B.Sc., M.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in partial fulfilment of the requirements

for the degree

Doctor of Philosophy

McMaster University

June 1969

DOCTOR OF PHILOSOPHY (1969)  
(Biology)

McMASTER UNIVERSITY  
Hamilton, Ontario

TITLE: Contact chemoreception in haematophagous tabanids: A study of the distribution and structure of tarsal and labellar taste receptors and their sensitivity to sugars and other chemicals.

AUTHOR: Suresh Behari Lall, B.Sc., (Rajasthan University, India)  
M.Sc., (Rajasthan University, India)

SUPERVISOR: Professor Douglas M. Davies

NUMBER OF PAGES: xii, 185

SCOPE AND CONTENTS: A study of the tarsi and mouthparts of females in three representative genera of tabanids revealed that the ventro-lateral surface of the fore-tarsi and the aboral margin of the labella were the principal loci of contact chemical sensilla. Of the four main types of aboral labellar trichodes (A,B,C and D), two (B and C) proved to be gustatory and then only at the tip. Two other types of trichodes (E and F) were identified on the anterior aspect of the labella. These were different from the aboral setae (type A,B,C, and D) in shape, size and function.

Extension of the proboscis was taken as the index of positive tarsal stimulation and the criterion of positive labellar stimulation was the spreading of the labellar lobes in an extended proboscis. This was observed to be the same in free as well as attached experimental flies. Newly emerged females showed similar response.

The frequency method generally employed in psycho-physical studies for estimating thresholds in mammals was successfully extended

to tabanids. Using this method the stimulative effectiveness and threshold of various sugars for the tarsal and labellar taste sensilla was determined. Comparison was made between the frequency method and the ascending method of estimating thresholds with regard to sucrose. The effect of starvation on responsiveness of tarsal and labellar contact chemoreceptors was determined. Newly emerged flies became increasingly sensitive to sucrose during the duration of tests when they were strictly maintained on water diet.

Intergeneric differences and similarities were seen in the behaviour immediately before feeding. The flies fed on "dry" sucrose as well as on solutions of appropriate concentrations. By analysing the crop contents of wild-caught tabanids, it was established that they fed on sugars and these findings were related to tabanid feeding behaviour and adaptation in nature.

Using whole blood, sugars and blood-sugar mixtures at various concentrations and proportions (in the case of blood-sugar mixtures), their dispatch to crop and/or mid-gut was studied. In addition, a select group of amino acids and nucleotides were also tested to determine if these acted as feeding stimulants for the deer-flies.

Wild-caught females of deer-flies lived on dry sucrose and water and on 1.0M glucose solution longer than on distilled water alone or without food and water.

#### ACKNOWLEDGEMENTS.

I thank Professor Douglas M. Davies for advice and laboratory facilities. Professors H. Kleerekoper and P.L. Newbigging as members of the original supervisory committee gave advice on the research project. Doctors A.D. Dingle, D.E.N. Jensen and J. Mills-Westermann as members of the new advisory committee critically re-examined the thesis and gave suggestions for its revision. Sections of the thesis were kindly examined by Doctors R.T. Yamamoto of North Carolina University, USA, L.E. Chadwick of Blue Hill Falls, Maine, USA, T.E. Mittler of University of California, Berkeley, USA and G.B. Fairchild of Canal Zone, Panama.

I am especially grateful to my wife Pushpa and my son Atul for their endurance during the difficult period of this study. Messrs. Jan Kula and Paul Henderson and Mrs. Helen Gyorkos gave technical assistance and Mr. Ralph Idema drew the illustrations under my guidance.

Appreciation is expressed to the staff of the IBM computer center, University of Saskatchewan, Regina campus for assistance in re-checking the statistical analysis of the results.

Research grants from the National Research Council of Canada and the Defence Research Board of Canada supported the study. The author was partly assisted by a teaching assistantship from the Department of Biology, McMaster University, during his tenure as a graduate student.

## TABLE OF CONTENTS

	Page
Acknowledgements .....	iv
I Introduction .....	1
II Materials and Methods .....	10
(i) Collection of tabanids and their maintenance ....	10
(a) Collection of adults .....	10
(b) Maintenance of adults .....	10
Part I <u>Morphological studies</u> .....	10
(a) Preparation of the flies for the head capsule and mouthparts .....	10
(b) Tarsal and labellar trichoid sensilla .....	11
Part II <u>Behavioural studies</u> .....	12
(a) Loci of contact chemoreceptors and the establishment of indices of stimulation .....	12
(i) Preparation of the flies .....	12
(b) Methods for determining the loci of contact chemoreceptors .....	13
(c) Methods for observing the behaviour of the free and attached experimental flies .....	14
(d) Sensitivity of the tabanid contact chemoreceptors to chemicals .....	15
(i) Preparation of the flies .....	15
(ii) Method of testing .....	16
(iii) Method of recording .....	18
(iv) Treatment of the data .....	18
(v) Test chemicals .....	19

TABLE OF CONTENTS (continued)	Page
(a) Sugars .....	19
(b) & (c) Amino acids and nucleotides .....	20
(d) Longevity of wild-caught tabanids .....	21
(e) Analysis of the crop fluid of wild-caught tabanids	22
(f) Dispatch of sugar solutions, whole human blood and blood-sugar mixtures .....	23
III Observations and Results .....	25
Part I <u>Morphological studies</u> .....	25
(a) Head capsule .....	25
(b) Mouthparts .....	27
<u>Morphology and distribution of trichoid sensilla</u> .....	31
(a) Antennal trichodes .....	31
(b) Labral trichodes .....	31
(c) Maxillary trichodes .....	31
(d) Labellar trichodes .....	32
(i) Distribution and number .....	32
(ii) Measurement of trichodes .....	33
(e) Tarsal trichoid sensilla .....	35
Part II <u>Behavioural studies</u> .....	37
(a) Sugar feeding .....	37
(b) Contact chemoreceptors of tabanids .....	40
(i) Loci .....	40
(ii) Ablation experiments .....	41
(iii) Point of stimulation on the sensillum .....	41
(c) Physiological state of the flies used in behavioural studies .....	42

TABLE OF CONTENTS (continued)		Page
	(d) Sensitivity of tabanid contact chemoreceptors ..	43
	(i) Tarsal gustatory sensilla .....	43
	(iia) Labellar contact chemoreceptors .....	44
	(iib) Amino acids .....	45
	(iic) Nucleotides .....	46
	(e) Dispatch of whole blood, glucose, sucrose and blood-sugar mixtures .....	46
	(f) Angiosperm flora of Beverley Swamp .....	48
	(g) Analysis of crop fluid .....	49
	(h) Longevity of wild-caught tabanids .....	50
IV	Discussion .....	51
Part I	<u>Morphological studies</u> .....	51
	(i) Comparative studies of the head capsule and mouthparts .....	51
	(a) Head capsule .....	51
	(b) Mouthparts .....	51
	(ii) Loci, structure and function of contact chemoreceptors .....	58
	(a) Tarsal contact chemoreceptors .....	59
	(b) Labellar contact chemoreceptors .....	59
	(i) Aboral trichodes .....	60
	(ii) Site of perception of the contact chemical stimuli .....	61
	(iii) Oral canalicular system .....	61
	(c) Other trichoid sensilla .....	62
	(i) Antennal trichodes .....	62
	(ii) Labral and maxillary trichodes .....	62



TABLE OF CONTENTS (continued)	Page
(d) Interrelationship of taste sensilla in relation to feeding behaviour.....	63
(iii) Indices of stimulation.....	64
Part II <u>Behavioural studies</u> .....	67
(i) feeding behaviour of female tabanids.....	67
(ii) General discussion of the methods for estimating thresholds of chemicals in insects.....	69
(iii) Sensitivity of the tabanid contact chemoreceptors to sugars and other phagostimulants.....	72
(a) Sensitivity of the tarsal contact chemoreceptors to sugars.....	73
(b) Sensitivity of the labellar contact chemoreceptors..	76
(i) Sugars.....	76
(ii) Amino acids and nucleotides as phagostimulants.....	80
(a) Amino acids.....	80
(b) Nucleotides.....	82
(iii) Comparison of the sensitivity of tarsal and labellar contact chemoreceptors.....	83
(iv) Dispatch of whole human blood, glucose, sucrose and blood-sugar mixtures ( the "switch mechanism" ) to crop or midgut.....	84
(v) Analysis of crop fluid.....	90
(vi) Longevity of the wild-caught tabanids.....	93
(vii) Some remarks on the relevance of the experimental results with the behaviour of the flies in nature and the further avenues of research as indicated from the present study.....	95
V Summary.....	98
VI References cited .....	102
VII Figures and Tables .....	118
VIII Appendices .....	174

LIST OF FIGURES

		Page
Fig. 1	Tabanids used in the study.....	118
Fig. 2-7	Head capsule and mouthparts of female tabanids..	119
Fig. 8-10	Antenna of tabanids.....	125
Fig. 11-13	Labrum-epipharynx of tabanids.....	128
Fig. 14-16	Mandible of tabanids.....	131
Fig. 17-19	Maxilla of tabanids.....	134
Fig. 20-22	Hypopharynx of tabanids.....	137
Fig. 23-28	Labium of tabanids.....	140
Fig. 29	Labella of female <u>Chrysops vittatus</u> showing the principal hair types.....	146
Fig. 30	Semi-diagrammatic representation of the loci of various types of labellar trichodes in females of <u>Chrysops vittatus</u> .....	147
Fig. 31	Longitudinal section of the labium of female <u>Chrysops vittatus</u> .....	148
Fig. 32-37	Foretarsi of tabanids showing the various types of sensilla .....	149
Fig. 38-39	Sugar feeding in female <u>Chrysops vittatus</u> and female <u>Hybomitra lasiophthalma</u> .....	155
Fig. 40	Apparatus for holding flies during behavioural studies .....	157
Fig. 41-43	Graphs showing the tarsal and labellar acceptance threshold for sugars in females of <u>Chrysops vittatus</u> .....	158
Fig. 44	Paper-partition chromatography of crop fluid of wild-caught females of <u>Chrysops vittatus</u>	161

LIST OF TABLES

		Page
Table I	Measurement studies of the head capsule and mouthparts of female tabanids	162
Table IIa	Measurement studies of the labellar trichodes for ten females of <u>Chrysops vittatus</u>	163
Table IIb	Variability in the number of labellar trichodes and psuedotracheae of ten females of <u>Chrysops vittatus</u>	164
Table IIc	Total number of labellar trichodes on the left and right labellum of 10 females of <u>Chrysops vittatus</u>	165
Table III	Sensitivity of tarsal contact chemoreceptors of female <u>Chrysops vittatus</u> to sugars (sample frequency method)	166
Table IV	Sensitivity of the labellar contact chemoreceptors of female <u>Chrysops vittatus</u> to sugars (population method)	167
Table V	Labellar acceptance threshold of female <u>Chrysops vittatus</u> for sugars	168
Table VI	Labellar sensitivity of female <u>Chrysops vittatus</u> to amino acids in aqueous solution	169
Table VII	Labellar sensitivity of female <u>Chrysops vittatus</u> to amino acids and nucleotides in phosphate buffer	171
Table VIII	Nucleotides as phagostimulants for females of <u>Chrysops vittatus</u>	172
Table IX	Dispatch of ingested diet by females of <u>Chrysops vittatus</u>	173
Appendix I	Effect of starvation on the sensitivity of tarsal and labellar contact chemoreceptors in <u>Hybomitra lasiophthalma</u> (Diptera : Tabanidae)	174

LIST OF TABLES (continued)		Page
Appendix II		
Table X	Representative angiosperms from the Beverley Swamp with type of nectar composition	179
Table XI	A protocol of the method of presenting test molar concentration of sucrose to the labella of female <u>Chrysops vittatus</u> and the manner in which response was recorded	180
Fig. 45	A protocol of the graph drawn for each fly (7-9) based on averaged data for a total of 10 trials (based on Table XI)	181
Table XII	Labellar response of seven females of <u>Chrysops vittatus</u> to sucrose, based on averages of ten trials at each concentration	182
Fig. 46	A protocol of the graph drawn for each sugar based on average data for the total number of flies tested (7-9) (based On Table XII)	183
Table XIII	The order of stimulative effectiveness of sugars for the labellar taste sensilla of females of <u>Chrysops vittatus</u> (A comparison of sample and frequency method for estimating thresholds)	184
Table XIV	Longevity of wild-caught females of <u>Chrysops vittatus</u> fed on sugars and water.	185

PUBLICATIONS FROM THE THESIS

LALL, S.B. & Davies, D.M. 1967, Tarsal sensitivity of female  
tabanid flies to sucrose and sodium chloride.  
Can.J.Zool. 45: 461-464

\_\_\_\_\_ & \_\_\_\_\_, 1968 Comparative studies on the sensitivity  
of labellar contact chemoreceptors in female tabanids  
(Diptera)  
Ann. Entomol. Soc. Amer. 61: 221-224

## I. INTRODUCTION

The class Insecta and the class Arachnida (order Acarina) contain many species that have become adapted, through convergent evolution, to feeding on blood from such vertebrate hosts as amphibians, reptiles, birds and mammals, including man. Although considerable research has been done on the orientation of the blood-sucking insects to their hosts, there is little information on contact chemoreception in haematophagous insects and of the factors that initiate, sustain and/or inhibit feeding, whether it is upon nectar or blood. Research on this aspect in haematophagous Tabanidae has been perfunctory and scarce.

The adult females of many dipteran species require a blood meal for oögenesis (anautogeny), although even in such haematophagous families as Culicidae and Simuliidae, there are exceptions. Autogeny in Nearctic Tabanidae has yet to be proven. Both sexes of many blood-sucking Diptera, including Tabanidae, visit flowers for nectar which supplies energy for activities, such as, flight, feeding, mating and oviposition, as well as for metabolic processes including oögenesis (cf: Wigglesworth 1965, Hocking 1953, Downes 1958). In fact, carbohydrates are necessary for survival in many adult Diptera, even in the presence of protein (Glaser 1923, Strangways-Dixon 1961a,b).

Specialised chemosensory (as well as photosensory) apparatus and mouthparts aid these blood-sucking flies in locating and feeding on vertebrate hosts as well as upon flower nectar. In addition, chemoreceptors may also play a critical role in locating appropriate habitats, mates and oviposition sites (Wallis 1954, Hodgson 1964). In social insects chemoreception reaches its highest development, where individuals must perform many sensory functions to maintain the complex organisation (cf: Dethier 1963, Hodgson 1964).

Chemoreceptors are specialised rather than general and are sensitive only to certain chemicals and not to others. They are particularly sensitive to the molecular sub-structure of certain chemicals. In fact, Dethier (1962) considers that eventually it may be meaningful to define them in precise categories, such as salt, sugar and sex-attractant receptors. However, since our knowledge of them is as yet inadequate, it is better to refer to them by the conventional ambiguous terms ---- receptors for olfaction, gustation and common chemical sense (Dethier and Chadwick 1948). The categories can be explained as follows:-

a. Sensilla sensitive to various classes of chemical compounds in the liquid and the gaseous phase. These are extremely sensitive to chemicals at very low concentrations and from a long distance,

and are called olfactory receptors. The loci of these receptors in insects are on the antennae, palpi and their homologues and the tarsi. The shape and structural organisation of olfactory receptors is extremely variable.

b. Sensilla responsive to chemicals when in actual physical contact.

Chemicals initially in the liquid or the solid phase are able to stimulate these receptors. Relatively high concentrations are generally required to excite them. Such receptors are called contact chemoreceptors, gustatory sensilla or simply organs of taste.

c. The receptors belonging to this category are called "organs of common chemical sense". Although specific receptor(s) of this category have not been identified, there is substantial experimental evidence to indicate their presence in a variety of insects. The main function of these sensilla is to detect the presence of deleterious substances in the environment and to elicit an avoiding reaction (Dethier 1953). Behavioural and electrophysiological work on Periplaneta americana L. (Roy 1953), grasshoppers (Slifer 1954) and Pieris (Dethier 1941) has provided convincing experimental evidence in support of the above. Dethier (1956) persuasively argues that while some repellents can act on senses other than olfactory and contact chemical sense, repellents at low concentration first induce reaction in the olfactory sensilla.



The organs concerned with olfaction are fundamentally different from the gustatory sensilla. They differ in their shape, size, distribution, histology, and also with respect to the threshold chemical concentrations for their stimulation.

Quantitative distinction between olfaction and taste has not been adequately demonstrated. The gustatory receptors can elicit a response on being stimulated by sugars and many other chemicals, while these chemicals may fail to evoke a response from olfactory receptors. Further, many organic compounds, e.g., alcohols, aldehydes and fatty acids, in liquid phase can stimulate oral and tarsal gustatory receptors and in the gaseous phase, olfactory receptors (Dethier and Chadwick 1948).

Snodgrass (1926) presented a detailed review embodying the known facts concerning the anatomy of insect sensilla and classified them into several categories on the basis of their structure rather than function. Since this time, several reviews based on structure (as well as partly on function) have appeared (cf: Eltringham 1933a, Dethier 1963, Wigglesworth 1965).

Intensive studies on several species of blow-flies and muscoid flies indicate that the tarsi and labella possess hairs responsive to a variety of chemicals (Minnich 1922, 1926, Grabowski and Dethier 1954, Dethier 1955a). These sensory hairs have been

stimulated individually and in groups by different chemicals to determine acceptance and rejection thresholds for various classes of organic and inorganic compounds. The complete sequence in the feeding behaviour of certain Diptera (e.g., muscoid flies and blow-flies) viz; extension of the proboscis, spreading of the labellar lobes and sucking were evoked by stimulation of a single gustatory hair (Dethier 1956). Feeding could be inhibited by the application of salts and a variety of non-carbohydrates and acids, which frequently cause regurgitation (Dethier 1955b). Sucking could be initiated and sustained by certain sugars, such as sucrose, glucose, fructose and others. Termination in feeding was suggested as due to (i) cessation of sensory input from the cells, either by the removal of the stimulus or by adaptation, (ii) central adaptation, (iii) inhibitory impulses from the gut, signalling satiation (Dethier and Bodenstein 1958) and (iv) stimulation of the rejection neuron in the tarsal or labellar chemoreceptor. Relationships between stimulative effectiveness and the properties of the test chemicals have been studied with limited precision in some insects (see von Frisch 1934, Haslinger 1935, Dethier 1955, Evans 1963, Salama 1966a,b for references). Four types of contact chemoreceptors have been identified in the blow-flies of which (i) the sugar receptor is sensitive only to certain carbohydrates and (ii) the non-sugar receptor, which is capable of being stimulated by many organic and

inorganic compounds (Dethier 1955). Some basic histological studies by Dethier (1955), Stuerckow (1961) and Peters (1963) and electrophysiological studies by Hodgson (1964), and den Otter and van der Starre (1967) have yielded the useful information in understanding the possible mechanism of contact chemoreception in blow-flies. The studies conducted by Dethier and his associates have amply demonstrated the greater suitability of the blow-fly, Phormia regina Meigen, owing to its size, easy rearing and a more consistent behavioural response, than any other fly species.

In contrast to the overwhelming research on contact chemoreception in the blow-flies, studies on the structure and function of taste sensilla in haematophagous insects have received little attention. Few workers have attempted to study these aspects in blood-sucking Diptera (see McGregor 1931, Day 1954, Frings and Hamrum 1950, Wallis 1954, Feir et al 1961, Owen 1963 for mosquito spp; Adams 1961, Hopkins 1964 for the stable-fly Stomoxys calcitrans L; Frings and O'Neal 1946, Lall and Davies 1967, 1968 for tabanids).

The family Tabanidae constitutes one of the most annoying group of blood-sucking flies inflicting painful bites and sucking blood thus sapping the vitality of the host and are often incriminated as vectors of parasites (Gordon and Crew 1953) and pathogens (cf: Anthony 1962).

Studies on the structure of the mouthparts, feeding behaviour, contact chemoreceptors and their function in feeding in haematophagous tabanids are few (cf: Bromley 1926, Snodgrass 1944, Bonhag 1951, Dickerson and Lavoipierre 1959, Frings and O'Neal 1946, Lall and Davies 1967, 1968) and no attempt has been made to approach the problem on a comparative basis. Nor has there been research devoted to the chemical factors (blood-borne and/or nectar sugars) that may act as feeding stimulants. The loci of contact chemoreceptors has yet to be determined as well as the type of hairs, their distribution, variability and function in any tabanid species. The solitary exception is a brief study by Frings and O'Neal (1946) on the female horse-fly, Tabanus sulcifrons Macquart. Knowledge of the feeding habits of species that are annoying pests and are vectors of parasites and pathogens should contribute significantly to the development of repellent materials, in the understanding of transmission of parasites and pathogens and in problems relating to host preference. A precise study of the various aspects of feeding behaviour of haematophagous flies is an integral part in the study of bionomics of these insects.

Unlike blow-flies and house-flies, which feed on plant juices and on decaying organic matter, adult females of horse-flies and deer-flies feed and subsist on the blood of a variety of

vertebrates in addition to flower nectar, plant sap and juices of ripe fruits, while the male feeds on nectar only (cf: Downes 1958). It seems probable that some of the factors involved in contact chemoreception are species specific in these flies.

The purpose of the present study is:-

- (i) To compare the female mouthparts in the genera Chrysops, Tabanus, and Hybomitra, in order to determine the similarities and differences in their piercing and sucking apparatus.
- (ii) To determine the loci, number and variability of contact chemoreceptors and to study their morphological and functional characteristics.
- (iii) To compare the mode of sugar feeding in the females of Chrysops vittatus Wiedemann, Tabanus lineola Fabricius and Hybomitra lasiophthalma Macquart.
- (iv) To analyse the crop contents of wild-caught females of C.vittatus with a view to relating these findings to the tabanid feeding behaviour and adaptation in nature.
- (v) To determine the criteria for the positive stimulation of tarsal and labellar contact chemoreceptors.
- (vi) To determine the sensitivity of tarsal and labellar contact chemoreceptors to sugars, amino acids and nucleotides.

(vii) To study the passage of ingested food, namely, sucrose, glucose, whole blood, blood-sugar mixture to crop and/or midgut.

Preliminary studies were undertaken to assess the effect of starvation on the sensitivity of tarsal and labellar taste sensilla to sucrose in newly emerged females of H.lasiophthalma maintained on water diet. Also studied was the longevity of wild-caught females of C.vittatus under laboratory conditions.

## II MATERIALS AND METHODS

### (i) Collection of tabanids and their maintenance

#### (a) Collection of adults

Adult females of Tabanus and Hybomitra spp. were trapped by using a modified Malaise trap with "dry" ice (CO<sub>2</sub>) as an attractant, as well as by leaving the door of a car open. Species of female Chrysops were collected in "profitable" numbers mainly during July by swinging an insect net around humans, who were attacked by swarms of these flies. On a warm day (temperature 18° - 24°C) with high humidity and a light or no wind, 200-400 individuals of female Chrysops spp. were collected in a 2-h. period by two men. Collections of tabanids used in this study were exclusively from Beverley swamp, near Hamilton, Ontario, Canada.

#### (b) Maintenance of the adults

Adult females of Tabanus lineola, Hybomitra lasiophthalma and Chrysops vittatus were maintained in batches of 10-15 in cardboard cylinders with screen tops and bottoms and were fed on dry sucrose and water at 19°C.

### Part I Morphological studies

#### a. Preparation of the flies for the study of head capsule and mouthparts

Fresh and alcohol preserved females of C.vittatus, T.lineola and H.lasiophthalma were used for the comparative studies

of the head capsule and mouthparts. The head after being detached from the body was boiled in 5% KOH for 15-20 min., rinsed in distilled water several times and put in 70% ethanol to which a few drops of acetic acid had been added. The mouthparts were dissected in 70% ethanol under a dissecting microscope and measurements made of these as well as the head capsule. A total of 5-8 flies were used for such measurements.

Drawings were made with the aid of a microprojector.

b. Tarsal and labellar trichoid sensilla

The general distribution of tarsal trichoid sensilla was studied in three tabanid species. Preliminary studies aimed at mapping the labellar trichoid sensilla of female C.vittatus revealed some variability in number among individual specimens. Accordingly it was considered necessary to examine many specimens in order to obtain average data. The labellar trichoid hairs of 10 females of C.vittatus were carefully measured with regard to their length, width of the base, tip and the length of the socket. A total of six hairs of each type were measured for each fly (6x10). At the suggestion of Dr. Thürm (in litt.) intra-vital staining with methylene blue following the method of Unna (1922) with certain modifications was employed. The methylene blue stain was used in two ways:-

(i) by injecting it into the live fly and then keeping the fly



immersed in methylene blue for 30-35 mins. or (ii) by immersing the live fly in the stain for 30-35 mins. Both gave somewhat satisfactory results, although heavy sclerotisation of the cuticle impeded in studying inner details of the sensillum. In either case, the detached appendages were rinsed in distilled water and fixed overnight in 8% ammonium molybdate at 4°C. After a washing in distilled water, they were quickly dehydrated in 95% ethanol and then in absolute ethanol. Final preservation was in methyl salicylate. For making permanent mounts the appendage was put in synthetic resin under a No. 1 coverslip. Measurements and the general study relating to the morphology of the sensilla were made from these preparations.

## Part II Behavioural studies

### a. Loci of contact chemoreceptors and the establishment of indices of stimulation

#### i) Preparation of the flies

Wild-caught females of C.vittatus were kept in batches of 10-15 in cardboard cylinders at 19°C and fed on dry sucrose and water for two days. This was believed to assist them in digesting their previously obtained nutrients and also to standardise the experimental conditions.

After two days the flies were chilled and thus immobilised

were individually attached by their wings or dorsa to a bayberry wax block (m.p.39°C) carried at the end of a glass rod or an applicator stick (fig. 41). Several were prepared at one time for a series of tests. Recovery of the flies averaged 2-3 mins. A period of 24 h. lapsed before testing, to give the flies time to recuperate and to acclimate to the experimental conditions. No food was offered to the flies during the 24 h. pre-test period, but each was allowed to drink water ab lib. A lens paper "dress" was applied around the thorax to contain the appendages which otherwise obstructed the testing procedure. A slit in the paper "dress" allowed the desired appendage to be drawn out for testing. All observations were made under a binocular microscope. The temperature of the room during experimentation was between 19-23°.

b. Methods for determining the loci of contact chemoreceptors

Fifteen flies attached by their dorsa or wings to waxed sticks were used for these experiments. A 1.0M sucrose solution was used to test those body parts, areas and specific sensilla which had contact chemoreceptive function. D-glucose and fructose were not used as 1.0M solutions gave inconsistent results in the preliminary tests. Testing involved "dipping" parts of the body or specific appendages suspected of bearing contact chemoreceptors into the 1.0M sucrose solution. In certain cases amputation of the part(s) or

segment of the appendage was performed to examine whether the loss of these, altered the response of the flies to the test solution. A period of 20-24 h. elapsed before tests began after the parts were amputated.

The flies were given 5 sec. to respond after which the part(s) were rinsed in distilled water. Tests were made at intervals of 10-15 mins.

A delta ejector and a microneedle mounted over a syringe were used to apply the test solution in determining the nature of the specific hair type and the site of perception of the stimuli. This facilitated single hair stimulation as well. A drop of sugar solution at the tip of the delta ejector was rolled along the length of the sensillum to ascertain the precise site of stimulation.

c. Methods for observing the behaviour of free and attached experimental flies

Ten to twelve "hungry", water-satiated, unmounted females of C.vittatus, T.lineola and H.lasiophthalma were used to observe the mode of feeding on sugar cubes and on filter paper impregnated with sucrose solution of appropriate concentration (0.5 to 1.0M). Newly emerged females of H.lasiophthalma (as well as other Tabanus and Chrysops spp.) were also observed as they fed on sugar and water. A series of drawings exhibiting the use of appendages, their deploy-

ment and the posture taken by the body during feeding, were made in the case of H.lasiophthalma and C.vittatus. Comparisons were also made between the feeding behaviour of free and attached flies. In the latter case, a strip of filter paper moistened with 1.0M sucrose solution was brought in contact with foretarsi or aboral margin of the labella or other appendages to see if this evoked a response. In some cases the appendages were amputated (partially or completely) to see if this resulted in a change of behaviour. This also facilitated observations on the importance of body parts and the appendages in the feeding behaviour of the flies.

d. Sensitivity of tabanid contact chemoreceptors to chemicals

(i) Preparation of the flies

Tests were made on flies (maintained on dry sucrose and water at 19°C for two days) attached by their dorsa or wings to waxed ends of applicator sticks or glass rods. They were allowed to imbibe water only during the 24 h. pre-test period. Before the beginning of each test the responsiveness of the fly to water was checked. Tests were made only when the flies responded negatively to water stimulation. Flies that extended their proboscis when touched with water were allowed to "drink" it ab lib., and were tested later when their response to water was negative.

(ii) Method of testing

The frequency method (cf: Woodworth and Schlosberg 1938) generally employed in psycho-physical studies by mammalian behaviourists and physiologists was extended to tabanids for estimating thresholds, and to observe the behaviour of the flies during successive trials over an extended period of time. Threshold estimations thus made, also permitted in getting some information on the question of stimulative effectiveness of sugars, their preference, acceptance and/or rejection by tabanids.

The number of flies used for testing each sugar was 7-9. Molar concentrations of test sugar were prepared in a manner such that the lower concentration was always half of the next higher. In order to avoid such biases as series effect, conditioning, habituation and/or anticipation, the test molar concentrations were offered in a random manner. The order of presentation varied with each trial. The number of tests for each of the 7-9 molar concentrations was 10. Before testing and between each concentration tested, distilled water was offered. If the fly's response to water became positive, then it was allowed to "drink" it to repletion and no further tests were made until its response to water became negative. A syringe was used to apply the test solution to a group of hairs that were earlier determined to be contact chemoreceptors. The fly was given 5 sec. to

respond to each test concentration, after which the labella or the tarsi as the case might be were rinsed in distilled water. A period of 10-15 min. lapsed between each test. After the completion of tests the flies were fed ab libitum on 1.0M sucrose solution. Tests were began next day and this procedure was repeated for several days on the same group of flies.

Using a population of (15-40)flies maintained under identical experimental conditions, the frequency method was used for estimating thresholds for the labella of female C.vittatus using a select group of five sugars (i.e., glucose, fructose, maltose, melezitose and fucose). This was intended to compare the response of a sample of flies (7-9) (see above) to that of the population (15-40) and to gain further confidence about the suitability of frequency method and the rank order of sugars tested. The method of presentation of test molar concentration in the population method was the same as in the sample method. However, each fly was tested only once or twice with the test molar concentration.

An examination of the ovarian development and of the fat body was made after the completion of behavioural studies.

In a preliminary study, using the ascending method of testing, the sensitivity of the tarsal and labellar contact chemoreceptors to sucrose and sodium chloride was compared for three

tabanid species (Lall and Davies 1967, 1968). This method was also used to observe the effect of starvation on the sensitivity of contact chemoreceptors in newly emerged females of Hybomitra lasiophthalma.

(iii) Method of recording

A + sign denotes a positive response, (i.e., extension of the proboscis (criterion of positive tarsal response) and the opening of the labellar lobes in an extended proboscis (index of positive labellar response). A ± sign means indefinite response and includes such overt reactions as partial extension of the proboscis with no opening of the labellar lobes, or quick extension and retraction of the proboscis. A "0" sign means no response.

(iv) Treatment of the data

The response of the fly during each trial was recorded separately. Determination of the percentage response was made for each molar concentration tested (in the 10-trial schedule). Preliminary graphs were made for each fly on this basis. The most probable acceptable concentration was considered to be one to which 50% of the responses were positive. This was depicted in the graph by drawing a line at the 50% level and calculations for the acceptance threshold were accordingly made for each fly. This also facilitated determination of range of responses in the test population of the

flies. The standard deviation was calculated by the following formula:

$$S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Where  $x$  = threshold of each fly in the test population

$\bar{x}$  = arithmetic mean

and  $n$  = number of flies used in the test.

In order to show the trend of acceptance threshold the data for all the 7-9 flies were averaged and exhibited in the form of a graph to show the percentage response with respect to each molar concentration. In the population method (with one or two trials) the most probable acceptable concentration was taken to be one to which 50% of the flies responded positively. The statistical analyses were rechecked and confirmed with the aid of IBM computer.

The order of stimulative effectiveness of test sugars as determined by the two above methods were compared.

#### (v) Test Chemicals

##### a. Sugars

A variety of D- sugars were used, including the ones that occur in flower nectar of angiospermic plants on which tabanids are



known to feed in nature (Hocking 1953). The sugars tested were: sucrose, glucose, fructose, maltose, melibiose, raffinose, melezitose and fucose.

Comparison of the sensitivity of tarsal and labellar taste receptors was made with respect to sucrose, glucose and fructose.

b. and c. Amino acids and nucleotides<sup>x</sup>

Aqueous solutions of 15 amino acids at 0.05M and 0.005M were used to determine their stimulative effectiveness on the labellar taste sensilla of water-satiated, hungry females of C.vittatus. Their pH value was precisely determined. Sucrose solution at 0.5M was the control. Seven amino acids dissolved in 0.133M phosphate buffer (pH 7.4) were tested with females of C.vittatus (of similar state as above) to determine the importance, if any, of the phosphate radical and the effectiveness of the amino acids in such a state. Phosphate buffer alone was used as a control. The amino acids used were: L-alanine, DL-aspartic acid, L-cysteine, DL-glutamic acid, Glycine, DL-histidine (HCl), L-isoleucine, DL-lysine (HCl), DL-methionine, arginine, DL-proline, DL-serine, DL-threonine, L-valine and DL-leucine. Solutions of ADP and ATP at 0.05M and 0.005M in 0.133M phosphate buffer (pH 7.4) were applied to the labella of hungry, water-satiated females of C.vittatus.

---

x Obtained from Nutritional Biochem., Cleveland, Ohio, U.S.A. and Cal. Biochem., California, U.S.A.

Feeding tests were conducted on starved flies fed ab lib on water, by releasing them in groups of 5-10 in 1500 ml beakers having a screen top and filter paper "carpeted" bottom. The test solution was offered by moistening thoroughly the cotton pad that emerged from the feeding bottle three-quarters filled with the test chemical. The solution contained small amounts of methylene blue. Solutions of AMP, ADP and ATP were offered at 0.05M and 0.005M dissolved in water, phosphate buffer (0.133M pH 7.4) and 0.15M NaCl. Controls were exposed to distilled water, buffer and 0.15M NaCl. After 30-45 mins., the flies were removed, chilled to immobilise and dissected under the binocular microscope. Presence of dye in the gut was used as the index of feeding.

d. Longevity of wild-caught tabanids

Wild-caught females of Chrysops vittatus were released in 1500 ml beakers containing the test diet to determine their longevity under laboratory conditions. A total of 20 flies were used for each experiment that involved maintenance of flies, (i) without food and water, (ii) on sucrose and distilled water, (iii) on 1.0M glucose and (iv) on water alone.

Checks were made after every 24 h. The dead flies were removed and their numbers recorded. Recording was suspended the day LD<sub>50</sub> was reached.

e. Analysis of the crop fluid of wild-caught tabanids

The crop fluid of wild-caught females of C. vittatus was analysed by paper-partition chromatography. Soon after being brought from the field, the flies were dissected and their crop removed and stored in 70% ethanol. A total of 5-7 crops (constituting one batch) were finely ground in a test tube by a pestle and the volume reduced by evaporation to a level suitable for application to Whatman No. 1 filter paper. The number of chromatograms run for such a batch was 2-3. A total of 12 such batches of crop fluid were analysed. In the preliminary run, some crops were removed and smeared on the paper for analysis and to ascertain the suitability of a solvent system.

After application of the crop or its contents, the sugars suspected of being present were applied in known volume as reference markers. They were prepared at 5% solutions in saturated benzoic acid. A period of 10-15 mins. elapsed before they were put in the chromatogram tank. In most cases, drying of applied sugars and crop fluid was accelerated by applying carefully controlled jet of air. A number of solvent systems were used to bring about an effective separation. The iso-propanol:water mixture (3:1 v/v) and the n-butanol:acetic acid:water mixture (5:1:4 v/v) gave desirable separation. After the chromatograms were run in the solvent by the ascending or descending techniques, they were dried and evenly

sprayed by the detecting benzidine reagent and heated to 100°C. All sugars reacted to heating within 4-5 mins. Their Rf values were precisely determined. Comparison of these values with the reference sugars assisted in identifying the crop sugars. Further confirmation was done by running two-way chromatograms. The quantitative estimation of the crop sugars was not attempted.

f. Dispatch of sugar solutions, whole human blood and blood-sugar mixture

Prior to the set up of the experiments for determining the acceptability of various liquid foods and their dispatch to the crop and/or midgut, several dissections were made to study parts of the alimentary canal in order to recognise more easily the location of test diet after its ingestion.

Fifteen freshly collected females of C.vittatus were dissected to study the structure of the crop and the nature of its contents (the latter to determine its attractiveness to other flies). Accordingly, smears of crop were made on the filter paper and exposed to females of Chrysops spp. including C.vittatus.

For the feeding experiments, the apparatus consisted of 1500 ml beakers with screen tops which contained feeding bottles, each containing a cotton wick thoroughly moistened with the test diet.

This permitted an uninterrupted supply of the chemical.

Citrated human blood was obtained from the Red Cross and also tested was the blood of a human volunteer to which heparin was added. The sugars used were sucrose at 0.5M and glucose at 0.25M and 0.5M. The latter was mixed with blood in various proportions (1:1, 1:2, 1:4, 1:6 v/v). The number of flies used for each set of experiment varied from 10-60.

### III OBSERVATIONS AND RESULTS

#### Part I Morphological studies

The general taxonomic features and distribution of the females of Chrysops vittatus, Tabanus lineola and Hybomitra lasio-phthalma (fig. 1) were described by Pechuman (1961).

The terminology followed in describing the head capsule and mouthparts is one that has been suggested by Peterson (1916) and Bromley (1926) and later modified by Snodgrass (1944) and Bonhag (1951).

##### a. Head capsule

The head capsule might be considered of importance to feeding only because it subtends the mouthparts. However, the outer capsule with the tentorium provides space and attachment for the muscles of the trophic appendages. Also, the eyes and the antennae are important sensory structures for vision and olfaction in host orientation from a distance. There are other structures on the head in the form of bristles, setae and hairs (fig. 2-7). In connection with feeding it seemed appropriate to provide some measurements of the head as well as of the mouthparts themselves.

The head is discoid being convex anteriorly (fig. 2-4) and slightly concave posteriorly (fig. 5-7). In relation to the rest of the body the head appears to be massive (see fig. 1). The

head capsule of C. vittatus excluding the mouthparts, measured 2.1 mm. in length from the vertex to the apex of the clypeus (Table I). In T. lineola and H. lasiophthalma the head is larger as is the whole body (fig. 1). The dichoptic compound eyes, composed of uniformly sized ommatidia, occupy the major portion of the tabanid head. The eyes present considerable variation with regard to their colour, presence or absence of stripes and hairs. In H. lasiophthalma and T. lineola, the eyes are greenish with reddish purple stripes; the eyes of C. vittatus are also somewhat striped or spotted. In H. lasiophthalma the eyes are hairy, whereas those of the other species are bare. Numerous hairs, setae and bristles project from various aspects of the head (fig. 2-4).

The paired antennae, in addition to the mouthparts are the only movable appendages connected with the head in tabanids (fig. 8-10). There are seven antennal segments: scape, pedicel and five flagomeres. In C. vittatus (fig. 8), the antennae are long and slender, each segment gradually diminishing in length till the last segment assumes a cone-like appearance at the tip. In T. lineola (fig. 9) and H. lasiophthalma (fig. 10) the antennal segments are broad and stouter. The third antennal segment, in both, forms a dorsal process and a slight concavity called an excision.

In the three tabanids studied, pegs and cones of various shapes and sizes lie scattered all over the surface of the antennal segment (fig. 8-10). Amongst these are groups of trichoid hairs that stand out prominently at the joints.

b. Mouthparts

In the females of C. vittatus, T. lineola and H. lasiophthalma, the mouthparts are produced into a well developed cylindrical proboscis, which extends downwards from the ventral aspect of the head carried vertically or horizontally in a backward direction (fig. 38-39).

From the complex of mouthparts, only the maxillary palps and labella are discernible, the rest lie concealed. The proboscis consists of three well-defined parts, rostrum, mediproboscis and distiproboscis, the last made up of spoon-shaped, fleshy structures - the labella. In the retracted proboscis of a fly, these paired structures lie folded against each other.

The paired mandibles and the maxillary galea are endowed with pointed ends, serrated edges, and marginal denticles. The apices of the palp bear tooth-like structures that have sharp ends. The unpaired hypopharynx includes a canal that is connected with the salivary duct.

The labrum-epipharynx of tabanids, is unpaired and forms the dorsal surface of the food canal. In addition, it provides cover for the remaining mouthparts that lie concealed in the labial groove.



The proximal portion of the labrum is broad and with the clypeus contributes to the formation of the roof of the cibarial pump (fig. 11-13). The inner aspect of this appendage bears a single row of microtrichae and of small-sized trichoid hairs (fig. 11-13). The broad base of the labrum gradually tapers distally into a blunt tip that has fimbriated margins. The labrum-epipharynx along with the hypopharynx (see below) enclose the cibarium. The inner surface of cibarium has a number of thin-walled hairs. The labrum is connected with the clypeus of the head. A suture called the clypeo-labral suture demarcates the boundary between the two. The labrum-epipharynx is smallest in T. lineola and longest in C. vittatus (Table I).

The mandibles in the three genera are strongly developed. They are paired, chitinous, sword-like structures suspending from the head capsule and having muscles that permit their movement in a transverse direction (fig. 14-16). The mandibles are serrated along the inner margin, and are broad at the base and sharply pointed at the tip. In all the three species they exhibit some differences in size. The mandibles are so arranged in the space (labial groove) provided by the labium that they press against the undersurface of the grooved labium and so contribute to the formation of the food canal.

The maxillae that lie behind the mandibles are paired, segmented structures having four parts in the three tabanid species:

the small cardo, the large stipes, the galea and the palpus. The palpi are smallest in C. vittatus and largest in H. lasiophthalma (Table I). The maxillae of the tabanids possess a variety of hairs and pegs (fig. 17-19).

The galea of the maxillary stylets is slender, blade-shaped, slightly flattened at the base and have a pointed tip. The recurved teeth occur all along the inner (posterior) face. The proximal end bears rows of minute projections (denticles) (see fig. 17-19). The galea present little variation in the extent of its development in the tabanids studied (Table I).

The unpaired hypopharynx shows little structural differences among the three tabanid studied (fig. 20-22). The base of the hypopharynx is broad and short, gradually broadening towards the middle and then tapering into a conical tip. The tip bears the well defined opening of the salivary duct. The caudal aspect exhibits a long internal groove (the salivary canal) running the length of the hypopharynx along the mid-line forming the canalicular structures that open to the tip (fig. 20-22). The proximal aspect of the canal continues into the head where it enlarges into the salivary pump before continuing as ducts to the two salivary glands. The size of the hypopharynx in the three genera shows little difference. (Table I).

The hypopharynx forms the ventral surface of the pharyngeal opening.

The unpaired labium is by far the longest of all the trophic appendages in the tabanids studied (Table I), and presents little variation in shape and size, although the mentum in C. vittatus is darker than in T. lineola and H. lasiophthalma. The labium is long and membraneous in C. vittatus (fig. 23) whereas in T. lineola (fig. 24) and H. lasiophthalma (fig. 25) it is short and broad. This is true for the labella as well which presents an intricate structure. The oral margin bears a system of interconnecting canals - the pseudotracheae (canaliculi) that occupy the entire length and width of the oral region. Minute chitinous projections stand out clearly on the canaliculi, in addition to transverse striations that give them the characteristic tracheated appearance (fig. 39). The aboral margin bears trichoid hairs, and pegs of various size and morphology. Variations in the number of trichoid hairs and pseudotracheae are observed amongst individuals and even between the left and right half of the labella (see Table IIb,c).

The individual canal of the pseudotracheal system joins with the fellow of the opposite side in such a manner to contribute to the formation of the left and right longitudinal canal that extends into the space between the mediproboscis and the distiproboscis (=labella) where the tip of the labrum rests.

In relation to the length of the head, the proboscis of tabanids studied exhibits variations. Thus, in C. vittatus, the proboscis is comparatively larger than T. lineola and H. lasiophthalma (see Table I).

Morphology and distribution of trichoid sensilla:

a. Antennal trichodes

The seven antennal segments of C. vittatus, T. lineola and H. lasiophthalma bears a variety of trichoid sensilla which stand out prominently at the joints. These are thin-walled hairs that arise from a socket with high rims. Many thick-walled, heavily pigmented bristles are also present, particularly on the scape and pedicel (fig. 8-10).

b. Labral trichodes

The inner aspect of the labral epipharynx bears a single row of microtrichia and of trichoid hairs (fig. 11-13). The labrum-epipharynx along with the hypopharynx encloses the cibarium, whose inner walls has a number of thin-walled hairs.

c. Maxillary trichodes

The maxillae of tabanids possess numerous hairs and pegs of varying shape and size (fig. 17-19). On the basis of length and morphological characters, three hair types are distinguished.

The III type of trichodes occupy the margins, arise from a high-rimmed socket and are setaceous, having an acute tip (fig. 17-19). The II type of setae are fewer in number and occupy the middle part of the maxilla, while the I type of hairs are limited to stipes. Of all the hair type, the type I are longest. There is little difference in the morphology of the three hair types.

d. Labellar trichodes

(i) Distribution and number

The labella of female C. vittatus presents an intricate structure. The aboral margin bore hairs and pegs of various morphological types, and the oral lobes have a system of interconnecting canaliculi.

The labellar trichodes appear to show some patterned arrangement which, however, does not seem to be very precise. On the basis of their size and shape the trichodes are designated as type A, B, C, D, E and F. The E and F type of hairs are present only on the anterior part of the labellum (fig. 30).

The A type of hairs are the longest on the aboral margin of the labella and have the largest basal diameter (Table IIa). When the proboscis is extended, they make the initial contact with the chemical, being facilitated in this by their large size and curvature (Table IIa and fig. 38).

The B and C type of hairs are located between A and D hairs, the latter being the smallest of all the aboral trichodes (Table IIa, fig. 30).

Pronounced individual variations are observed in the number and position of labellar trichodes (Table IIb and fig. 30). Differences are also observed in the right and left halves of the labella with respect to the total number of hairs (Table IIc). The counts of the individual hair types and also of pseudotracheae for 10 females of C.vittatus are presented in Table IIb. The grand total is given in Table IIc.

As is clear from Table IIa-c, the left and right labellum are not mirror images even when the sum of hairs on both sides is approximately same. For instance female No. 6 and 8 have just about the same number of trichodes (see Table IIc) on the left and the right labellum, but the number of particular hair type is different (Table IIb). The other 8 females show variation in the individual number of trichodes as well as the total number.

(ii) Measurements of trichodes

The data shown in Table IIa are from the measurements of a total of 6 hairs of each type for 10 females (6x10). Because of their position, length and morphology it is relatively easier to

distinguish between the trichodes type A,B,C and D. Their diagnostic features are as follows:-

Type A:- are longest of all the marginal hairs measuring on an average  $0.154\text{mm}$  ( $\pm 0.0174$ ). At its point of emergence the hair is wide ( $0.0053\text{mm}$   $\pm 0.00058$ ) but gradually tapers into a slightly pointed tip (width at the tip is  $0.00159 \pm 0.00038$ ). The number of these hairs varies from 10-20 in the left labellum and 10-18 in the right (range for 10 flies). The width of the socket is  $0.012\text{mm}$   $\pm 0.00066$ .

Type B:- differs from A in being medium-sized, curvaceous and in having a biluminate structure, one thick-walled and the other thin-walled. Also, the socket had high rims and the tip was obtuse. In methylene blue preparations, strands of nerve fibres are seen and can be traced up to the tip, where they to open through a pore to the exterior.

On the average it measures  $0.0989\text{mm}$  ( $\pm 0.0088$ ) in length.

The total number of B type of labellar trichodes varies from 8-15 in the left labellum and 9-14 in the right (range for 10 flies).

Type C:- The morphology of these trichodes is similar to type B, but they differ from them in being shorter and narrower, and in emerging from a narrower and shorter socket. (see Table Ia for details). The total number of these trichodes varies from 12-18 in the left labellum

and from 14-21 in the right.

Type D:- of all the aboral marginal hairs these are most numerous, varying from 25-44 in the left labellum and 18-45 in the right.

These hairs seem to be distributed along the margin of the labellum and are (see fig. 30) the smallest of all (Table IIa).

Type E and F:- These are present on the anterior aspect of the labellum (see fig. 30). The E type are fewer in number (9-29 in left and 8-27 in right) than the F. The latter is approximately half the length of E and also differs in other measurements (see Table IIa).

The oral margin of the labella bears an intricate system of interconnecting canals the pseudotracheae (canaliculi) that occupy the entire length and width of the oral region. Minute chitinous projections stand out clearly on the canaliculi, in addition to transverse striations that give them the characteristic tracheated appearance (fig. 39). The total number of canaliculi in C. vittatus varies (Table IIb).

e. Tarsal trichoid sensilla

The forelegs of C. vittatus, T. lineola and H. lasiophthalma have the same basic structure as other Diptera. The fore tarsi contain a large number of trichodes of various shapes and sizes. Of these the largest hairs stand out prominently and project outwards.



These appear to be similar in morphology to labellar trichodes of type A. In addition there are medium-sized trichoids which are biluminate and blunt-tipped. Numerous setae, pegs and cones lie scattered on the foretarsi. Scattered population of these structures also occur on the tibiae (see fig. 32-37).

PART II BEHAVIOURAL STUDIESa. Sugar feeding

Comparative studies on the mode of sugar feeding in females of Chrysops vittatus and Hybomitra lasiophthalma revealed differences as well as similarities. Some observations were also made on the feeding behaviour of females of Tabanus lineola. After release in the container and immediately upon settling down upon a surface both species raised their forelegs and tapped the substratum in a wide arc. In C.vittatus the proboscis in the non-feeding stage is partially retracted and folded back or carried vertically with the labellar lobes tightly pressed against each other (fig. 38), while in H.lasiophthalma it is always carried vertically (fig. 39). The palpation of the foreleg continued until they contacted the sugar. This resulted in an immediate lowering of the proboscis (fig. 38-39). The contact of the marginal hairs of the labellum with the sugar solution stimulated the spreading of the labellar lobes and sucking was initiated. The response of the flies that had first been fed ab lib. on sugar was weak. Flies with amputated foretarsi failed to respond, and even walked over the test chemical with the other two pairs of legs. The stages in the act of sugar feeding of a male of C.vittatus were similar to the females.

While searching for the food, adults of C.vittatus and H.lasiophthalma both raised the right and left forelegs alternately and tapped the substrate in a rhythmic fashion. The entire weight of the body during this operation was supported by meso- and meta-thoracic legs. The antennae assumed a straight position at right angles to the head and occasionally exhibited jerky movements. The maxillary palps were held at an angle to the labrum and the head, the other trophic appendages remained hidden in the labial groove and did not participate actively before or during the feeding on sugar. Usually, in C.vittatus, neither the head nor the thorax exhibited any movement during feeding. At this time the head was always in a lowered position between the forelegs, which were occasionally raised and rubbed against the labrum or the head itself.

In both H.lasiophthalma and C.vittatus, the preparatory (pre-feeding) activities viz; palpation of the forelegs, extension of the proboscis and the spreading of the labellar lobes were similar (fig. 38-39), but the subsequent stages showed differences. Thus in H.lasiophthalma the spread labellar lobes were pressed against the sugar to such an extent that a slight bend or flexure was visible from the lateral view, at the level of the sclerite between the labellum and the main body of the labium. The head gradually

lowered presumably to create more pressure on the labellar lobes accompanied by the lowering of the thorax. This continued until the thorax almost touched the sugar cube or the cotton pad as the case might be, and the head was correspondingly lowered. Sometimes the head and the body of the fly were elevated and repeatedly thrust on the sugar cube. Such a behaviour was not observed for C.vittatus. However, in both species accidental loss of contact with the food often resulted in a repetition of the entire act of pre-feeding behaviour. This was also observed when the fly endeavored to explore other parts of the container as a suitable locus for feeding.

No behavioural differences were observed between wild-caught and newly emerged flies. Attached flies extended their proboscis on tarsal stimulation and sucking was "triggered" when the marginal labellar hair contacted the acceptable diet. No observation could be made on the presumed mechanism of "liquefaction" of the dry sucrose or the dry crop smear because of the heavy pigmentation of the labella and its close application to the test chemical. However, pulsation of the head and proboscis indicated that food in the liquid state probably was sucked up. Flies that fed on 0.5M and 1.0M sucrose solution (containing methylene blue) dispatched the ingested diet to crop only (as evidenced by the presence of dye in the crop) (see section on dispatch of blood, sugar etc.).

b. Contact chemoreceptors of tabanids

(i) Loci

Females of C.vittatus, T.lineola and H.lasiophthalma were used to identify areas, parts, groups of hairs and specific hair types concerned with the perception of contact chemical stimuli. Precise determination of the hair types by testing with 1.0M sucrose was made with respect to the labellar receptors of C.vittatus.

Only the ventro-lateral surface of the foretarsi, and not the dorsal surface were shown to possess gustatory sensilla. Stimulation of these receptors stimulated an extension of the partly retracted proboscis. Only the first four segments of the foretarsi, when touched with sucrose solution, mediated a positive response, the last segment bearing the pulvillus and the claw elicited no response. With the midtarsi and hindtarsi a positive response was elicited only with a higher concentration of sucrose solution, namely 2.0M, and also with 3.0M and 4.0M.

In addition to the experiments with the cephalic and thoracic appendages, other parts of the body were tested as well but with negative results, namely, the wing base, ovipositor, and ventral surface of the thorax and abdomen.

Flies that were deprived of water responded to stimulation by water in a manner similar to 1.0M sucrose solution.

Of the four main types of (A,B,C and D) marginal labellar hairs only type B and C evoked extension of the proboscis and spreading of labellar lobes when stimulated by 1.0M sucrose solution. Type A and D did not react to sucrose stimulation, nor did the E and F type.

(ii) Ablation experiments

Ablation of the parts having the contact chemical sensilla was performed to see if this effected any change in the behaviour of the flies. The response after tarsal and labellar stimulation in flies, which had their antennae, palpi, mandibles, labrum, galea, meso- and meta-tarsi removed was similar to that in intact flies.

Extension of the proboscis was elicited when the fore-tarsi contacted 1.0M sucrose solution. However, tarsalectomy (fore-tarsi) resulted in complete lack of response, even though other parts of the legs were moistened with 1.0M sucrose solution or the flies walked over the test chemical.

Labellactomised flies showed no response to stimulation by sucrose.

(iii) Point of stimulation on the sensillum

Only the tip of the hair was observed to be sensitive to stimulation. Application of the drop of 1.0M sucrose solution along the length of the hair elicited no response. Single hair stimulation

in the case of either tarsi or labella resulted in a complete behavioural response, i.e., when the tarsal hair was stimulated, proboscis was extended and when the labellar hair was stimulated, the lobes opened as well, in an extended proboscis. The flies sucked the acceptable liquid when permitted to do so.

c. Physiological state of the flies used in behavioural studies

In the experiments on the sensitivity of the flies to different chemicals it was considered important to establish as much uniformity as possible in the test insect. This study of the feeding apparatus, behaviour and contact chemoreceptors was conducted on flies collected exclusively from Beverley Swamp, near Hamilton, Ontario, Canada. It was assumed that this restriction would ensure, to a considerable extent, the similarity of the flies with regard to their ecological niche (in terms of oviposition sites, larval and pupal habitat) and also, with regard to the emerged adults having access to similar plant and animal hosts. To determine the nutritional state and reproductive development of the species used in the present study, several hundred females of C.vittatus, T.lineola and H.lasiophthalma were dissected during various phases of this study. Glucose and less commonly fructose were the principal sugars present in the crop of wild-caught females of C.vittatus. Preliminary studies aimed at determining the longevity of wild-caught C.vittatus under labor-

atory conditions revealed that the flies had enough reserves to sustain life for sometime without further feeding, as those on water alone lived for 16 days (see Appendix II Table XIV), although the wild-caught flies fed readily on sucrose (Table IX). In all but one case the ovaries of wild-caught females of C. vittatus had undeveloped eggs.

The flies attacked humans in large numbers in the field and were presumably in a state of ovarian development that demanded a peak uptake of blood meal to meet the nutritional demands of egg-development.

Whether they were inseminated was not determined.

d. Sensitivity of tabanid contact chemoreceptors

(i) Tarsal gustatory sensilla

Studies on the sensitivity of tarsal contact chemoreceptors of female Chrysops vittatus to three principal nectar sugars (i.e., sucrose, glucose and fructose) revealed differences in acceptance threshold (as estimated by the frequency method) Table III. The flies also exhibited variations in the daily response to a particular molar concentration. Variations were also observed in the response of the flies from trial to trial. Of the three sugars tested, sucrose was the most effective and required a relatively lower concentration, than for glucose and fructose, to elicit a positive response. Glucose was more stimulating than fructose as shown by its threshold value.



However, the three sugars were similar in their effect since they evoked an increased percentage of positive response with increasing molar concentration (fig. 41).

The response of [redacted] flies during the entire period of experimentation was vigorous and reproducible.

In an earlier study using the ascending method of testing the sensitivity of tarsal taste sensilla of females of C.vittatus, Tabanus lineola and Hybomitra lasiophthalma was compared. Species differences were indicated. The order of sensitivity of tabanid flies to sucrose was C.vittatus > H.lasiophthalma > T.lineola (cf: Lall and Davies 1967).

(iia) Labellar contact chemoreceptors

Using the frequency method (sample and population) of estimating threshold, the sensitivity of labellar contact chemical sensilla of C.vittatus to eight sugars was determined. All the test sugars proved to be effective as feeding stimulants. The labellar acceptance threshold for sugars exhibited variation (Table IV, also Table XI [redacted] for a protocol of recording the [redacted] response of a fly in the 10-trial schedule). Thus, among monosaccharides, glucose was slightly more stimulating than fucose the latter was more effective than fructose. Of the three disaccharides tested (i.e., sucrose,

maltose and melibiose), sucrose proved to be a superior stimulus, followed by maltose and melibiose. Melezitose was more stimulating than raffinose among the trisaccharides tested (Table IV). Of all the eight sugars tested, sucrose and maltose had the lowest acceptance threshold (see Table IV and fig. 42-43 for trend of acceptance threshold).

The order of stimulative effectiveness of the sugars as determined, by sample and population frequency method, indicated an identical rank order.

Species differences were indicated in the labellar acceptance threshold of C.vittatus, T.lineola and H.lasiophthalma (as determined by the ascending method) (see Lall and Davies 1968).

The flies dissected after the completion of behavioural studies showed partly differentiated ovaries and moderately developed fat bodies.

(iib) Amino acids

Aqueous solutions of 15 amino acids with precisely determined pH were tested at 0.05M and 0.005M to test their effectiveness on the labella of C.vittatus. The results were largely negative (Table VI). Stimulation of the labella by 0.05M concentration generally resulted in a "quick" withdrawal of the proboscis from the

surface of the test chemical. When offered water the flies readily imbibed it.

The response of the flies to a select group of amino acids dissolved in 0.133M phosphate buffer (pH 7.4) showed some response. Only histidine, leucine, lysine and methionine induced some activity (Table VII). Tests with phosphate buffer alone were largely negative.

(iic) Nucleotides

Of the tests with ADP and ATP dissolved in phosphate buffer (0.133M - pH 7.4) at 0.05M and 0.005M, ATP induced some activity as compared to the control (Table VII).

Feeding experiments were conducted with these nucleotides (i.e., ADP and ATP as well as with AMP). Flies released in containers having the test diet showed little response towards them. The nucleotides were offered as solutions in distilled water, in phosphate buffer and in 0.15M NaCl. The results are shown in Table VIII.

e. Dispatch of whole blood, glucose, sucrose and blood-sugar mixture

Earlier studies on the nature of the crop fluid in wild-caught females of C.vittatus revealed that the crop contains only sugars, although the flies are known to feed on blood. Dissected flies showed fresh and partly digested blood meal in the midgut. This led to the assumption that the flies may have sugars and the blood meal in separate section of the midgut. Experiments were,

therefore performed with whole human blood, sucrose, glucose and blood-sugar mixture to see their transport immediately after ingestion.

Experimental flies were observed to "spend" much of their time in random flying and/or remaining on the filter paper which "carpeted" the bottom of the beaker. The approach to the feeding bottle was either by a single "hop" or by a slow "walk". Flies, that fed, extended their proboscis when the foretarsi established contact with the cotton wick moistened with test diet. Sucrose (at 0.5M) and glucose (at 0.25M) were always passed to the crop and the percentage of feeding was high (Table IX). However, the flies fed only on whole blood when it was pre-warmed to 37°C, and then only partially. Of the 60 flies tested for the whole human blood, 18 partially fed on it and passed it to the midgut.

The observations that sugars were more attractive to the flies than the whole blood, led us to devise further experiments to see if the addition of sugar would enhance the attractiveness and acceptability of the blood. For this purpose, glucose was chosen as it occurs in the plant and animal hosts of the tabanids. With 0.5M glucose as dilutant a 1:1 (v/v) whole blood:glucose mixture fed to 60 flies increased the percentage of feeding (78.3%). The mixture was transported mainly to crop. However, with 0.25M glucose as the dilutant the picture was different. Several dilutions of

this mixture were offered to the flies (Table IX). As is clear from the table, the percentage of feeding was considerably increased with glucose in the mixture as compared to the whole blood alone. The ingested diet was dispatched to the mid-gut. In only one out of nine flies did the mixture (1:6 v/v) go to the crop.

Of the seven controls out of 30 that fed on water, the crop was the locus of ingested fluid (Table IX).

f. Angiosperm flora of Beverley Swamp

The tabanid collecting area had a variety of flowering plants which were in full bloom during June, July and earlier part of August. Some of these, e.g., Daucus carota, Solidago spp. and Potentilla spp. formed thick beds and were spread in large areas. Although actual feeding was not observed, some Chrysops spp. (including C.vittatus) were collected by sweeping an insect net on the plants. A single male of C.vittatus was also collected in this manner. Males of Tabanus and Hybomitra spp. were generally observed hovering over these plants and were netted.

The flowers were of diverse colors, occupying different loci on the plant (inflorescence of many types), and had their corolla variously modified. Shuel (1968 in litt.) suggested that nectar composition of these plants also varied (see Appendix II, Table X). Some

had glucose-dominated nectar, while others had equal volumes of sucrose, glucose and fructose. (also for further observations on nectar composition of plants see Wykes 1952 and Percival 1961).

g. Analysis of crop fluid

The gut of female Chrysops vittatus is sub-divided into several parts, namely foregut, midgut and hindgut. The crop arises as a diverticulum of the foregut and is connected with the latter by a long, narrow duct. In the wild-caught females the crop was generally filled with a hyaline or pale yellow fluid containing several air bubbles. The crop consisted of two unequal lobes which had several marginal elevations. When full it occupied a considerable space in the body and extended from the thorax into the abdomen. However, when empty, the crop, with collapsed walls, lay as a mass of crumpled tissue in the thorax.

Crop fluid (wet as well as dried) of C.vittatus was attractive to other Chrysops spp., including females of C.vittatus. The flies vigorously palpated their foretarsi on filter paper impregnated with the crop fluid and the proboscis was extended. The flies fed avidly on crop contents when dry as well as wet. During feeding the labella were closely applied to the impregnated filter paper and this prevented observations on the mechanism of liquefaction of the dry material. However, pulsatory movements of the

head and the body indicated that the flies were feeding.

Paper-partition chromatography of the crop fluid of wild-caught females of C.vittatus revealed that glucose and fructose were the only sugars present, the former in larger quantities as evidenced by the intensity of spots (fig. 40). There was no trace of other sugars.

h. Longevity of wild-caught tabanids

Of the two sugars tested i.e., dry sucrose and 1.0M glucose, the flies lived longest on the former (25 days). Distilled water alone sustained life for 16 days (Appendix II Table XIII). However, lacking water and sugar all flies died within 24 h. ( $LD_{100}$ ).

IV DISCUSSIONPart I      Morphological studies(i) Comparative studies of the head capsule and mouthpartsa. Head Capsule

Comparative studies on the head capsule of the three female tabanids, Chrysops vittatus, Tabanus lineola and Hybomitra lasiophthalma revealed basic morphological similarities (fig. 2-7). However, the length and width of the head in T.lineola and H.lasiophthalma was more than that of C.vittatus (Table I).

The heavy head of T.lineola and H.lasiophthalma, possibly compensates for the disadvantages accrued because of a short and broad labium (see Table I), that restricts the flies to feeding on certain types of plant and animal hosts and not on others.

b. Mouthparts

In the present study the structure of the mouthparts of representatives of three female tabanid genera was compared, emphasizing C.vittatus, T.lineola and H.lasiophthalma. These had mouthparts adapted for cutting the skin and lacerating the blood vessels of the host and for sucking the exudates from the wound. It is likely that the flies suck exudates from plant hosts in a manner somewhat similar to the feeding on blood of vertebrates. This should



happen often in nature, for the female tabanids are known to feed from plant sources and the males entirely subsist on such a diet, as they lack a cutting (=piercing) apparatus (Downes 1958 and personal observations of the author on the tabanids in McMaster University collection). That only the female has feeding structures attuned to a haematophagous mode of life is not limited to tabanids. In all blood-sucking Nematocera and Brachycera only the female sucks blood, although in certain cyclorrhaphous flies, e.g., Stomoxys, Lyperosia (=Haematobia) and Glossina, the males as well as the females, pierce the vertebrate skin and suck blood (cf: Snodgrass 1944).

The acceptance of the term "haematophagous" is rather unfortunate for the blood-sucking Diptera, such as Tabanidae, Culicidae, Ceratopogonidae and Simuliidae, as they are known to feed on flower nectar (of various types; cf: Percival 1961), exudates of injured plants, ripe fruits and in many cases their importance in various overt activities of the insect and in the metabolic processes has been demonstrated (see Hocking 1953 and Downes 1958 for bibliography). Further studies on sugar feeding, host flower preference etc., may eventually lead us to accept the idea that these insects are indeed polyphagous in nature (sensu stricto).

In the female tabanids, the mouthparts are produced into a well-developed proboscis, which descends from the lower

aspect of the head, partially retracted and held vertically or horizontally in a backward direction (fig. 2-4). The proboscis was extended when the tarsal and/or labellar hairs were stimulated by such acceptable substance as sucrose (fig. 38-39).

The total length of the proboscis in C. vittatus, almost equal the height of the head, but in T. lineola and H. lasiophthalma it is only a little more than half the height of the head (Table I). Unusually long proboscis were observed in Indian and Russian tabanids belonging to the genus Pangonia (Mitter 1917, Olsufjev 1937). In other tabanids, e.g., Chrysops, Tabanus, Hybomitra, Haematopota, Goniops and Atylotus, the proboscis is relatively shorter in comparison to Pangonia (Bonhag 1951, Lavoipierre 1958 and author's observation on tabanid collection of McMaster University).

It seems possible that some relationship exists between the shape and size of the proboscis and the host(s) on which the flies may feed in nature. In C. vittatus, the labium is long, slender and broad and the same was true of the labella. It is suggested that a long feeding apparatus such as the one possessed by C. vittatus and other members of the sub-family Chrysopinae (author's observations) is probably suited to feed on flowers with deep-seated nectaries as well as on animals with thick fur. Thus, the flies may be able to

feed on flowers with modified corollas (as found in Labiatae, Leguminosae) as well as on ones with little or no modifications (e.g., Umbelliferae and Compositae). These families were well represented in the tabanid collecting area (see Appendix II Table X). In addition, the flies may feed on flowers with open nectaries (also on extrafloral sources) and on vertebrates with little or no fur. This obviously would affect their distribution, a fact supported by collection of this fly species by the author from diverse localities. These observations are further supported by collection data of Pechuman (1961) and other North American workers. On the other hand, a short, broad labium as possessed by females of T. lineola and H. lasiophthalma may restrict the flies to feeding only on certain types of plant and animal hosts, thus affecting their range. In fact, I always netted and trapped several hundred times more Chrysops spp. than Tabanus or Hybomitra during my three years of tabanid collection in various localities including Beverl y Swamp.

Oldroyd (1966: in litt.) suggested that the species of Chrysops are primitive owing to the retention of the long proboscis that was adapted to feed on flower nectar. Blood feeding was considered to have arisen secondarily. But Downes (1958) and Fairchild (1967: in litt.) disagreed. The last author considers Chrysopinae and Tabaninae to be more closely related to each other than to

Pangoninae. The flower feeding habit is probably shared, to some extent by all Tabanidae, but has become an exclusive source of nutrients in only a few groups. The presence of mandibles was considered to be a primitive character, as Diptera are believed to be descendants of mandibulate ancestors, and they have been lost or degenerate in a few groups which are either strictly nectar feeders or do not feed at all as adults. Blood feeding, according to Fairchild (1966: in litt.), is the primitive condition in the family Tabanidae. However, there is little doubt about the importance of proboscis length in relation to feeding on host(s). Its importance has also been stressed for Simuliidae (Wenk 1960) and Tachinidae (West 1953) in relation to selection of flowers.

The piercing fascicles in females of C.vittatus, T.lineola and H.lasiophthalma presented basic similarities. Each consists of paired mandible, maxillary galeae. These serve as apparati for piercing the host skin and in lacerating the blood vessels. The row of denticles on each galea likely serve as "holdfast" structures in facilitating the cutting action of the mandibles and in the insertion of other trophic appendages into the deeper layers of the host tissues. Piercing structures of varying lengths have been recorded in Chrysops univittatus (Matheson 1950), Tabanus spp. (Cameron 1942), Corizoneura (=Pangonia) longirostris (Mitter 1917). The row of denticles on the

galea as described in the present study were considered to be sensory structures by Bromley (1926) for the females of Tabanus atratus and as piercing structures by Bonhag (1951) for the females of Tabanus sulcifrons. In Chrysops silacea Aust. they were shown to be piercing structures by histological studies (cf: Gordon and Crewe 1953).

The labrum-epipharynx and hypopharynx are the only other unpaired stylets of the tabanids studied (in addition to labium), the former serving as a protective cover for the mouthparts that lie concealed in the labial "gutter", and the latter carrying the secretion of the salivary glands, probably with anti-coagulant for ensuring a free flow of blood. Some generic size differences were noticed in the tabanid fascicles (Table I) although their shape was similar (fig. 14-16). An elongated labrum and grooved hypopharynx have been described in other tabanids, e.g., Pangonia (Mitter 1917), Tabanus sulcifrons (Bonhag 1951). Gordon and Crewe (1953) demonstrated that the labrum of Chrysops silacea is the only trophic appendage that is involved in actual sucking of blood from the vertebrate host. This has been demonstrated for other tabanids as well (cf: Dickerson and Lavoipierre 1959).

The labium of the tabanids showed some differences in shape and size (Table I). In the non-feeding stages the labella remain closed and the labium itself lies retracted under the head

(fig. 38-39). Interestingly enough the labium of other Diptera of diverse feeding habits shows a somewhat similar structure (cf: Graham-Smith 1930, Snodgrass 1944, West 1953 and Wenk 1960).

The oral margin of the labella in the three tabanids studied had an intricate system of canaliculi; which probably serves for "mopping up" exudates and is also capable of sucking. A longitudinal section of the labium of wild-caught female Chrysops vittatus (fig. 31) showed the presence of what appeared to be nucleated blood cells. These may represent the participation of labella in the sucking of blood. However, it is not certain whether the labella enters the wound made by the piercing stylet or that it is used to "mop up" the exuding blood from the host skin. Fallis (1967: in litt.) suggested that these might be avian, reptilian or amphibian blood erythrocytes, but certainly not mammalian. Controversy still prevails on the role of tabanid labium in the feeding behaviour of tabanids. Thus, Snodgrass (1944) conceded that females of Tabanus atratus collected nutrients by labellar channels and convey it to the food canal between the labrum and the mandibles. Other workers believed that the labrum alone functions as a sucking apparatus and that labium is used only to "lick" up exudates of plants or to "slake" the thirst (Mitter 1917, Olsufjev 1937, Bonhag 1951).

In blood-sucking cyclorrhaphous flies, namely Stomoxys and Glossina, the labella are greatly reduced and the prestomial teeth of the labella are used for piercing the host skin (Snodgrass 1944). In Musca cassirostris the prestomial teeth are so large that they are capable of "scraping" down to the blood vessels. The labella are, however, unable to pierce (Crampton 1942). Snodgrass (1944) classified the piercing mouthparts of Diptera into two categories: one in which the mandibles and maxillary stylets were the principal piercing organs (called styletovulnerant type) and the other in which the labium was the piercing organ (called labiovulnerant). Tabanidae thus fall in the category of styletovulnerant type of mouthparts.

(ii) Loci, structure and function of contact chemoreceptors

The presence of contact chemical sensilla in insects is of definite value in detecting acceptable chemicals, deleterious substances and they play a critical role in a variety of activities relating to feeding, courtship, and in the selection of mates and oviposition sites (cf: Dethier 1963, Hodgson 1964 for references). In the present study the females of Tabanus lineola, Hybomitra lasio-phthalma and Chrysops vittatus were observed to possess taste sensilla only on the ventro-lateral surface of the foretarsi and the aboral margin of the labella.

(a) Tarsal contact chemoreceptors

of female tabanids agree in their morphology with the similar sensilla described for the foretarsi of Stomoxys calcitrans (Adams 1961) and Phormia regina (Dethier 1954). In the present study they were shown to be important in monitoring the initial stages of feeding namely, probing the substrate for acceptable chemical(s) and in "triggering" extension of proboscis, on contact with sucrose (dry and wet). Evidently these sensilla are involved in sending "appropriate signals" indicating the definite loci of acceptable food on the substratum, after odour had directed the insect from afar. This may be assisted by other stimuli, such as, humidity, temperature and touch. Although tarsal taste sensilla have been reported in several Diptera and their function in various overt activities emphasized, some species are reported not to have them, e.g., Glossina spp. (Dethier 1954), Rhagoletis spp. (Middlekauf 1941). It is probable that in these insects other sensory mechanisms control the initial stages of feeding (i.e., exploration, detection of chemicals and extension of feeding apparatus) thus bringing other receptor areas in contact with stimulating chemical for further qualitative and quantitative "screening").

(b) Labellar contact chemoreceptors



(i) Aboral trichodes

of female C.vittatus are of four main types (i.e., A,B, C and D) that vary in number not only amongst individuals, but also in the right and left labellum. Females of T.lineola and H.lasiophthalma also possess taste sensilla on the aboral aspect of the labellum.

For the sake of brevity the labellar trichodes of C.vittatus are compared with Phormia regina (Evans and Mellon 1962, Wilczek 1967), as information on others is inconclusive or at best perfunctory. Interestingly enough the labellar trichodes of C.vittatus show some degree of patterned arrangement as in P.regina (Wilczek 1967). However, the hair type and their arrangement in Calliphora erythrocephala L. (Peters 1963) is without any specific arrangement.

The total number, size and variability of the labellar trichodes in female C.vittatus (see Table II a,b,c) differs considerably from other dipteran species, e.g., Calliphora erythrocephala (Peters 1963) and Phormia regina (Wilczek 1967). Information on other dipterans is lacking. It is possible that the differential sensitivity of various insect species to chemicals may be related to the number of taste sensilla present. The gustatory receptors may differ from each other in their thresholds at a given time (under a certain

physiological condition). Thus a single taste sensillum may have a lower threshold than the group because of the interactions of the receptor sensitivity (see Dethier 1955).

(ii) Site of perception on contact chemical sensilla

In the three tabanids studied the tip of the medium-sized labellar and tarsal hair was the site of perception of stimuli. Although few insects have been studied to determine this point for obtaining a generalised picture, Dethier (1955) showed that amputation of hair tip of Phormia regina, resulted in a permanent loss of responsiveness of the hair to sugar and water. It has been suggested that the tip of the hair has either a cuticular covering that permits penetration of stimulating molecules or else the nerve fibres end blindly or are open at the hair tip thus communicating with the external environment (cf: Adams 1961).

(iii) Oral canalicular system

The oral margin of the female C.vittatus, T.lineola and H.lasiophthalma consists of interconnecting system of pseudotracheae (fig. 23-25). The number of these in C.vittatus was 22 (average of 10 flies) with variation in the left and right labellum and amongst individuals (see Table IIb). Such variations were observed in Calliphora:28-34 (average 30) (Peters 1930, Graham-Smith 1930) and in Phormia:25-28 (average not given) (Wilczek 1967).

By comparison Chrysops vittatus has lesser number of canaliculi. It seems that the number of these may have some relationship to the volume of nutrients imbibed, which evidently is related to the size of the animal and its gut.

(c) Other trichoid sensilla

(i) Antennal trichodes

were observed in C.vittatus, T.lineola and H.lasiophthalma, but behavioural studies negated their being functional as taste sensilla. On the basis of his morphological and cytological study of the antennal trichodes of Tabanus quinquevittatus Wiedeman, Scudder (1953) concluded that these were indeed gustatory. In all the other Diptera studied thus far there is no evidence for the presence of antennal contact chemoreceptors. It is quite possible that these are in fact olfactory rather than contact chemical sensilla. However, antennal contact chemoreceptors have been demonstrated in other insects; e.g., Rhagium spp. and the cricket, Liogryllus campestris (Kunze 1933), Apis mellifera (Marshall 1935) and certain butterflies (Weisman 1962).

(ii) Labral and maxillary trichodes

Although stimulation of various parts of the labrum-epipharynx and maxilla elicited no response in the tabanids studied,

in other insects, e.g., mosquito spp. (Christophers 1960, von Gernet et al 1966) and Simulium spp. (Yang 1968) labral chemosensory spines have been described and their importance in the detection and dispatch of ingested food, indicated, although Hosoi (1959) and Owen (1963) have questioned their role in mosquitoes.

d. Interrelationship of taste sensilla in relation to feeding behaviour

Tabanids agree with other Diptera (and some insects) with regard to the loci of taste sensilla. Thus, the tarsi (in main fore-tarsi) and labella (oral and aboral marginal receptors) are the principal site of gustatory sensilla that are concerned with monitoring preliminary aspects of feeding (Lall and Davies 1967, 1968, Dethier 1955, Adams 1961). In insects other than Diptera (e.g., Lepidoptera, Hymenoptera) antennal contact chemical sensilla (in addition to tarsal and ones present on the mouthparts) have been described (see Frings and Frings 1949 and Weisman 1962 for references). The cibarial and foregut taste sensilla (as reported in certain Diptera see Day 1952, Gelperin 1966a,b) form another class of receptors that are probably concerned with qualitative "screening" and quantitative aspects of incoming fluid. In synergism with neural, humoral or endocrinal factor(s) these sensilla may be involved in threshold regulation, crop emptying and other related aspects of feeding (see Dethier 1963).

The presence of taste sensilla at several loci in tabanids is probably indicative of the potential of "receptor reservoir" that may be useful under a given situation or could effectively take over the role of part(s) that are accidentally severed. Such hazards may not be uncommon in nature. This may be true for other insects as well.

An integrated hierarchial system of taste sensilla involving those on external appendages (e.g., tarsi, labella, henceforth called exteroceptors) and the ones internal (e.g., cibarial, foregut called interoceptors) is of obvious survival and adaptive value. For, while the exteroceptors may detect and discriminate between acceptable and toxic chemicals, the interoceptors may precisely control and regulate the quality and quantity of the incoming acceptable fluid, corresponding to the physiological state of the insect and possibly commensurate with the nutritional requirements.

A distinction should be made between various phases of feeding, e.g., pre-ingestion, ingestion and post-ingestion. It is conceivable that the sensilla might function differently under these phases.

(iii) Indices of stimulation

The presence of extensible mouthparts (=haustellate) in Diptera and other insects, such as Lepidoptera and Hemiptera affords a satisfactory means of judging the positive or negative response of

the insect to test chemicals. Stimulation of taste sensilla situated on certain appendages of the body generally evokes these responses in these insects (cf: Frings and Frings 1949). These behavioural manifestations serve as useful parameters in determining the stimulative effectiveness and thresholds of test chemicals. In the present study, females of C.vittatus, T.lineola and H.lasiophthalma extended their proboscis when a single or group of tarsal gustatory sensilla were stimulated by 1.0M sucrose solution. Likewise, positive stimulation of a labellar sensillum or group of them evoked an extension of the proboscis and opening of the labellar lobes and sucking was initiated if the flies were allowed to "drink". The former was taken as the index of positive tarsal stimulation and the latter of labellar stimulation.

Although many haematophagous and non-blood sucking Diptera possess tarsal and labellar taste sensilla, and respond similarly as tabanids do, there are few that lack tarsal contact chemoreceptors, e.g., Rhagoletis spp. (Middlekauf 1941); Glossina spp. (Dethier 1954).

Proboscis activity (i.e., extension or retraction) has been used as an index of stimulation in several dipteran species, e.g., Tabanus sulcifrons (Frings and O'Neal 1946), Phormia regina (Minnich 1926a,b, Dethier 1955), Stomoxys calcitrans (Adams 1961),

Musca domestica (Deonier and Richardson 1935), certain mosquito species (Feir et al 1961, Salama 1966b), and other haustellate insects (see Frings and Frings 1946: for a review). In the mandibulate insects, movement of the mouthparts (sideways) is generally taken as the index of stimulation, e.g., in Periplaneta americana (Frings 1946).

Some workers have used duration of feeding and measurements of crop loads as the **criterion** for evaluating the acceptance and rejection threshold of test chemicals (cf: Dethier and Chadwick 1948a). However, it seems that such a **criterion** is meaningful only when the threshold of gorging or related phenomena is being investigated, for while a certain molar concentration of the test chemical may be detected by the tarsal and/or labellar taste sensilla, it is quite likely that it may not induce gorging owing to its being at a subliminal level.

## Part II Behavioural Studies

(i) Feeding behaviour of female tabanids

Tabanids are often incriminated as vectors of parasites (Gordon and Crewe 1953) and pathogens (cf: Anthony 1962). Although blood is the principal source of nutrients for the development of eggs (Olsufjev 1937), the flies do indeed imbibe carbohydrates from flower nectar of angiospermic plants (Hocking 1953, Downes 1958). The energy thus obtained is used for a variety of behavioural activities, e.g., flight feeding, mating and oviposition and metabolic processes such as oogenesis (cf: Hocking 1953). Despite the considerable importance of carbohydrates in the life processes of tabanids, little or at best perfunctory attention has been given to the tabanid behaviour in selecting the host, procuring their sugar meal and the manner in which it is imbibed.

Many adult Diptera and insects show a strong positive response to sucrose and to several other sugars that occur in flower nectar (Percival 1961). According to all published reports tabanids ingest food that is liquid (nectar and/or blood) and there is no reference in the literature to their being able to feed on semi-liquid or dry acceptable diet (Fairchild 1967:in litt.)

In the present study a member of the sub-family



Chrysopinae:Chrysops vittatus and one of the sub-family:Tabaninae:  
Hybomitra lasiophthalma both fed on sucrose solution (1.0M) and the  
sugar cube, although exhibiting structural differences in the mode  
of applying the proboscis to the carbohydrate diet in preparation  
to sucking (fig. 38-39). The pre-feeding behaviour of the two  
tabanids studied was largely mediated by the forelegs and the taste  
sensilla present on them. Positive stimulation of the foretarsal  
gustatory sensilla evoked extension of the proboscis bringing the  
marginal labellar hairs in contact with the sugar solution which  
triggered opening of the labellar lobes and sucking ensued when  
permitted. Actual feeding involved close application of the labella  
and probably action of the food pump that directed the ingested food  
to the alimentary canal. The essential stages in the tabanid feeding  
on sugar resemble the ones observed for other Diptera, e.g., Chrysops  
silacea (Gordon and Crewe 1953), Musca domestica (Deonier and  
Richardson 1938), blow-flies (Minnich 1926a,b, West 1953). However,  
not all Diptera feed on sugars, e.g., Glossina spp. (Glasgow 1963).

The mechanism of "liquefaction" of dry acceptable nutrients  
has been little understood. In the tabanids studied this could not be  
ascertained owing to the close application of the labella, but it  
seems possible that salivary secretion(s) assist in "liquefaction"

of dry sugar thus facilitating its imbibition. Certain mosquito spp. (Eliason 1963) and the house-fly, Musca domestica (Ascher and Hirsch 1965) salivate sugar before sucking.

By comparing our observations on sugar feeding in tabanids with those of blood sucking, as described by other workers (cf: Dickerson and Lavoipierre 1959), it seems possible to suggest that blood feeding may be similar to sugar feeding once laceration of the host skin and blood vessels has occurred and blood flows. Tabanids are generally regarded as "pool" blood feeders (=telmophage of Lavoipierre 1965) (cf: Gordon and Crewe 1953, Oldroyd 1966 in litt.).

(ii) General discussion of the methods for estimating thresholds of chemicals in insects

In studies concerning the feeding behaviour of insects, the use of a satisfactory method for estimating thresholds of test chemicals is a prime requisite. Other factors of some importance which affect the results are: breeding conditions, maintenance of the adult, nutritional background of the test insect, age, sex, reproductive development and laboratory conditions during the tests (cf: Dethier and Chadwick 1948a).

In insects until now, the chosen methods for assessing the stimulative effectiveness and thresholds of chemicals have been:-

- a. Tests in which individual insects in a sample population of 200 or more are offered molar concentrations of the test chemical in an ascending order (i.e., from subliminal to supraliminal) until a response is obtained. This method has been extensively used by many workers (Minnich 1921, 1926, Dethier 1955, Lall and Davies 1967, 1968).
- b. Each insect is presented with molar concentrations of the test chemical in a descending order until an appropriate response has been mediated. An extension of this has been in terms of using both a and b (cf: Marshall 1935, Dethier 1952).
- c. The insects are randomly sampled, i.e., a different group is tested at each concentration and note taken of the percentage of each class which responds (Dethier 1952).

Several authors have undertaken the onerous task to compare the above methods for determining the thresholds and of asserting the suitability of the method that they used in their studies (cf: reviews by Marshall 1935, von Frisch 1934, Dethier 1952). Evans and Barton-Browne (1960) devised a method of working with smaller populations of insects and considered it suitable, as a replacement for the method (a - c) discussed above, only when a comparison of two or more thresholds on the same fly is a part of the experimental design. It was also considered to be appropriate for a group of flies where

sensitivities of two or more compounds were to be compared. However, in this method as in others, the molar concentration of the test chemicals were presented in a series, i.e., ascending or descending. The magnitude of the values, thus obtained depended upon the order in which the stimuli were presented. Time relations were of considerable importance. The data of various workers showed that response to chemicals was greatly affected by previous stimulation.

The methods discussed above have a number of inbuilt biases, namely, series effect, conditioning, habituation, anticipation and perseverance of excitatory and/or inhibitory state as the case may be. To further clarify these points the following observations of Dethier, Solomon and Turner (1965) are pertinent: "A water-satiated blow-fly, Phormia regina, does not extend its proboscis when water touches a labellar hair. However, if another labellar hair is stimulated by sucrose directly prior to stimulation, water stimulation alone causes proboscis extension. This induced responsiveness to water reflects a perseverating central nervous excitatory state (CES) which decays slowly in time, its intensity and decay rate are a function of concentration of sucrose stimulation and degree of food deprivation". It is obvious that the methods (a - c) of estimating thresholds have biases that cannot be eliminated satisfactorily.

In reviewing the literature on various methods of determining thresholds, notice was taken of the "frequency" method that has been used by mammalian behaviourists and physiologists (see Materials and Methods and Woodworth and Schlosberg 1938 for details). Offering molar **concentrations** of test chemicals in a random order dismisses the interference of the factors outlined above and assists in getting an unbiased and more precise order of effectiveness and thresholds of test chemicals. In the present study a comparison was made between the ascending method and the frequency methods (sample and population methods) with respect to sucrose and between the two frequency methods with respect to five principal sugars that occur in flower nectar on which tabanids feed in nature or which are otherwise of interest.

(iii) Sensitivity of the tabanid contact chemoreceptors to sugars and other phagostimulants

The comparative approach in research on contact chemoreception has received limited use owing to the erroneous but sustained belief that stimuli were all important and the kind of organism was rather secondary. This led to the inference that all organisms respond in an identical manner when exposed to the same chemical (cf: Kare and Ficken 1963). That this is invalid has been demonstrated

As sucrose is the principal nectar sugar on which tabanids feed in nature and also as this has been used by various workers, for the sake of brevity the results obtained with Chrysops vittatus are compared with other Diptera. The tarsal acceptance threshold of female C. vittatus as determined by the frequency method was 0.0677M ( $\pm$  0.0597). Using the ascending method of estimating threshold it was computed to be 0.0125 - 0.0625M (Lall and Davies 1967). These results agree closely and this gives added confidence in the tarsal acceptance threshold of this fly for sucrose. Also the tarsal acceptance threshold of Tabanus sulcifrons was 0.063M (Frings and O'Neal 1946). However, when these tabanid thresholds are compared with other Diptera, sharp species differences are reflected. Thus in Culiseta inornata was 0.0135M (Feir et al 1961) and of Phormia regina was 0.019M (Dethier and Chadwick 1948). The lowest threshold for any single hair (taste sensillum) of P. regina was 0.00001M. Hassett et al (1950) gave 0.098M as the tarsal acceptance threshold for sucrose in P. regina. This is at considerable variance with the value obtained by Dethier and Chadwick (1948) for the same fly species. By comparison, P. regina (Dethier and Chadwick 1948) is the most sensitive of all to sucrose.

The order of stimulative effectiveness of the three sugars

tested in female C.vittatus was sucrose > glucose > fructose. This is at variance with other dipterans. Thus, the order of stimulative effectiveness for sugars in Calliphora erythrocephala was: sucrose > fructose > glucose (Minnich 1926a), for Aedes aegypti, Sarcophaga bullata Parker and Musca domestica it was: sucrose > fructose > glucose (Galun and Fraenkel 1957) and in the blow-fly, Phormia regina it was: sucrose > fructose > glucose (Dethier 1956). Although sucrose appears to be the most effective stimulant of all the sugars tested for the tarsi of C.vittatus and other dipterans, the former differs from others in the fact that glucose is more stimulating than fructose.

The greater stimulative effectiveness of glucose for the foretarsi of Chrysops vittatus seems to be in conformity with our other results. For, glucose was the predominant sugar in the crop fluid of wild-caught female C.vittatus, the nectar content of the flowers collected in the tabanid area were rich in glucose (Shuel 1968: in litt. also see Percival 1961 and Wykes 1952), glucose is the predominant sugar in the mammalian blood on which the flies feed in nature and also that glucose sustained considerable longevity under laboratory conditions (see Appendix II Table XIV).

It seems possible to suggest that the greater effectiveness of glucose over fructose may be due to the feeding habits of C.vittatus (the flies may show preference for flowers rich in glucose and may

selectively feed on them), as well as the fact that other sources of nutrients (e.g., juices of ripe fruits, vertebrate blood) are also rich in glucose. The sensory system of the insect may have been adapted to feeding on flowers containing a particular type of nectar composition during the long process of evolution. Further, the chemical structure and configuration of glucose is similar to sugars that are highly stimulating (cf: Dethier 1955, Evans 1963).

b. Sensitivity of labellar contact chemoreceptors

(i) Sugars

Using the sample and population frequency method, the acceptance threshold of eight sugars (that occur in flower nectar or are otherwise of interest) for the labellar taste sensilla of female Chrysops vittatus was determined. Sucrose proved most effective of all and had the minimum acceptance threshold followed by maltose (Table IV). Since sucrose has been the chosen sugar of many workers, for the sake of brevity the results obtained with C. vittatus are compared with other Diptera. In C. vittatus the mean labellar acceptance threshold was  $0.0067 \pm 0.00372$ . The labellar acceptance threshold of the same fly species as computed by the ascending method was between 0.0025 - 0.005M (Lall and Davies 1968). Species variations are indicated in the acceptance response to sucrose in which either



effectiveness of two of the monosaccharides (i.e., glucose and fructose) for the labella is the same as that for the foretarsi. Sucrose was the superior stimulus of all the sugars tested for the labella as it was for the foretarsi.

The order of stimulative effectiveness of the five sugars as determined by the population frequency method was similar to the one determined by the sample method (see Table V for thresholds).

Various other methods (e.g., ascending, descending, crop loads: for a review see Dethier and Chadwick 1948) have been used for determining the stimulative effectiveness of sugars for the labella of Diptera. Thus, selecting only the sugars used in the present study, the rank order of sugars for the oral lobes (=labella) of Calliphora vomitoria was: saccharose (=sucrose) = ca maltose > glucose (Minnich 1931)., for Culex pipiens var. pallens it was: sucrose = maltose > glucose (Hosoi 1959), for Phormia regina it was: mono-  
saccharides: fructose = fucose > glucose; disaccharides: sucrose = maltose (Haslinger 1935) and for Aedes aegypti it was: monosaccharides: D-fructose > L-fucose and disaccharides: sucrose > maltose > melibiose and trisaccharides: melezitose > raffinose (Salama 1966b).

A comparison of the above results with Chrysops vittatus indicates several species differences. Thus, in C.vittatus glucose is more stimulating than fucose and both are more than fructose among

the monosaccharides (Table IV). Species differences are also indicated in the order of effectiveness of sucrose and maltose and also in the thresholds for sugars (cf: Salama 1966b, Hosoi 1959, Dethier 1963).

Despite the considerable differences in the acceptance threshold for sugars tested in the present study and as observed by other workers, there seems to be a similar general trend. Thus, disaccharides are more stimulating than mono- or trisaccharides. However, the sharp species differences in thresholds and effectiveness of sugars are probably indicative of the different feeding habits, host preference, selection (especially in haematophagous species such as tabanids) and the type of nutrient materials imbibed. An interesting feature of the present study is the role of melibiose as stimulant for the labella of Chrysops vittatus. It lacks the glucosidic ring considered to be important for being a superior stimulus (cf: Evans 1963, Dethier 1963), but is present in small quantities in certain flower nectars (cf: Wykes 1952, Percival 1961). While one out of seven flies responded to 0.031M (in the ten trial schedule), the other six had a very high acceptance threshold. The maxima of acceptance threshold were reflected at 1.32M. In other insects, melibiose is either non-stimulating at all concentrations (cf: Dethier 1963) or it requires a very high molar concentration to evoke a positive response (Arab 1957, Salama 1966b). Fucose is an effective

stimulant in C.vittatus as in other insects; although it is considered to be non-nutritive (von Frisch 1934, Hassett et al 1950). It is of some interest to state that while melibiose occurs in flower nectar, fucose is inaccessible to the flies in their natural diet.

Besides the evidence accumulating in recent years to support the idea of molecular specificity of test chemicals in the stimulation of receptors (e.g., Carbohydrates: see Evans 1963), the idea of attunement of insect sensory system to flowers and nectar type (Beutler 1945, Wykes 1952) continues to be of some interest.

(ii) Amino acids and nucleotides as phagostimulants

Studies on the phagostimulants of haematophagous and non-blood sucking insects has revealed considerable species variation (cf: Hosoi 1959, Robbins et al 1954, Galun 1963, Friend 1965) Dethier (1966) suggested that feeding responses are primarily due to the interaction between the chemical sense organs and the stimulating substances.

There is no information on the role of amino acids and nucleotides as phagostimulants for haematophagous tabanids.

a. Amino acids

Although sugars are necessary for energy, exogenous proteins are essential for reproduction in many insects (in haematophagous insects: blood proteins cf: Wigglesworth 1965). The

question generally asked is: Can the complex sensory system of insects enable them to distinguish between various amino acids, nucleotides and proteins from blood or other sources, that can be utilised in oogenesis? Also, can the amino acids serve as substitute for proteins and be utilized in oogenesis?

Amino acids in aqueous solution induced little feeding response from the labella of female Chrysops vittatus (Table VI) and a similar situation was observed with respect to a select group of amino acids in 0.133M phosphate buffer (pH 7.4:approximately same as that of human blood) (Table VII). The low percentage of response for aqueous solutions of amino acids is probably due to the acidic state of the solution, its molar concentration (see Table VI), temperature and probably because of the physiological state of the insects (the flies had undifferentiated ovaries and moderately developed fat bodies). The question of aminoacids as chemostimulants for insects is highly controversial. Thus, the present findings on haematophagous C.vittatus agree with those of Wolbarsht and Hanson (1965) who failed to observe any response as a result of stimulation by amino acids (concentration used 0.05M and 0.005M) from saprophagous blow-flies, Phormia regina, that were in a known state of ovarian development during which they imbibe proteins. However, Dethier (1961) in an earlier study suggested that Phormia regina may detect some of the

non-carbohydrate solutes in a brain-heart infusion. Robbins et al (1965) showed that the house fly, Musca domestica L. can detect certain phosphate salts of amino acids (prepared in 0.133M phosphate buffer pH 7.2), the buffer itself was shown to be non-stimulating. Some ixodid ticks, however, have been shown to possess chemosensory hairs in which one neuron is sensitive to amino acids, e.g., asparagine and glutamic acid (Elizarov 1965). Dethier (1966 in litt.) suggested that the question of detection of free amino acids in aqueous solution and buffers is still open and unresolved. Schoonhoven (1968) expressed similar views. Current opinion subscribes to the view that it is only by electrophysiological methods that one can gain any meaningful information about the above subject. Further, "special" credence should be given to the physiological state of the insect, especially with regard to age, reproductive development and nutrition in such a study.

b. Nucleotides

In haematophagous insects and others the chemical factors that evoke feeding responses and engorgement seem to be species specific (cf: Hosoi 1959, Owen and Reinholz 1968). Solutions of ATP and ADP in phosphate buffer induced little activity (Table VII) when touched to the labella of female Chrysops vittatus. Feeding experiments with ATP, ADP and AMP in aqueous solution, buffer and 0.15M NaCl likewise failed to evoke a significant level of feeding activity (Table VIII).

Like amino acids, the question concerning the role of nucleotides as phagostimulants in Diptera is equally variable. Thus, Hosoi (1959) tested several nucleotides and blood fractions from human and bovine (ox) blood as possible feeding stimulants for the mosquito, Culex pipiens var. pallens Coq. Adenosine - 5' - phosphate, adenosine diphosphate, adenosine triphosphate, 4 - deoxyadenylic acid and adenosine - 5 - sulphate were effective as feeding stimulants in a decreasing order. However, in another species of mosquito, Culiseta inornata Willis., Owen and Reinholz (1968) did not get a positive response on stimulation by adenylic acid, ADP and ATP in tris buffer. This is in agreement with our results for Chrysops vittatus. There is no doubt of the importance of adenine moiety and the number of phosphate radicals for insects in which nucleotides have been shown to be feeding stimulants, e.g., Rhodnius prolixus Stahl (Friend 1965).

### III. Comparison of the sensitivity of tarsal and labellar contact chemoreceptors.

Studies on the tarsal and labellar taste sensilla of haematophagous tabanids indicated that despite similarity in their morphology, the functional characteristics of the two types of receptors in terms of their threshold varied (see Table III, IV). Both were, however, sensitive to water, sodium chloride, sucrose (Lall and Davies 1967, 1968) and seven other test sugars. The

trend of acceptance of the three principal nectar sugars, namely: sucrose, glucose and fructose was similar for both the tarsi and labella. Glucose was more stimulating than fructose and sucrose most stimulating of the three. Although, the labellar gustatory sensilla in Diptera are known to be more sensitive, e.g., Culiseta inornata (Feir et al 1961), Tabanus sulcifrons (Frings and O'Neal 1946) and Calliphora erythrocephala, in few other dipterans, the tarsi are more sensitive than the labella; e.g., Calliphora vomitoria (Minnich 1931) Musca domestica, Cochliomyia americana C. and P. (= hominivorax Coq.) (Deonier 1938).

The cause of this differential sensitivity is not understood. The number of gustatory sensilla stimulated may be one answer or the fact that there are some intrinsic differences among the receptors of insect species.

In tabanids studied the taste sensilla on the tarsi and labella are sensitive to a variety of chemicals that play a major role in ascending series in the complex chain of feeding behaviour.

IV. Dispatch of whole human blood, glucose, sucrose and blood-sugar mixtures (the "switch mechanism") to crop or midgut.

Most adult female tabanids are known to obtain their blood meal from mammals (cf: Anthony 1962) and reptiles (Surcouf 1921) but not from amphibians and rarely from birds (Bennett 1960). In addition

they also feed on carbohydrate food (see Hocking 1953, and Downes 1958 for sources of this food).

In the present study females of Chrysops vittatus fed on whole human blood (partially), glucose, sucrose and blood-glucose mixtures (in various proportions and concentrations). Sugars were dispatched to the crop and whole blood to the midgut, immediately after ingestion. That the crop in C.vittatus contained certain sugar was further confirmed by paper-partition chromatography of the crop fluid of wild-caught females in which glucose and fructose were present (see section on crop analysis for details). There are no comparable records for tabanids on this aspect in the literature, and the opinions about the distribution of blood and nectar are largely speculative or at best observational, unsubstantiated by experiments.

The modifications of the gut into a foregut diverticulum (=crop) that receives sugar meal and the midgut where blood is dispatched has also been noticed in species of mosquitoes (Tremblay 1952, Clements 1960), Stomoxys calcitrans (Lotmar 1949) and certain simuliid spp. (Yang 1968). Most workers agree with the concept (Tremblay 1952) that it is the nature of the food and not the method of feeding which determines the passage of food to the crop and/or midgut. However, Hosoi (1959) presented evidence contrary to this in Culex pipiens var. pallens. This adaptation to receive the carbohydrate and blood meal



in two different loci of the gut in Chrysops vittatus is in accordance with the duality that exists in the feeding habits of these flies, and is of considerable survival value. For, while the fullness of the crop with nectar or sugar fluid ensures a sustained source of energy for activities, such as, flight that must be performed in "search" of a suitable host, the empty stomach ensures space to receive the blood meal, whenever an opportunity is presented.

Although blood sucking is the principal target of the female Chrysops vittatus, the flies feed but reluctantly in the laboratory, which provided controlled environment and stimuli considered to be important in relation to blood-feeding. Only 18 out of 60 flies fed on whole blood (Table IX) and only when it was pre-warmed to 37°C. Laarman (1960) encountered a similar situation in Aedes aegypti and suggested that the "poor" response of the insect to blood may be an indication of certain fixity of this last part of the host "seeking" behaviour. Dethier (1957) considered heat as a primary factor in blood sucking in Glossina spp. Like Aedes (Laarman 1960) and Glossina (Dethier 1957), the mosquito, Culiseta inornata (Owen 1963) does not feed on "naked" blood. Hopkins (1964) suggested that this may be due to the absence of blood sensitive external receptors. This is in contrast to the stable-fly, Stomoxys calcitrans which readily feeds on naked blood in the laboratory (Hopkins 1964).

The "poor" response of female Chrysops vittatus to blood should not be taken to mean that blood is unacceptable when offered in a "withdrawn" state, but to the fact that factor(s) that evoke ingestion of blood are probably missing. Other factors such as physiological state of the flies and the previously obtained nutrition (blood or nectar and their state of digestion) may also influence blood feeding (or even response to blood) under laboratory conditions. Some Canadian and German black-flies also show "poor" response to withdrawn blood (Wenk 1965, Yang 1963).

Since sugars evoked a maximum feeding in C.vittatus, (i.e., 98% with sucrose and 100% with glucose: see Table IX) it was considered meaningful to employ glucose (which is the predominant sugar in the mammalian blood, in some flower nectar and was also found in the crop of wild-caught females of C.vittatus) as a dilutant to see if this increased the attractiveness of the whole blood, influenced the dispatch mechanism and the concentration of the glucose required to do this.

Flies fed on 1:1 (v/v) mixture of whole blood and 0.5M glucose and passed the ingested food mainly to the crop (Table IX), indicating the importance of molar concentration and possibly the fact that glucose level was appropriate to excite the receptors(?) in the cibarium (concerned with the detection of sugars: proposed as

sugar receptors). The presence of blood in the mixture was either "ignored" (not detected) or else was not sufficient to stimulate the taste sensilla (different from sugar receptors----henceforth called blood receptors). Day (1954) determined the stimulatory component of the blood and blood sugar mixture and the approximate threshold of stimulation in Aedes aegypti. Addition of small quantities of blood to 0.5M glucose resulted in the mixture going partly to the crop and partly to the midgut. Over a wide range of concentration of blood in mixtures of glucose solution, the mixture went to midgut and crop. However, it must be emphasized that such a distribution of ingested diet may not represent the correct situation, owing to the delay in the dissection of flies. It is possible that the flies may have dispatched "slugs" of food to the midgut during this period. In female Chrysops vittatus, the concentration of glucose seemed to have a decisive role in the dispatch of ingested mixture. Thus, with 0.25M glucose as the dilutant for offering blood at various proportions, the mixture went to the midgut and only in one case of 9 (1:6 v/v) did it go to the crop (Table IX). Evidently, glucose below this concentration is not detected or else fails to excite the "switch mechanism". In the haematophagous simuliids, there have been few attempts to resolve this question. Earlier reports indicated that crop may also receive ingested blood in Simulium spp. (Vargas 1942),

but later experimental evidence have negated this (Blacklock and Lewis 1953, Yang 1968). The results obtained with female C.vittatus in the present study are of considerable interest as glucose (used in the present study as dilutant) is present in the natural diet (blood and nectar) of these insects. It is possible that the amount of glucose along with other factors in the blood may determine the volume of blood that may be imbibed or it may determine the very process of blood feeding.

A number of authors have suggested the role of sensilla in the dispatch of nectar and blood to crop and/or midgut in a few haematophagous dipteran species. From his study on the distribution of ingested food in Aedes aegypti, Day (1954) was able to locate, identify and study the structure of these sensilla in the cibarium and bucco-pharyngeal region. These were considered to detect and discriminate sugar, blood and blood-sugar mixtures. However, these sensilla have also been identified in other Diptera, e.g., Glossina (Jobling 1933), Culiseta inornata (Owen 1963) and certain mosquito spp. (von Gernet and Buerger 1966).

#### V. Analysis of crop fluid

In accordance with the duality that exists in the feeding habits of female Chrysops vittatus (i.e., feeding on blood and nectar), the alimentary canal seems to be modified to receive the ingested food in two different loci (nectar to the crop and blood in the midgut) (see p. 84 for details).

The crop of C.vittatus agrees in its origin and structure with higher Diptera, in that it arises as a diverticulum from the foregut and is typically bilobed as in the blow-fly, Phormia regina (Gelperin 1966a,b), Musca domestica (West 1953), Stomoxys calcitrans (Lotmar 1949), Aedes aegypti (Clements 1960).

In addition to the fluid, the crop of C.vittatus contained several air bubbles. This disagrees with Hocking (1953) who found no air bubbles in the crops of Northern Simuliidae and Tabanidae. However, in Aedes spp., McGregor (1930) and Marshall and Staley (1932) observed air bubbles and stated that these are displaced more or less completely when the mosquito feed on fluids other than blood. The functional importance of air bubbles in the crop is unknown.

Paper-partition chromatography of the crop fluid of wild-caught females of Chrysops vittatus revealed that glucose and fructose were the only sugars present, the former in greater quantity as

evidenced by the intensity of spots (fig. 44). It is probable that the crop serves as a reservoir for sugars only and that imbibed blood is dispatched elsewhere (see section VIII for details). The presence of these sugars in the crop also indicates that the flies do indeed feed on sugars in nature, that are probably derived from plant sources. The angiospermic flora of tabanid collecting area had flowers, some of which are reported to have glucose-dominated nectar (see Appendix II: Table X and Percival 1961, Shuel 1968 in litt.) with little or no sucrose. These flies probably derived their sugar meals from flowers that are rich in glucose and fructose or may have fed on nectar at a time when these sugars were predominant.

Alternatively it is possible that tabanids did imbibe sucrose containing nectar and that enzyme(s) reduced it to glucose and fructose. However, this needs experimental verification. Yang (1968) fed sucrose to several Simuliid spp., but paper-partition chromatography of the crop fluid revealed only glucose and fructose, although he failed to obtain any demonstrable enzymatic activity in the crop and could see no gland cells histologically. However, it seems unlikely that the crop with its chitinous lining can elaborate enzymes or that the sugars are transported from other parts of the gut to the crop (Lewis 1968: in litt.).

Recently some studies have been made to analyse the crop sugars of haematophagous Diptera that are known to feed on nectar in nature with a view to relate these findings to flower preferences. Thus, Kirk and Lewis (1951) (as cited by Lewis and Domoney 1966) showed that glucose and fructose were the principal sugars in the crop of the sand-fly, Phlebotomus papatasi and sucrose was only rarely present. Lewis and Domoney (1966) analysed the crop contents of wild-caught females of Simulium damnosum Theobald, S. adersi, S. griseicolle and sand-flies. Glucose, fructose and sucrose were the predominant sugars in the crop along with traces of maltose, raffinose and melibiose in the females of S. damnosum. This exactly tallies with the nectar composition of large number of angiospermic plants that have been analysed in different parts of the world (cf: Percival 1961 for references). The presence of such trace sugars as maltose, melibiose and raffinose in S. adersi and S. griseicolle was considered to indicate their having different feeding habits to those of S. damnosum (Lewis and Domoney 1966). The females of Chrysops vittatus differ from the above insects in having no sucrose nor traces of other sugars, but rather only glucose and fructose. This indicates that their feeding habits and preferences for flower nectar of certain types differ from those of black-flies and sand-flies mentioned above.

Literature on the sugar meal and its source in haemaphagous Diptera and other insects (except honey bee) is scanty and few attempts have been made to relate these findings to the adaptation and behaviour of the insects in nature. Hocking (1953) summarised the available evidence of flower feeding in blood sucking Diptera. Eighteen out of the forty four records for tabanids were on Umbelliferae. Species of Chrysops and Tabanus were observed to feed on Potentilla fruticosa, Daucus carota and others. These and other angiosperms were also recorded by me in the tabanid collecting area. Wenk (1965) showed that German simuliids show a marked preference for the white willow, Salix alba in spring, for the yellow blossoms of the ivy, parsnips (Pastinaca sativa) in summer and the green blossoms of Hedera helix in autumn. Available evidence indicates that mosquito spp. are relatively less catholic in their feeding preference for flowers than tabanids and simuliids (cf: Haeger 1955, Breland and Pickard 1961 and Sandholm and Price 1962).

#### VI. Longevity of wild-caught tabanids

The study of longevity of tabanids is of considerable interest as it is linked with the epidemiology of the disease transmitted, population biology, frequency and number of tabanid attack on host animal and consequently blood feeding on which depends the number of gonotrophic cycles. Little is known of the longevity of tabanids in nature and none under laboratory conditions.



In the present study wild-caught females of C.vittatus lived longest on 'dry' sucrose and water ( $LD_{50}$ :25 days), followed by glucose (19 days) and water (16 days). This seems to suggest the long life span of the flies in nature. That the flies lived for 16 days on water alone seems to suggest that either they had fed in nature and had sufficient reserve to sustain life for a considerable period of time or that larval reserves augmented by adult feeding contribute to this. Moreover, the temperature of their environment (the flies were maintained at 17°C) may have some role (by reducing the rate of metabolic processes). However, the flies died within 24h. without food and water.

Proof attesting to the long span of life of female C.vittatus in nature comes from several sources. I collected this species in Beverley swamp in the last week of June and occasionally collected a few until the first week of September (for three consecutive years). This is further supported by other tabanid records (see Pechuman 1961, for references).

On the basis of available evidence on the longevity of wild-caught haematophagous dipterans, it seems that black-flies live longest, e.g., Similium decorum lived for over 60 days in captivity (Davies 1959). A summary of the longevity of various Diptera and other insects was presented by Clark and Rockstein (1964). In general,

it appears that females live longer than males and that the longevity of reared adult females is greater than wild-caught (Galun and Fraenkel 1956), although Davies (1953) disputed the latter point in black-flies.

Temperature, humidity, light, sugar and blood feeding, excessive handling in the laboratory and presence of parasites and pathogens are the factors listed as affecting longevity (Hocking 1953, Lavoipierre 1958, Greenberg 1960).

VII. Some remarks on the relevance of the experimental results with the behaviour of the flies in nature and the further avenues of research as indicated from the present study.

The results of the present study on the feeding apparatus, loci, structure and function of taste sensilla (tarsal and labellar) and the chemical factors that initiate, sustain and/or inhibit feeding in haematophagous tabanids serve to contribute basic information on various aspects of feeding behaviour. Studies of sugar feeding in nature and under laboratory condition have demonstrated the critical value of carbohydrates. The structure of mouthparts, the manner and duration of feeding on sugars have intimate relationships with host preference and selection (animal and plant, disease transmission and the frequency of attack on the host).

Blood feeding may be similar to sugar feeding once the mouthparts pierce the host skin, lacerate the blood vessel and cause flow of blood. Presence of crop sugars in wild-caught females lends the first experimental support to the view that tabanids do indeed feed on nectar in nature and retain it temporarily in the crop, just as they feed on sugars in the laboratory. Experimental feeding indicated that blood is dispatched to midgut after ingestion. The tabanid collecting area had a preponderance of flowers with glucose as the principal sugar (in some cases sucrose as well) thus supporting the idea that tabanids derive their carbohydrate meals from plant sources. Preliminary studies on the longevity of flies lend further evidence to this. Extension of the work along above lines is likely to yield significant information on flower and animal host preference and selection. The present study clearly indicates the critical role of taste sensilla (tarsal and labellar as well as others) in the feeding behaviour. It is desirable to investigate the cytology and electrophysiology of the receptor cell associated with each sensillum (tarsal, labellar, cibarial and foregut). The hypothesis that the flies possess a hierarchical system of sensilla that monitor various phases of feeding (pre-ingestion, ingestion and post-ingestion), should be further tested to obtain additional experimental proof. A study of the responses of the flies on stimulation by two opposing

stimuli will yield valuable information on the evaluation of excitatory and/or inhibitory impulses by the central nervous system and their interactions. This may be extended to obtain anatomical and cytological information on the question of neural centers in the brain which receive impulses from taste receptors. Study of the fine structure of the sensillum and the brain may substantiate or negate these. Obviously, such a discovery is intimately linked with the physiology of the receptors and should assist in a better understanding of various behaviour patterns.

Application of frequency method as used in the present study (for the first time in insects) should assist in obtaining unbiased threshold values of chemicals thus aiding in a better understanding of the question on stimulative effectiveness, rank order, preference, acceptance and/or rejection of chemicals.

Studies of chemical factors that act as feeding stimulants and serve to initiate, sustain and/or inhibit feeding is helpful in devising effective deterrents and repellents thus assisting in formulating effective control measures for the extermination of immature and adult tabanids.

The failure of amino acids and nucleotides to evoke significant feeding activity is probably indicates that some of the stimulatory factor(s) are missing, or the physiological state of the

flies is such that these substances are not imbibed. It is also probable that the sensory mechanisms are not attuned to detect these chemicals or else receptor(s) sensitive to them are absent. However, a categorical answer to the question of stimulation by these chemicals can best be obtained by electrophysiological research.

The increased sensitivity of the newly emerged females of Hybomitra lasiophthalma maintained on water diet seems to suggest that the physiological state of the insect may be critical in initiating or sustaining changes in the sensitivity of receptors. It remains to be seen whether or not the factors are neural, endocrinal or both.

The present study, therefore, serves as a base line report for further studies on the intricate problems of feeding behaviour in haematophagous tabanids.

#### V SUMMARY

1. The head capsule and feeding apparatus of female Chrysops vittatus, Tabanus lineola and Hybomitra lasiophthalma, conformed to a basic structure, although exhibiting differences in size. The flies possess piercing and sucking fascicles that adapt them to feed upon blood and nectar. The possession of a long, slender and scaly proboscis in C.vittatus as compared to that of other two species, was considered to indicate their being able to feed on thick-furred animals and

deeper-seated nectaries, as well as on unspecialised hosts. This was suggested to effect their distribution. In contrast to this, females of T.lineola and H.lasiophthalma, which possess a broad and short proboscis, were considered to have lesser latitude with regard to selection and feeding on plant and animal hosts.

2. The three tabanids fed on "dry" sugar as well as on sugar solutions of appropriate concentrations. No behavioural differences were manifested by C.vittatus and H.lasiophthalma during their pre-feeding activity, but the method of feeding (application of the proboscis) and consequent patterns of behaviour varied.

3. The ventro-lateral surface of the foretarsi and the aboral margin of the labella were the principal loci of gustatory sensilla in the three tabanids. The two types of tarsal and labellar contact chemoreceptors appeared to have similar morphology: each was medium-sized, blunt-tipped, with two lumina and arose from a high-rimmed socket. Besides, both were sensitive only at the tip.

4. Extension of the proboscis was the index of positive tarsal stimulation, and the spreading of the labellar lobes in an extended proboscis, the index of positive labellar stimulation.

5. A preliminary study aimed at assessing the physiological state of the flies used in behavioural studies, revealed that most had fed on sugar and some on blood in nature. The fat bodies were well developed

but the ovaries showed little differentiation. In a large number of cases they consisted of hollow sac of cells.

6. Contact chemoreceptors on the foretarsi and aboral margin of the labella were sensitive to chemostimulation by water, amino acids, nucleotides and sugars. The two together play an important role in the complex chain of feeding behaviour in the proposed hierarchical system of taste sensilla.

7. The order of stimulative effectiveness of the three sugars tested on the foretarsi was: sucrose > glucose > fructose., and the eight sugars tested on the labella by the sample and population frequency method had the similar rank order: monosaccharides: glucose > fucose > fructose., disaccharides: sucrose > maltose > melibiose and the trisaccharides: melezitose > raffinose.

8. Starvation affected the sensitivity of the tarsal and labellar taste receptors of newly emerged females of H.lasiophthalma. The flies became increasingly sensitive to stimulation by sucrose when maintained on a water diet.

9. The flies fed on whole human blood partially and transported it to the midgut. Glucose and sucrose, after ingestion were located in the crop. The flies seemed to possess a "switch mechanism" that determines the passage of ingested food to crop or midgut. Addition

of glucose enhanced the attractiveness of the whole blood and the flies avidly fed on the mixture. A higher concentration of glucose (0.5M) was required in the blood-glucose mixture for its dispatch to crop after ingestion. With a lesser concentration of glucose (0.25M), the flies passed the ingested diet to midgut.

10. Paper-partition chromatography of the crop fluid of wild-caught C.vittatus revealed glucose and fructose to be the only sugars present, the former in larger quantity as evidenced by the intensity of the spot. This is the first experimental evidence of tabanids feeding on specific sugars and retaining them in the crop. A survey of the angiospermic flora in the tabanid collecting area indicated flowers of various types and nectar composition. These findings were considered to have important bearing on flower preference, selection and feeding.

11. Wild-caught females of C.vittatus lived longest on "dry" sucrose and water followed by 1.0M glucose and distilled water. That they could survive for a long time on water alone was believed to indicate that the flies had retained sufficient reserves from the larva and/or had fed previously in nature so that sustenance was ensured for a considerable length of time.



VI. REFERENCES CITED

- Adams, J.R., 1961, The location and histology of the contact chemoreceptors of the stablefly, Stomoxys calcitrans L. Ph.D. Thesis, Rutgers Univ., New Brunswick, N.J.
- Anderson, A.L., 1932, The sensitivity of the legs of common butterflies to sugars, *J. Exp. Zool.*, 53(1):235-259.
- Anthony, D.W., 1962, Tabanidae as disease vectors, in "Biological transmission of disease agents", pp. 93-107 - Maramorsch K. ed., Academic Press, New York.
- Arab, Y.M., 1958, Behavioural responses to electrical stimulation of the blow-fly, *J. Insect Physiol.*, 2:324-329.
- Ascher, K.R.S. & Hirsch, J., 1965, The house-fly and the cube of sugar. *Wld. Rev. of Pest Control*, 4:103-111.
- Bennett, G.F., 1960, On some ornithophilic blood-sucking Diptera in Algonquin Park, Ontario, Canada. *Can. J. Zool.* 38:377-389.
- Beutler, R., 1953, Nectar. *Bee World* 34:106-116, 128-136, 156-162.
- Blacklock, B.D., 1926, The further development of Onchocerca volvulus Leuckart in Simulium damnosum Theob., *Am. Trop. Med. Parasitol.*, 20:203-218.
- Bonhag, P.F., 1951, The skeleto-muscular mechanism of the head and abdomen of the adult horse-fly (Diptera:Tabanidae). *Trans. Amer. Entomol. Soc.*, 77:131-202.

- Bradley, G.H., 1931, Feeding tests of anopheline mosquitoes with leguminous plants. *J. Econ. Ent.*, 24:1229-1233.
- Breeland, S.G. & Pickard E., 1961, Observations on mosquito feeding activity on the flower heads of Eupatorium and Solidago (Compositae). *Mosq. News*, 21:32-34.
- Bromley, S.W., 1926, The external anatomy of the black horse fly Tabanus atratus Fab. (Diptera: Tabanidae). *Ann. Entomol. Soc. Amer.*, 19:440-460.
- Christophers, Sir S.R., 1960, Aedes aegypti (L). The yellow fever mosquito. Its life history, bionomics and structure. Cambridge Univ. Press, London.
- Clark, A.M. & Rockstein, M., 1964, Aging in Insects, in "Physiology of Insecta", Vol. I ed. M. Rockstein, Academic Press Inc., N.Y., Chapter 6.
- Clements, A.N., 1963, The Physiology of Mosquitoes. *Internat. Ser. Monogr.*, The MacMillan Co., N.Y.
- Cragg, F.W., 1912, The structure of Haematopota pluvialis (Meigen). *Sci. Mem. Med. Sanit. Dept. India*, 55:33 pages; 7 plates.
- Davies, D.M., 1953, Longevity of blackflies in captivity. *Can. J. Zool.* 31:304-312.
- Day, M.G., 1954, The mechanism of food distribution to midgut or diverticula in the mosquito. *Aust. J. Biol. Sci.* 7:515-524.

- Demianowicz, D., 1964, Das Zuckerbild blütenveinei Sortenhonige.  
Ann. de l'Abeille, I:289-99.
- den Otter, G & van der Starre, H., 1967, Response of tarsal hairs of  
the blue bottle, Calliphora erythrocephala Meig., to sugar  
and water. J. Insect Physiol., 13:1177-1188.
- Deonier, C.C. & Richardson, C.H., 1935, The tarsal chemoreceptor  
responses of the house-fly, Musca domestica L. Ann.  
Entomol. Soc. Amer., 28:467-474.
- Deonier, C.C., 1938, The gustatory nature of the chemotarsal  
stimulation in the house-fly, Musca domestica L.  
J. Exp. Zool., 79:489-500.
- Dethier, V.G., 1941, Function of antennal receptors in lepidopterous  
larvae. Biol. Bull., 80:403-414.
- \_\_\_\_\_, 1952, Adaptation to chemical stimulation of tarsal  
receptors of the blowfly. Biol. Bull., 193(2):178-189.
- \_\_\_\_\_, 1953, Summation and inhibition following contra-lateral  
stimulation of the tarsal chemoreceptor of the blow-fly.  
Biol. Bull., 195:257-268.
- \_\_\_\_\_, 1954, Notes on the biting responses of tsetse flies.  
Amer. J. Trop. Med. Hyg., 3:160-171.
- \_\_\_\_\_, 1955a, Tarsal chemoreceptors of the house-fly. Proc. Roy.  
Soc. Lond. (A), 3:87-90.

- Dethier, V.B., 1955b, The physiology and histology of the contact chemoreceptors of the blow-fly. *Quart. Rev. Biol.*, 30:348-371.
- \_\_\_\_\_, 1956, Chemoreceptor mechanisms in molecular structure and functional activity of nerve cells. *A.I.B.S.*, Wash. D.C., 1-30.
- \_\_\_\_\_, 1961, Behavioral aspect of protein ingestion by the blow-fly, *Phormia regina* Meigen. *Biol. Bull.*, 121:456-470.
- \_\_\_\_\_, 1962, Chemoreceptor mechanism in insects. *Symp. Soc. Exp. Biol.*, 16:180-196.
- \_\_\_\_\_, 1963, *The Physiology of the insect senses*. Methuen & Co., London.
- \_\_\_\_\_ & Bodenstein, D., 1958, Hunger in the blow-fly. *Zeitschr. f. Tierpsychol.*, 15:129-140.
- \_\_\_\_\_ & Chadwick, L.E., 1948, Chemoreception in insects. *Physiol. Rev.*, 28(2):220-254.
- \_\_\_\_\_, Solomon, R.L., & Turner, L.H., 1965, Sensory input and central excitation and inhibition in the blow-fly. *J. Comp. Physiol. Psychol.*, 60:303-313.
- Dickerson, G. & Lavoipierre, M.M.J., 1959, Studies on the method of feeding blood sucking arthropods. III. The method by which *Haematopota pluvialis* (Diptera:Tabanidae) obtains its meal from the mammalian host. *Ann. Trop. Med. Parasitol.* 53:465-472.

- Downes, J.A., 1958, The feeding habits of biting flies and their significance in classification. *Ann. Rev. Entomol.* 3:249-266.
- Eliason, D.A., 1963, Feeding adult mosquitoes on solid sugar. *Nature, Lond.* 200:289.
- Elizarov, Y.A., 1965, Investigation of chemoreception of insects and ticks. Physiology of contact chemoreceptory sensilla in ixodid ticks. *Zool. Z.* 44:1461-1472.
- Eltringham, H., 1933a, The senses of insects. Methuen & Co., London. ix - 126 p., 25 figs (1,2,8,60).
- Evans, D.R., 1963, Chemical structure and stimulation by carbohydrates. Proc. I. Internat. Symp. Olfaction and Taste (Stockholm), I:165-176.
- \_\_\_\_\_ & Dethier, V.G., 1957, The regulation of taste thresholds for sugars in the blow-fly. *J. Insect Physiol.*, I:3-17.
- \_\_\_\_\_ & Barton-Browne, L., 1960, The physiology of hunger in the blow-fly. *Amer. Midl. Natur.* 64:282-300.
- Fair, D., Lengy, J.I. & Owen, W.B., 1961, Contact chemoreception in mosquito, Culiseta inornata (Williston): Sensitivity of the tarsi and labella to sucrose and glucose. *J. Insect Physiol.*, 6:13-20.

- Frings, H., & O'Neal, 1946, The loci and threshold of contact chemoreceptors in females of the horse-fly, Tabanus sulcifrons Macq. J. Exp. Zool., 103:61-79.
- \_\_\_\_\_ & Frings, M., 1949, The loci of contact chemoreceptors in insects: A review with new findings. Amer. Midl. Natur., 41:602-658.
- \_\_\_\_\_ & Hamrum, C.L., 1950, The contact chemoreceptors of adult yellow fever mosquitoes, Aedes aegypti. J. N.Y. Entomol. Soc., 58:133-142.
- \_\_\_\_\_ & Frings, M., 1959, Studies on the antennal contact chemoreceptors of the wood nymph butterfly, Cercyonis pegale. N.Y. Entomol. Soc., 67:97-105.
- Galun, R. & Fraenkel, G., 1957, Physiological effects of carbohydrates in the nutrition of a mosquito Aedes aegypti and two flies, Sarcophaga bullata and Musca domestica, J. Cell. Comp. Physiol., 50:1-23.
- \_\_\_\_\_, Avi-Dor, Y. and Bar-Zeev, 1963, Feeding responses in Aedes aegypti: Stimulation by adenosine triphosphate. Science, N.Y., 142:1674-1675.
- Gelperin, A., 1966a, Control of the crop emptying in the blow-fly. J. Insect Physiol., 12:331-345.

- Gelperin, A., 1966b, Investigation of a fore-gut receptor essential to taste threshold regulation in the blow-fly. ibid., 12:829-841.
- Glaser, R.W., 1923, The effect of food on longevity and reproduction in flies. J. Exp. Zool., 38:383-412.
- Glasgow, J.P., 1963, Distribution and abundance of tsetse flies. Internat. Ser. Monogr. Appl. Biol., Vol. 20. MacMillan.
- Gordon, R.M. & Crewe, W., 1953, The deposition of infective stages of Loa loa by Chrysops silacea and the early stages of its migration to the deeper tissues of the mammalian host. Ann. Trop. Med. Parasitol., 47:74.
- Grabowski, C.T. & Dethier, V.G., 1954, The structure of the tarsal chemoreceptors of the blow-fly, Phormia regina Meigen. J. Morphol., 94:1-20.
- Graham-Smith, G.S., 1930, Further observations on the anatomy and function of the blow-fly, Calliphora erythrocephala. I. Parasitology, 22:47-115.
- Greenberg, B., 1959, House-fly nutrition. I. Quantitative study of the protein and sugar requirements of males and females. J. Cell. Comp. Physiol., 53:169-177.
- Haeger, J.S., 1955, The non-blood feeding habits of Aedes taeniorhynchus (Diptera: Culicidae) on Sanibel Island, Florida. Mosquito News, 2-:136-147.

- Haslinger, F., 1935, Über den Geschmacksinn von Calliphora erythrocephala Meigen, und über die Verwertung von zuckern und zuckernkoholen durch diese Fliege. Z. Vergl. Physiol. 22: 6140640.
- Hassett, C.C., Dethier, V.G. & Gans, J., 1950, A comparison of nutritive values and taste threshold of carbohydrates for the blow-fly. Biol. Bull., 99:446-453.
- Hocking, B., 1953, The intrinsic range and speed of flight in insects. Trans. Roy. Entomol. Soc., Lond. 104:233-345.
- Hodgson, E.S., 1964, Chemoreception in "Physiology of Insects", Rockstein, M. editor, Academic Press, Vol. I.
- Hopkins, B.A., 1964, The probing response of Stomoxys calcitrans (L.) (the Stablefly) to vapours. Anim. Behav. XII:513-523.
- Hosoi, T., 1954, Mechanism enabling mosquito to ingest blood into the stomach and sugar fluids into the oesophageal diverticula. Annot. Zool. Jap. 27:82-90.
- \_\_\_\_\_, 1958, Adenosine-5'-phosphate as the stimulating agent in blood for inducing gorging of the mosquito. Nature, 181:1664-1665.
- \_\_\_\_\_, 1959, Identification of the blood components which induce gorging of the mosquito. J. Insect Physiol., 3:191-218.



- Kare, M.R., Ficken, M.S., 1963, Comparative studies on sense of taste. Proc. 1st Internat. Symp. on Olfaction and Taste, Lond., N.Y., pp. 285-97.
- Kirgjsman, B.J., 1930, Reizphysiologische untersuchungen an blutsaugenden Arthropoden im Zusamsenhang mit ihrer Nahrungswahl. I. Teil. Stomoxys calcitrans L. Zeit. Vergl. Physiol. 11:702-729.
- Kunze, G., 1933, Einige Versuche <sup>"</sup> über den Antennegeoch-mackssinn der Honigbiene. Zool. Jahrb., Abt. Allg. Zool. Physiol., 52:465-512.
- Laarman, J.J., 1959, Host seeking behaviour of malaria mosquitoes. Proc. XV Internat. Congr. Zool. (1958):648-649.
- Lall, S.B. & Davies, D.M., 1967, Tarsal sensitivity of female tabanid flies to sucrose and sodium chloride. Can. J. Zool. 45:461-464.
- \_\_\_\_\_ & \_\_\_\_\_, 1968, Comparative studies on the sensitivity of labellar contact chemoreceptors in female tabanids (Diptera). Ann. Entomol. Soc. Amer. 61:221-225.
- Lavoipierre, M.M.J., 1958a, Blood feeding, fecundity and ageing in Aedes aegypti var. queenslandensis. Nature, Lond. 191:575-576.

- Lavoipierre, M.M.J., 1958b, Presence of a factor inhibiting biting in Aedes aegypti. Nature, Lond. 182:1567-1568.
- \_\_\_\_\_, 1965, Feeding mechanism of blood sucking arthropods. Nature, Lond. 208:302-303.
- Lewis, D.J. & Domoney, G.R., 1966, Sugar meals in Phlebotominae and Simuliidae (Diptera). Proc. Roy. Entomol. Soc. Lond. 41:175-179.
- Lotmar, R., 1949, Beobachtungen "über Nahrungsaufnahme und Verdaung bei Stomoxys calcitrans (Diptera). Mitt. Schweiz. Entomol. Ges. 22:97-115.
- Marshall, J., 1935, On the sensitivity of the chemoreceptor of the antennae and fore-tarsus of the honey-bee, Apis mellifera. J. Exp. Biol., 12:17-26.
- \_\_\_\_\_, & Staley, J., 1932, On the distribution of air in the oesophageal diverticula and intestine of mosquitoes. Its relation to emergence feeding and hypopygeal rotation. Parasitology 24:308-381.
- Matheson, R.H., 1950, Medical Entomology, pp. 421-427. Comstock Publ. House, Inc., N.Y.
- McGregor, M.E., 1930, The artificial feeding of mosquitoes by a new method which demonstrates certain function of the diverticula. Trans. Roy. Soc. Trop. Med. Hyg. 23:329-331.

- McGregor, M.E., 1931, The nutrition of adult mosquitoes: Preliminary Contribution. Trans. Roy. Soc. Trop. Med. Hyg. 24:465-472.
- Middlekauf, W.W., 1941. Some biological observations of the adults of the apple maggot and the cherry fruit flies. J. Econ. Ent. 34:621-624.
- Minnich, D.E., 1921, An experimental study of the tarsal chemoreceptors of two nymphalid butterflies. J. Exp. Zool., 33(1): 173-203.
- \_\_\_\_\_, 1922, The chemical sensitivity of the tarsi of the red admiral butterfly, Pyrameis atalanta Linn. J. Exp. Zool., 35(1):57-81.
- \_\_\_\_\_, 1926, The chemical sensitivity of the tarsi of certain muscid flies. Biol. Bull., 51(3):166-178.
- \_\_\_\_\_, 1929a, The chemical sensitivity of the legs of the blowfly, Calliphora vomitoria Linn., to various sugars. Zeitschr. vergl. Physiol., Julius Springer, Berlin.
- \_\_\_\_\_, 1931, The sensitivity of the oral lobes of the proboscis of the blowfly, Calliphora vomitoria Linn., to various sugars. J. Exp. Zool., 60(1):121-139.
- Mitter, J.L., 1917, Note on the method of feeding of Corizoneura (Pangonia) longirostris Hardwick, with a description of the mouthparts. Ind. J. Med. Res., 5:523.

- Oldroyd, H., 1954, The horse-flies (Diptera: Tabanidae) of the Ethiopian region. II. *Tabanus* and related genera. Brit. Mus. Natur. Hist., 341 pages.
- Olsufjev, N.G., 1936, Dipterous insects. Horseflies (Tabanidae). Vol. VIII Publ. Press of Acad. Sci., U.S.S.R., Moscow, Leningrad.
- Owen, W.B., 1963, The contact chemoreceptor organs of the mosquito and their function in feeding behaviour. J. Insect Physiol. 9:73-87.
- \_\_\_\_\_ and Reinholz, S., 1968, Intake of nucleotides by the mosquito Culiseta inornata in comparison with water, sucrose and blood. Exp. Parasitol. 22:43-49.
- Pechuman, L.L., 1961, The Tabanidae of New York. Proc. Roy. Acad. Sci. 19(3):121-179.
- Percival, S.M., 1961, Types of nectar in the angiosperms. New Phytol. 60:35-281.
- Peters, W., 1963, Die Sinnesorgane an den Labellen von Calliphora erythrocephala Mg. (Diptera). Z. Morph. Ökol. Tiere 55: 259-320.
- Peterson, A., 1916, The head capsule and mouthparts of Diptera. Illinois Biol. Monogr. 3:112 pages.

- Robbins, W.R., Thompson, M.A., Yamamoto, R.T. & Shortino, T.J.  
1965, Feeding stimulants for the house-fly, Musca domestica  
L. Science, N.Y. 147:628-630.
- Roy, C., 1954, Olfactory nerve potentials in direct measure of chemo-  
reception in insects. Ann. N.Y. Acad. Sci. 58:250-255.
- Salama, H.S., 1966a, Taste sensitivity of some chemicals in Rhodnius  
prolixus Stål and Aedes aegypti L. J. Insect Physiol.  
12:583-589.
- \_\_\_\_\_, 1966b, The function of mosquito taste receptors, ibid.,  
12:1051-1060.
- Sandholm, H.A. & Price, R.D., 1962, Field observations on the nectar  
feeding habits of some Minnesota mosquitoes. Mosq. News  
22:346-349.
- Schoonhoven, L.M., 1968, Chemosensory bases of host plant selection.  
Ann. Rev. Entomol. 13:115-136.
- Scudder, H.I., 1953, Cephalic sensory organs on the female horse-fly,  
Tabanus quinquevittatus Wiedemann (Diptera: Tabanidae)  
Ph. D. Thesis, Cornell Univ., Ithaca, N.Y.
- Slifer, E.H., 1954, The reaction of the grasshopper to an odorous  
material when held near one of its feet (Orthoptera:  
Acrididae). Proc. Roy. Entomol. Soc. London (A) 29:177-179.

- Smith, J. ed., 1958, Chromatographic techniques: Clinical and Biological applications. Heinemann, London.
- Snodgrass, R.E., 1926, The morphology of the insect sense organs and the sensory nervous system. *Smithson. Misc. Coll.* 77:1-80.
- \_\_\_\_\_, 1944, The feeding apparatus of biting and sucking insects affecting man and animals. *Smithson. Misc. Coll.* 104:1-113.
- Strangways-Dixon, J., 1961a, The relationship between nutrition, hormone and reproduction in the blow-fly, Calliphora erythrocephala (Meigen). I. Selective feeding in relation to the reproductive cycle, the corpus allatum volume and fertilization. *J. Exp. Biol.* 38:225-235.
- \_\_\_\_\_, 1961b, The relationship between nutrition, hormone and reproduction in the blow-fly, Calliphora erythrocephala (Meig.) II. The effect of removing the ovaries, corpus allatum and the median neurosecretory cells upon selective feeding and the demonstration of the corpus allatum cycle. *ibid.* 637-646.
- Stüerckow, B., 1961, Histologische Untersuchungen am labellaren Marginalborste den Fliegen, Calliphora und Phormia. XIth Internat. Congr. Entomol., Wien, 1960, I:410-411.
- Surcouf, J.M.R., 1921, Diptera: Fam. Tabanidae. Wytman's genera *Insectorum. Fasc.* 175:182pp.

- Tremblay, H.L., 1952, The distribution of certain liquids in the esophageal diverticula and stomach of mosquitoes. Amer. J. Trop. Med. Hyg., 1:693-710.
- Unna, P.G., 1922, Centlb. Bakt. Parasitol. 1, Abt. 88:59.
- Vargas, L., 1942, Algunas consideraciones sobre el desarrollo de Onchocerca volvulus en los Simulides. Rev. Inst. Salubr. Enferm. Trop., Mex. 3:57-65.
- von Frisch, K., 1934, "Über den Geschmackssinn der Biene. Zeit. Vergl. Physiol. 21:1.
- von Gernet, G. & Buerger, G., 1966, Labral and cibarial sense organs of some mosquitoes. Quaestiones Entomol. 2:259-270.
- Wallis, R.C., 1954, The effect of population density and of NaCl concentration in test series in laboratory experiments with ovipositing Aedes aegypti L. Mosq. News. 14:200-204.
- Weisman, R., 1962, Untersuchungen über den Sitz des kontaktchemischen Sinnes bei Lepidoptera. Z. Angew. Zool. 49:123-133.
- Wenk, P., 1965a, "Über die Biologie blutsaugender Simuliidae (Diptera) I. Z. Morph. Okol. Tiere, 55:656-670.
- \_\_\_\_\_, 1965b, "Über die Biologie blutsaugender Simuliidae (Diptera) II. Z. Tropen. Med. Parasit. 16:207-226
- West, L.S., 1951, The housefly. Comstock Publ. Co., Ithaca, N.Y.

- Wigglesworth, V.B., 1965, The principles of Insect Physiology.  
Methuen & Co., London.
- Wilczek, M., 1967, The distribution and neuro-anatomy of the labellar sense organs of the blow-fly, Phormia regina Meigen.  
J. Morphol., 122:175-202.
- Wolbarsht, M.L. & Hanson, F.E., 1965, Electrical and behavioral responses to amino acid stimulation in the blowfly.  
Olfaction and taste. II. Academic Press.
- Woodworth, R.S. & Schlosberg, H., 1938, Experimental Psychology  
P:200-218.
- Wykes, G.R., 1952a, An investigation of the sugars present in the nectar of flowers of various species. New Phytol.,  
51:210-215.
- \_\_\_\_\_, 1952b, The preference of honeybee for solution of various sugars which occur in nectar. J. Exp. Biol., 29:511-599.
- Yang, Y.J., 1968, A study of ingestion and digestion emphasizing the peritrophic membrane and digestive enzymes in the adult black-flies (Diptera:Simuliidae) fed blood, blood-sucrose mixtures and sucrose. Ph.D. thesis, McMaster University, Hamilton, Canada.



Fig. 1. Female tabanids used in the study. Top: Tabanus lineola,  
center: Hybomitra lasiophthalma and bottom: Chrysops  
vittatus (3.7 x life size).



Fig, 2. Anterior view of the head capsule and mouthparts  
of a female Chrysops vittatus

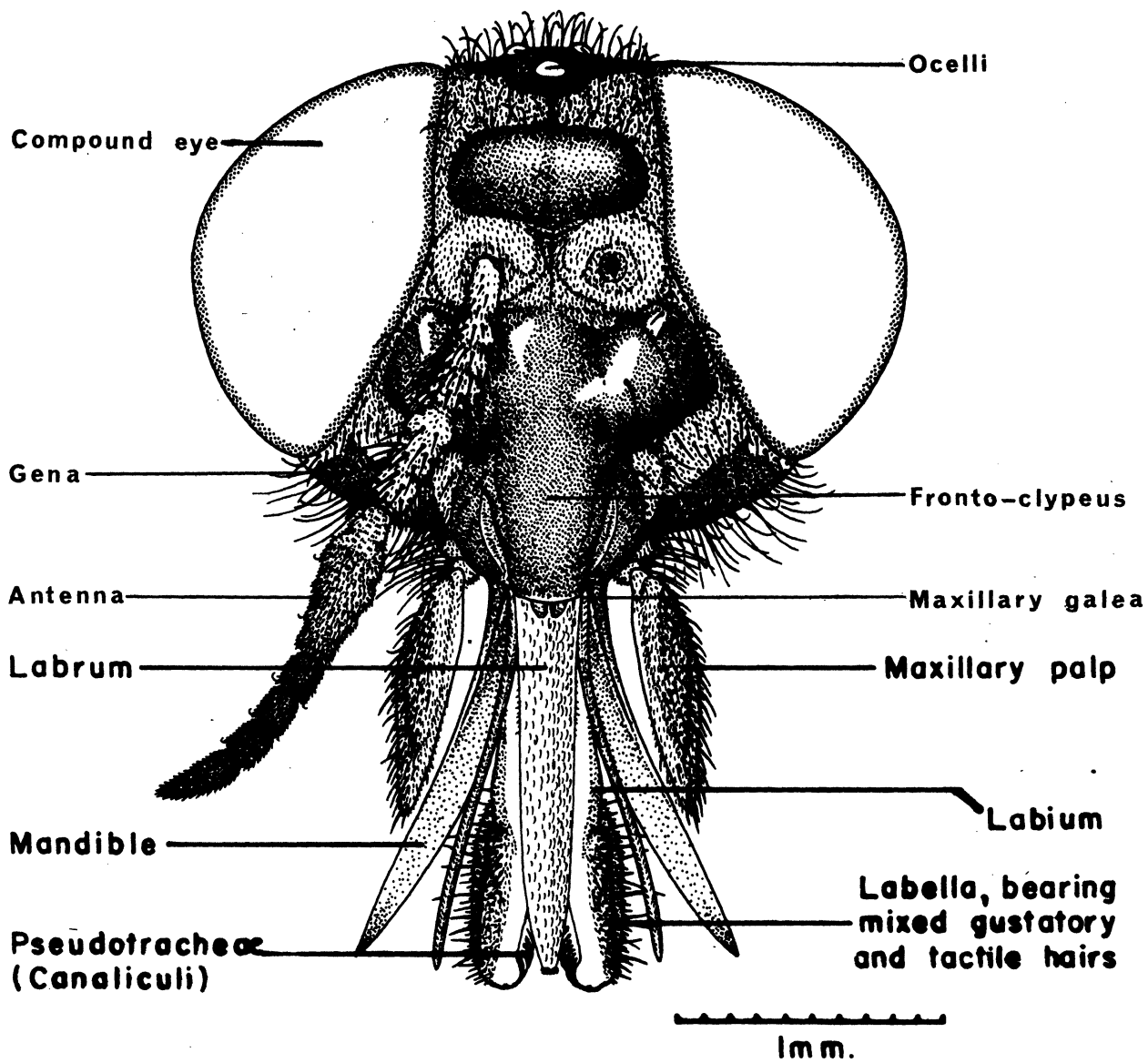


Fig. 3. Anterior view of the head capsule and mouthparts  
of a female Tabanus lineola

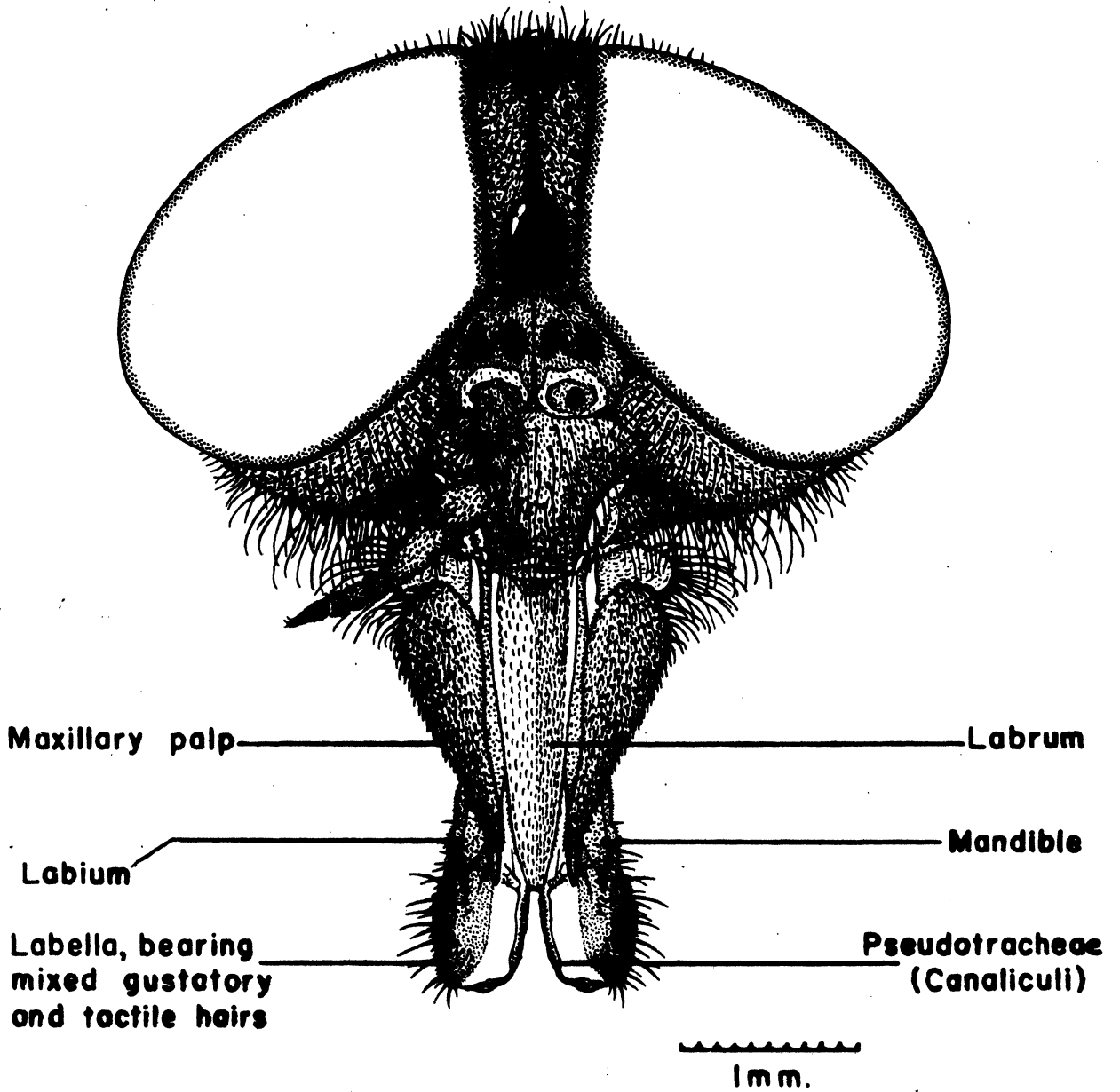


Fig. 4. Anterior view of the head capsule and mouthparts  
of a female Hybomitra lasiophthalma

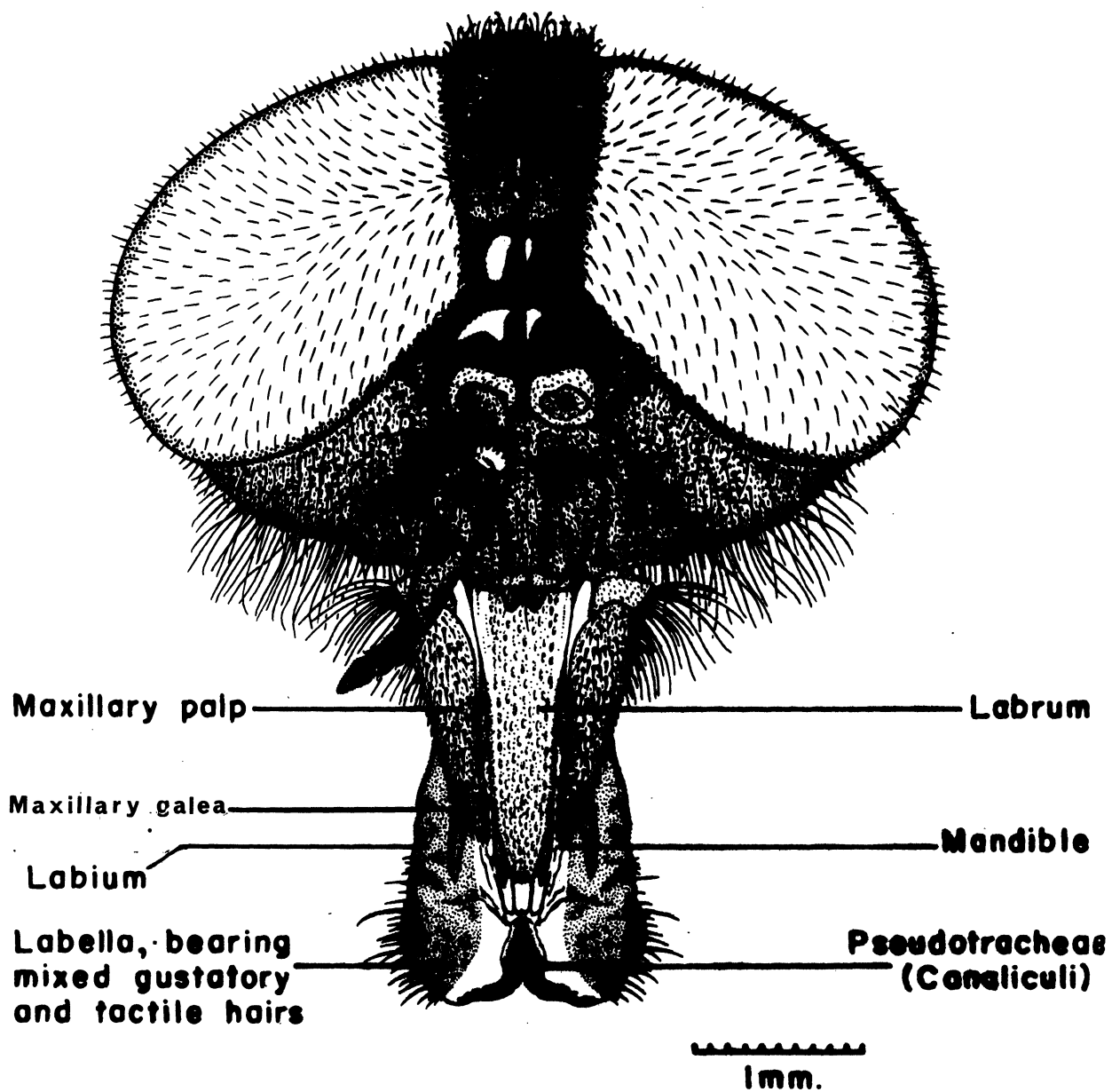




Fig. 5. Posterior view of the head capsule and mouthparts of  
a female Chrysops vittatus.

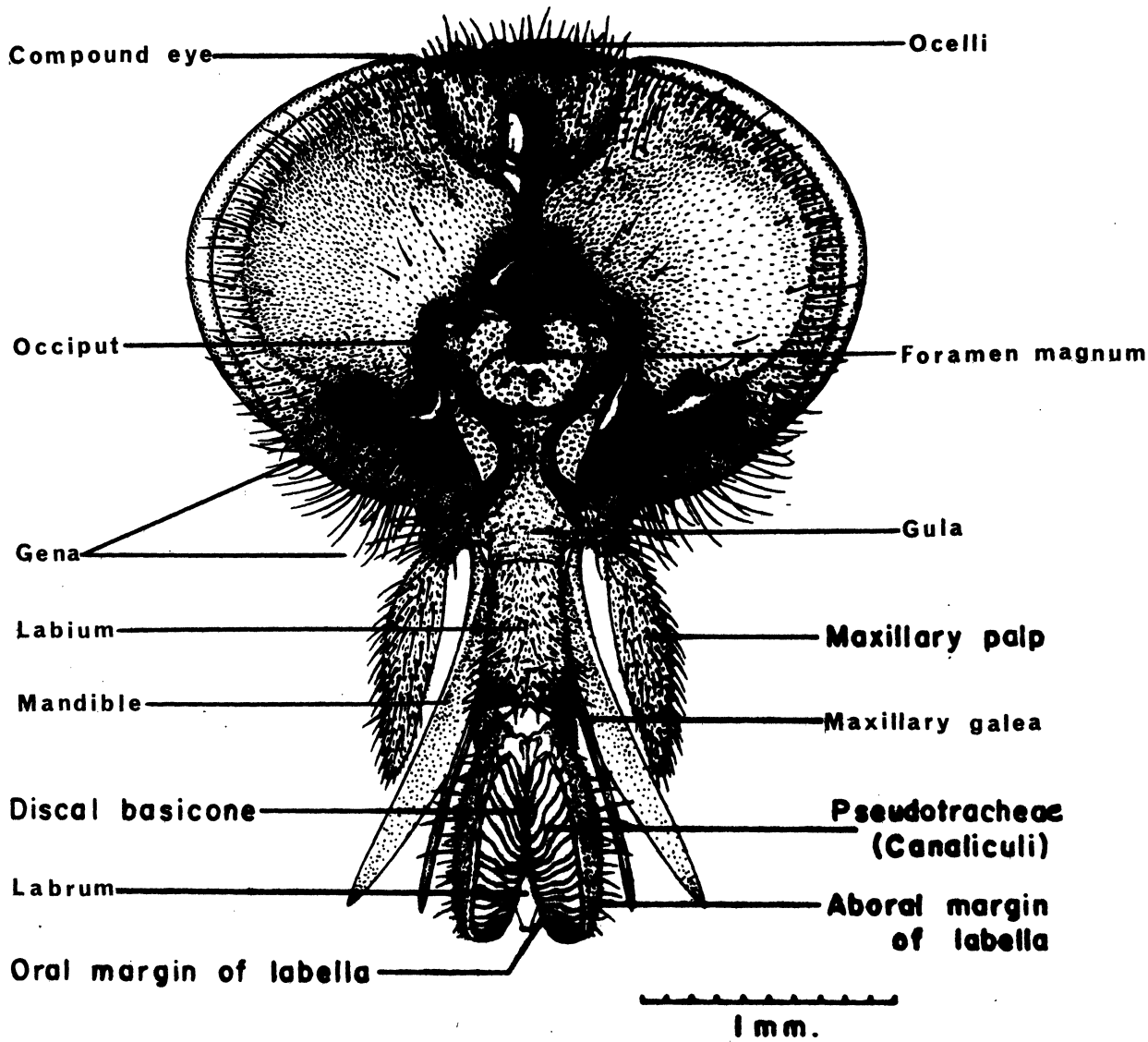


Fig. 6. Posterior view of the head capsule and mouthparts  
of a female Tabanus lineola

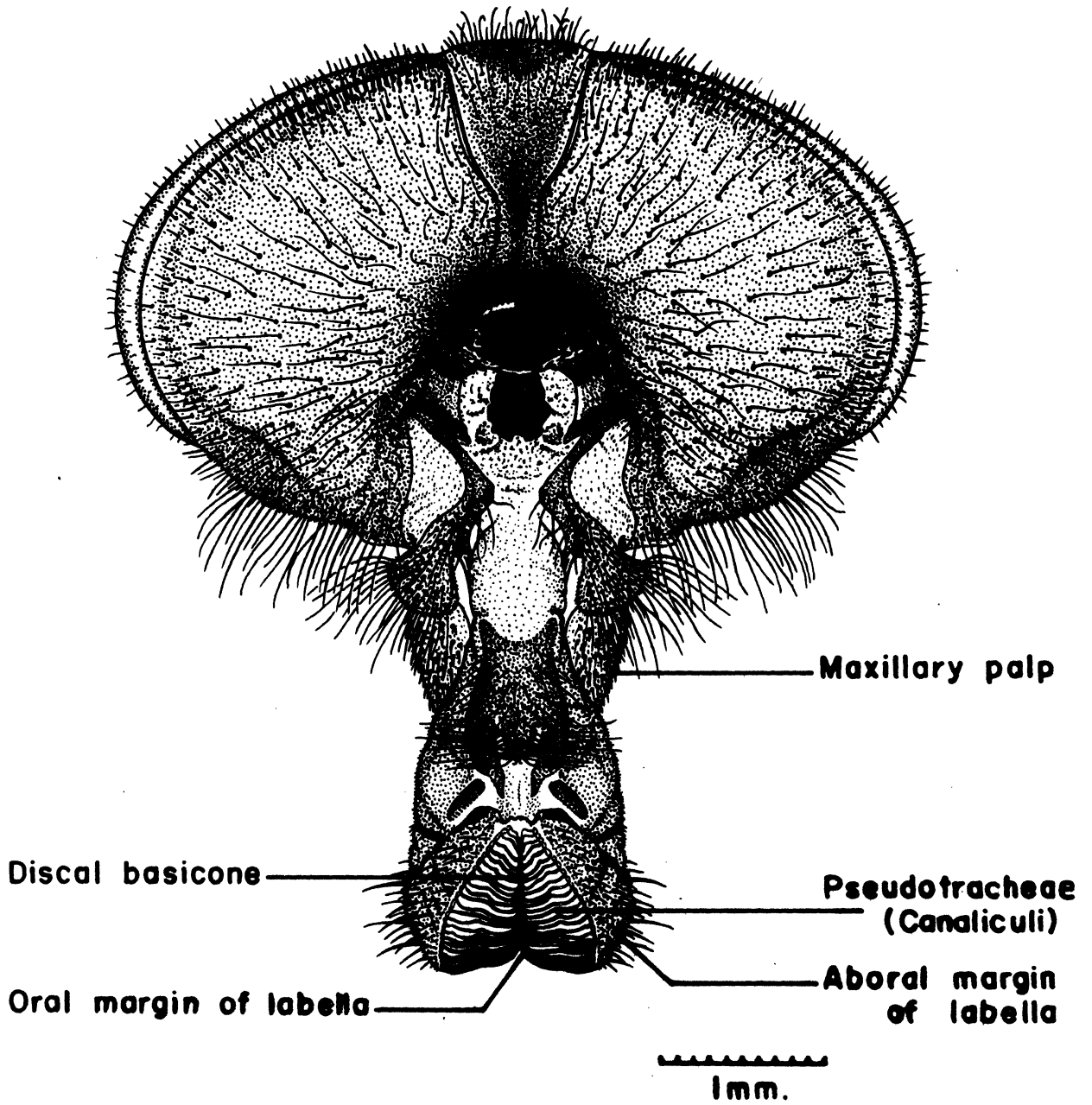
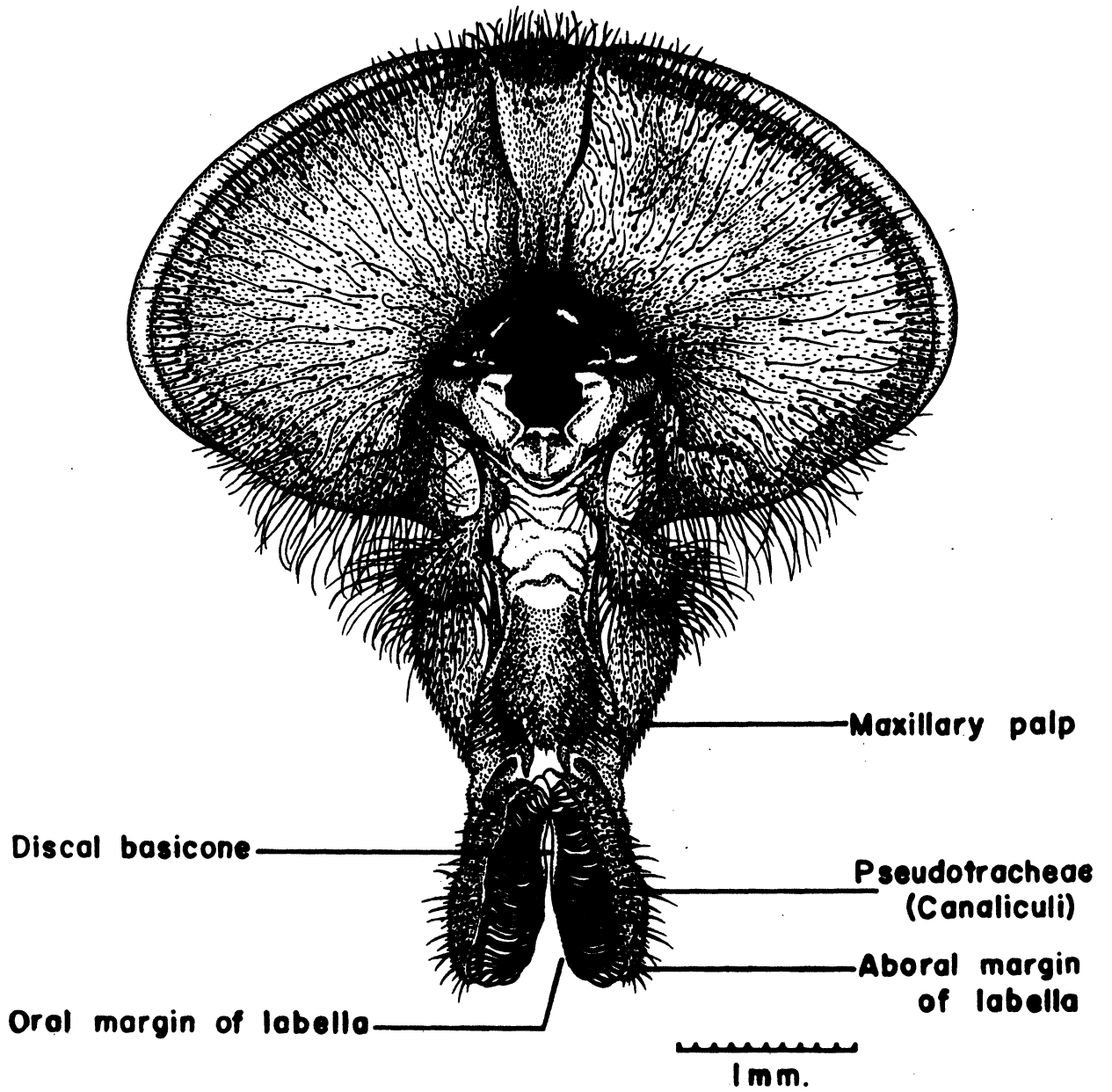


Fig. 7. Posterior view of the head capsule and mouthparts  
of a female Hybomitra lasiophthalma



Antennae of tabanids

Fig. 8. Antenna of a female Chrysops vittatus



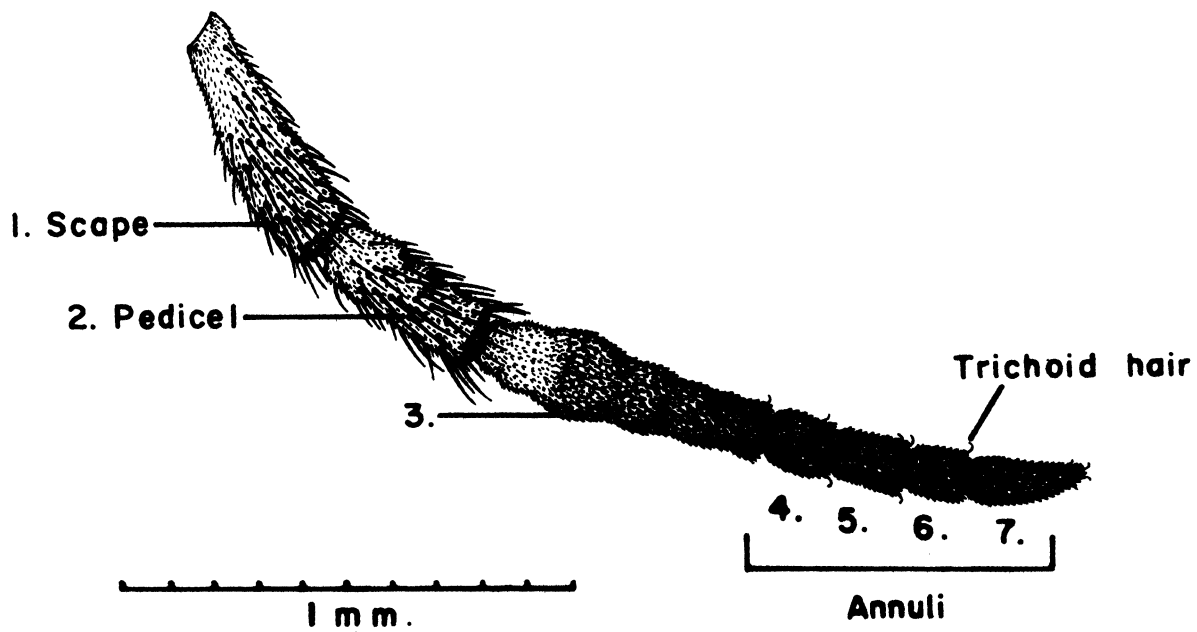


Fig. 9. Antenna of a female Tabanus lineola

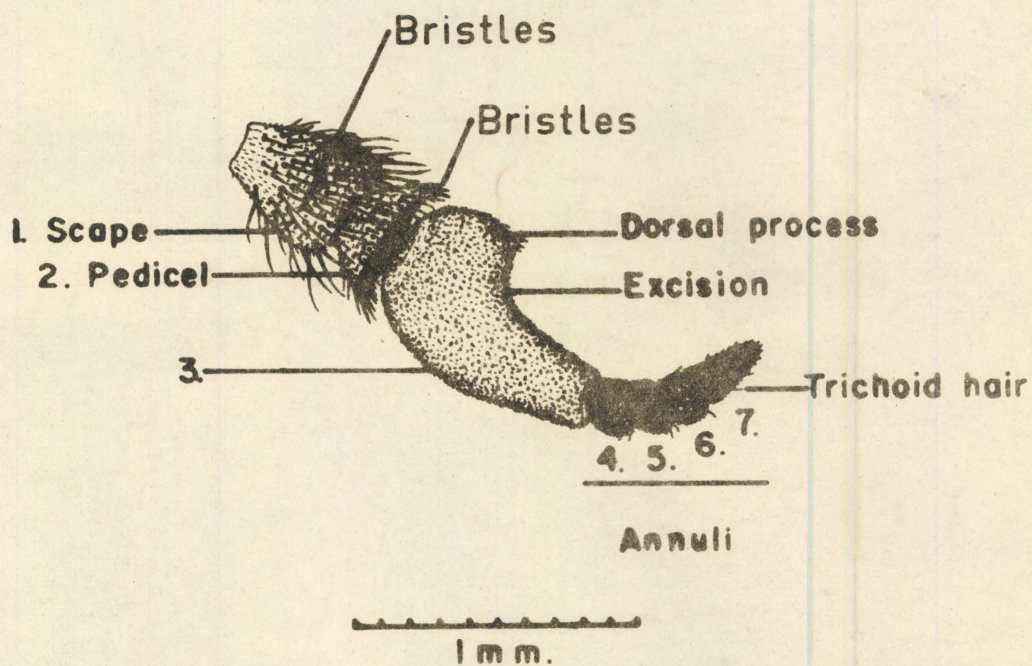
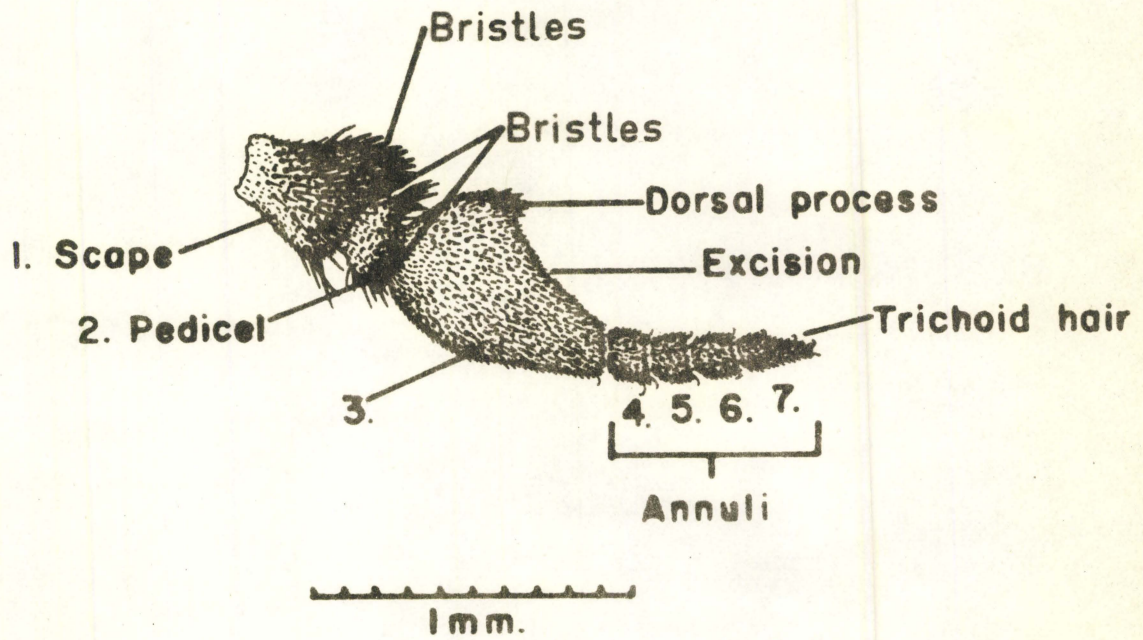


Fig. 10. Antenna of a female Hybomitra lasiophthalma



Mouthparts of tabanids

Fig. 11. Labrum of a female Chrysops vittatus:

A. Anterior view. B. Posterior view.

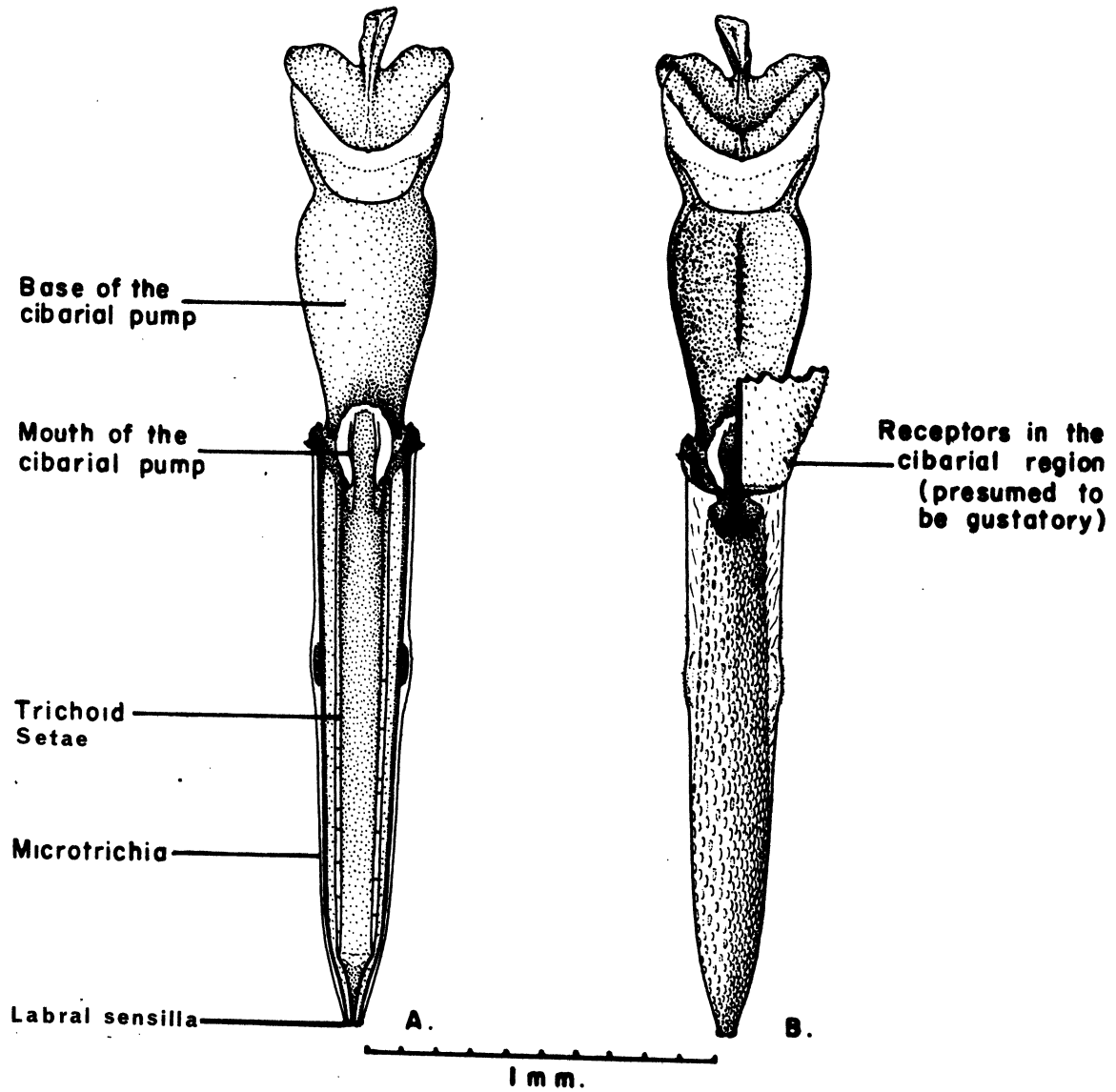




Fig. 12. Labrum of a female Tabanus lineola:

A. Anterior view. B. Posterior view.

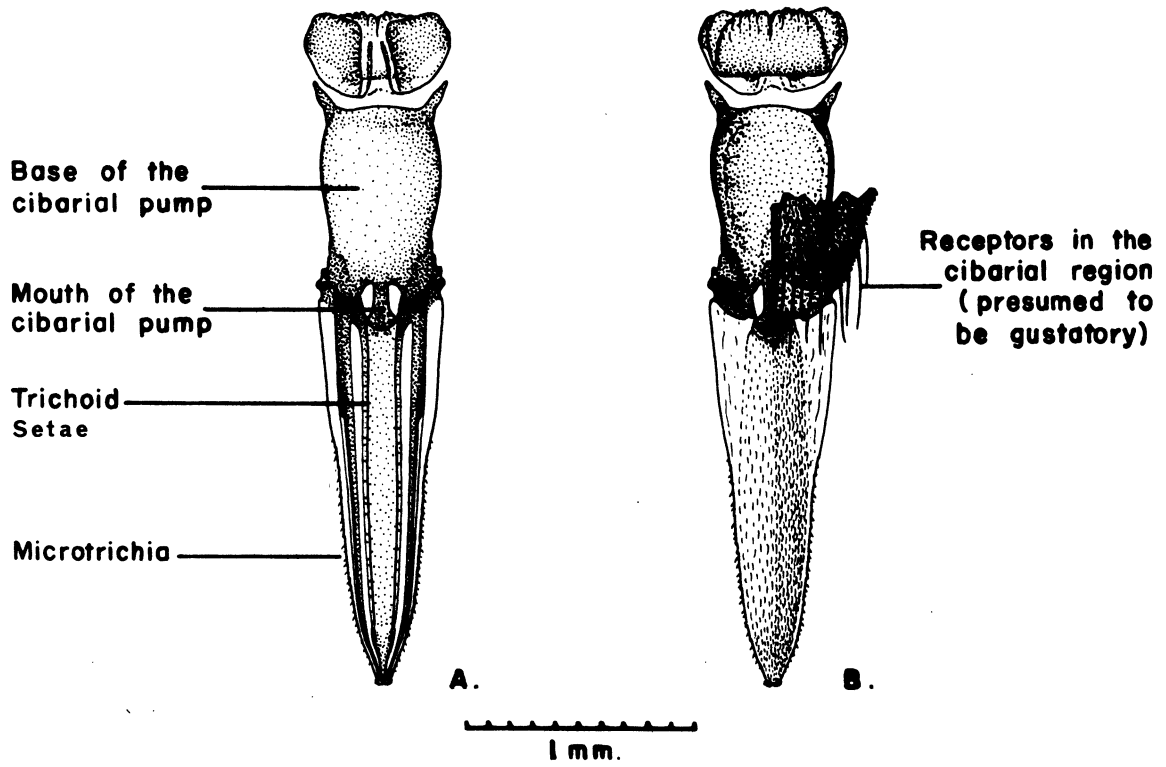


Fig. 13. Labrum of a female Hybomitra lasiophthalma

A. Anterior view. B. Posterior view.

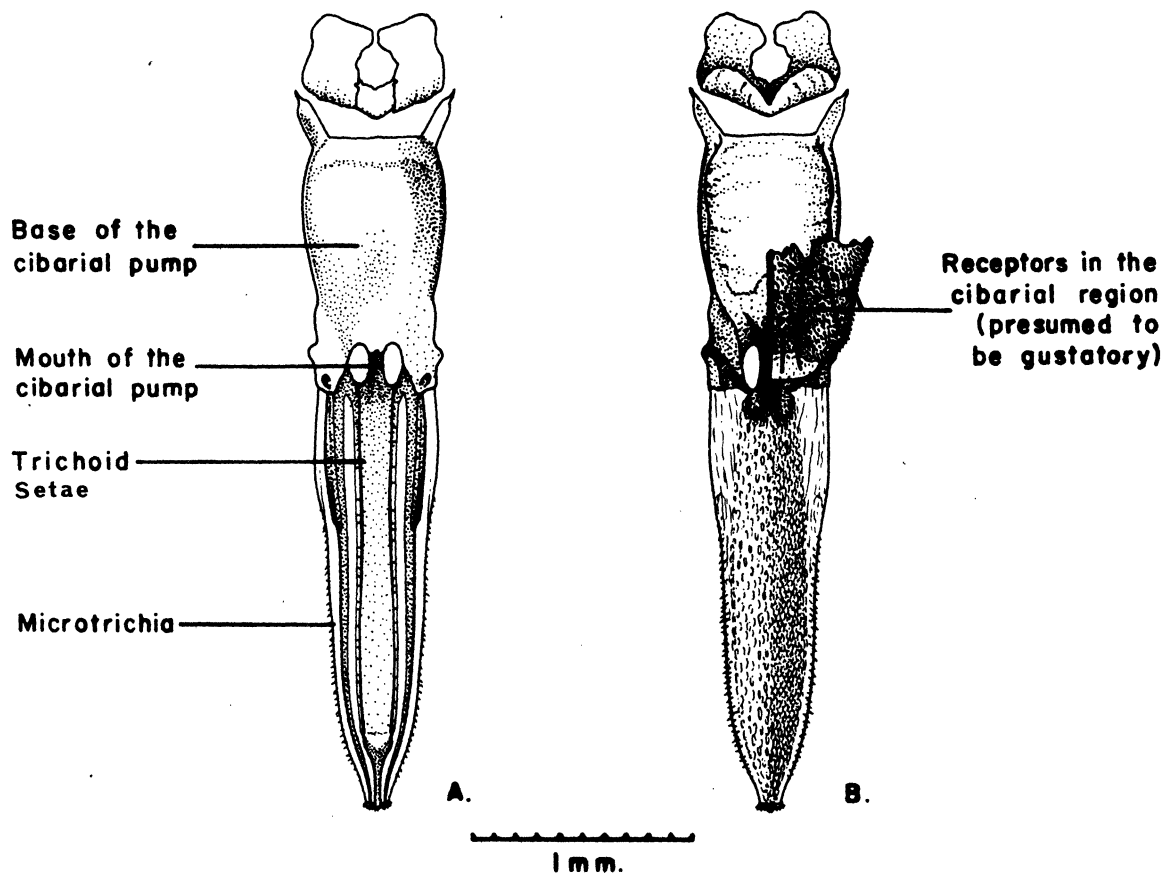
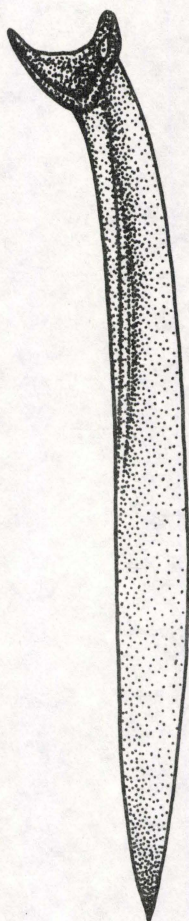


Fig. 14. Right mandible of a female Chrysops vittatus  
(anterior view)



1 mm.

Fig. 15. Right mandible of a female Tabanus lineola  
(anterior view)

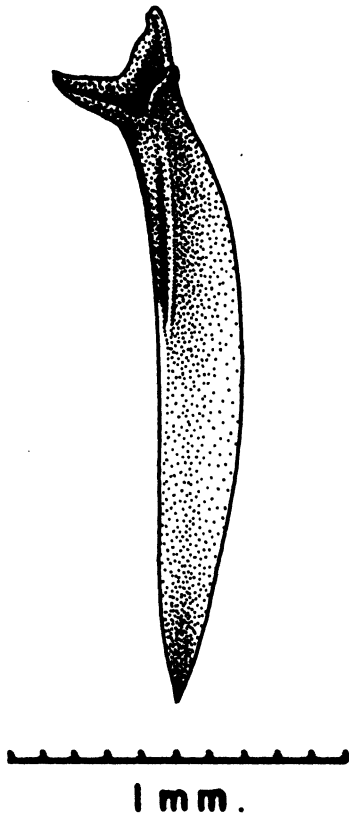
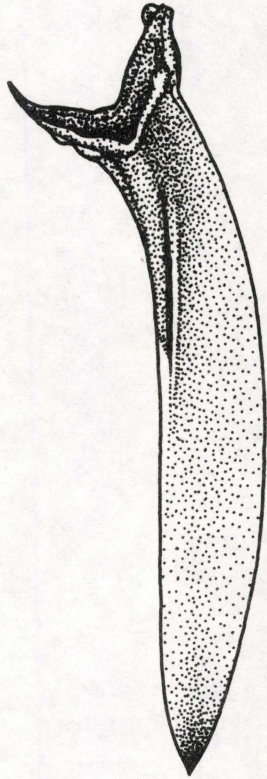




Fig. 16. Right mandible of a female Hybomitra lasiophthalma  
(anterior view)



1 mm.

Fig. 17. Maxilla of a female Chrysops vittatus:

A. Right maxillary palp and galea (right side)

B. Left maxillary galea (right side)

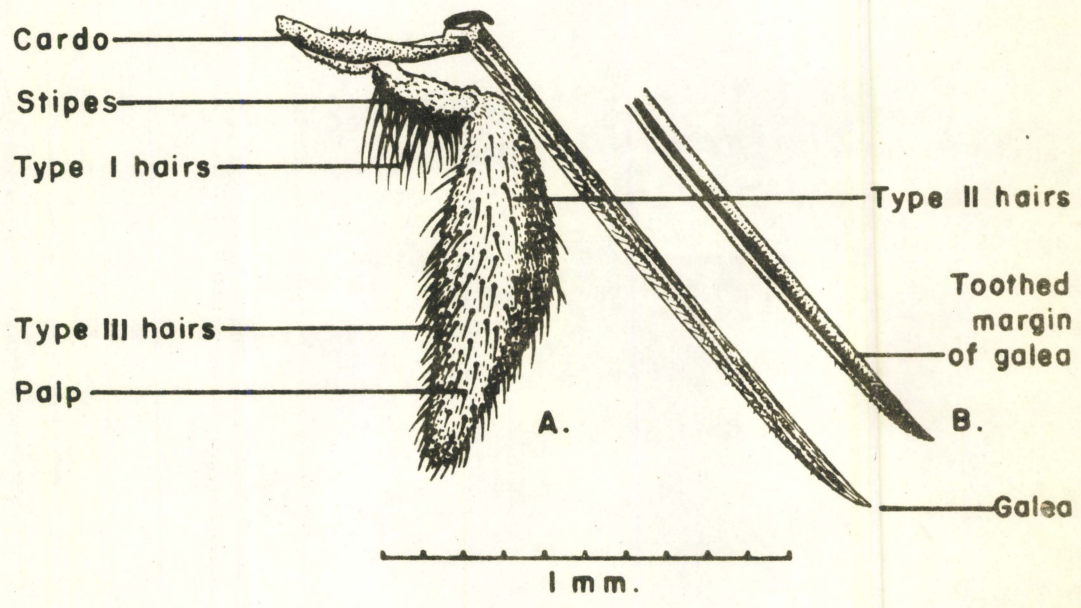


Fig. 18. Maxilla of a female Tabanus lineola:

A. Right maxillary palp and galea (right side)

B. Left maxillary galea (right side)

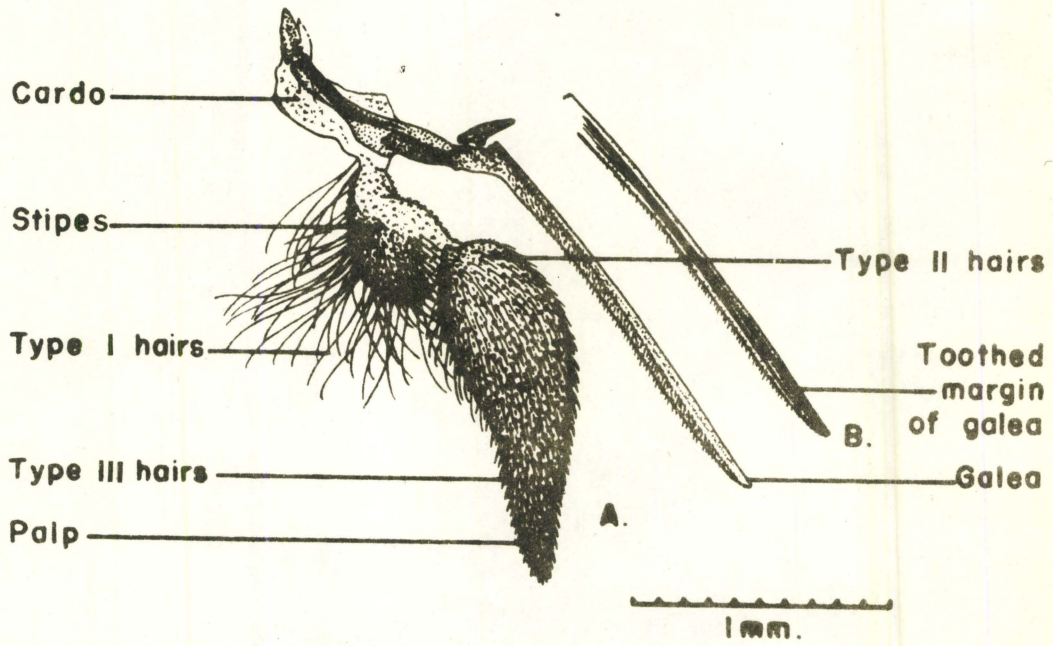


Fig. 19. Maxilla of a female Hybomitra lasiophthalma:

A. Right maxillary palp and galea (right side)

B. Left galea (right side)

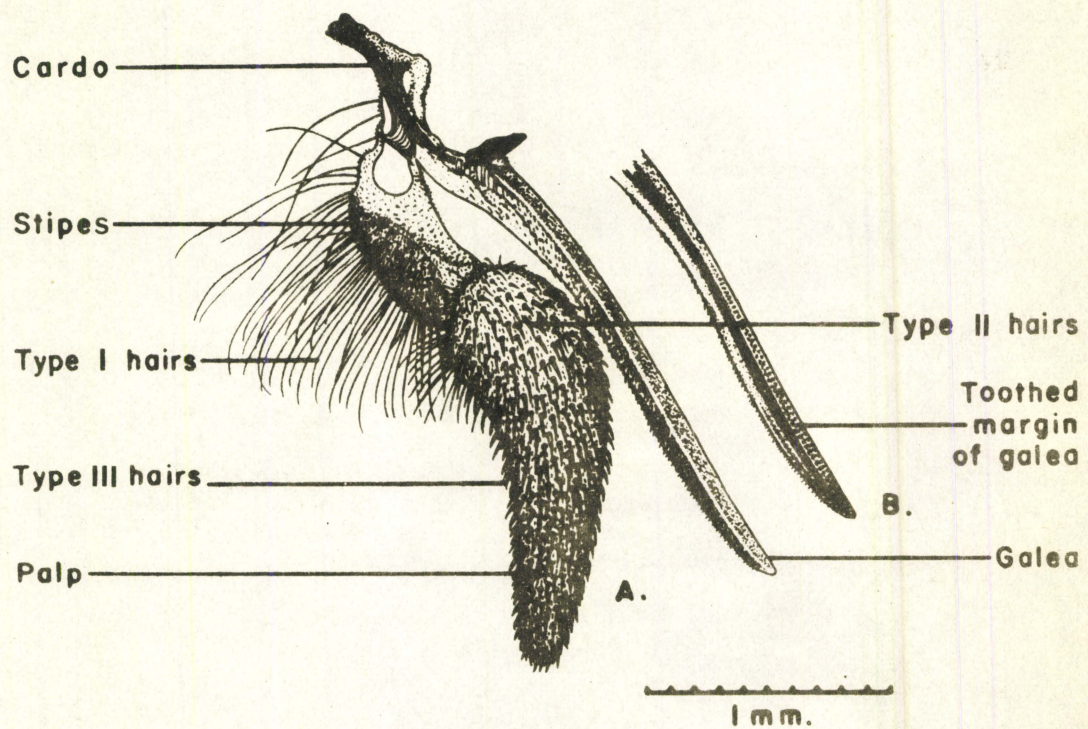




Fig. 20. Hypopharynx of a female Chrysops vittatus  
(anterior view)

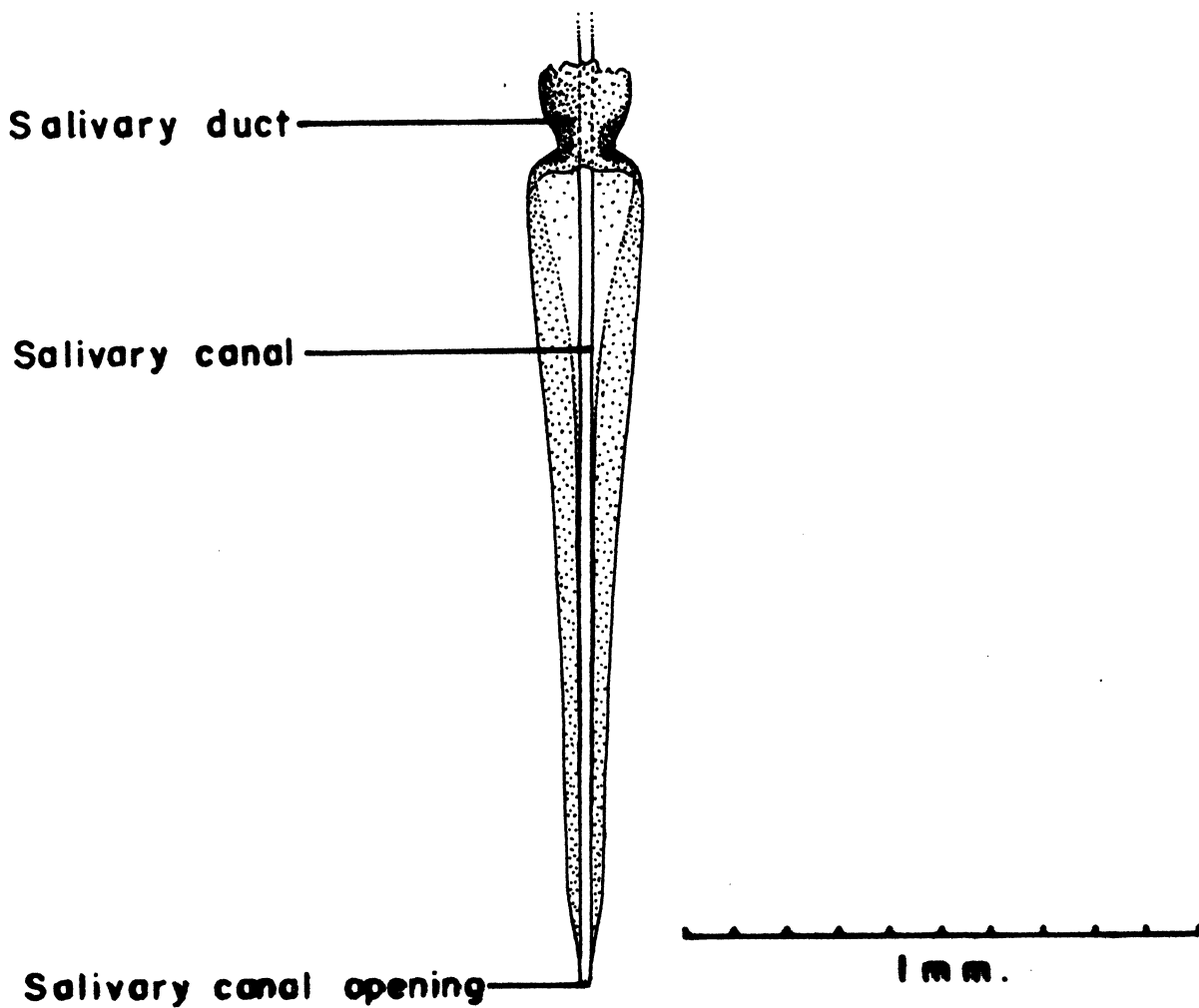


Fig. 21. Hypopharynx of a female Tabanus lineola

(anterior view)

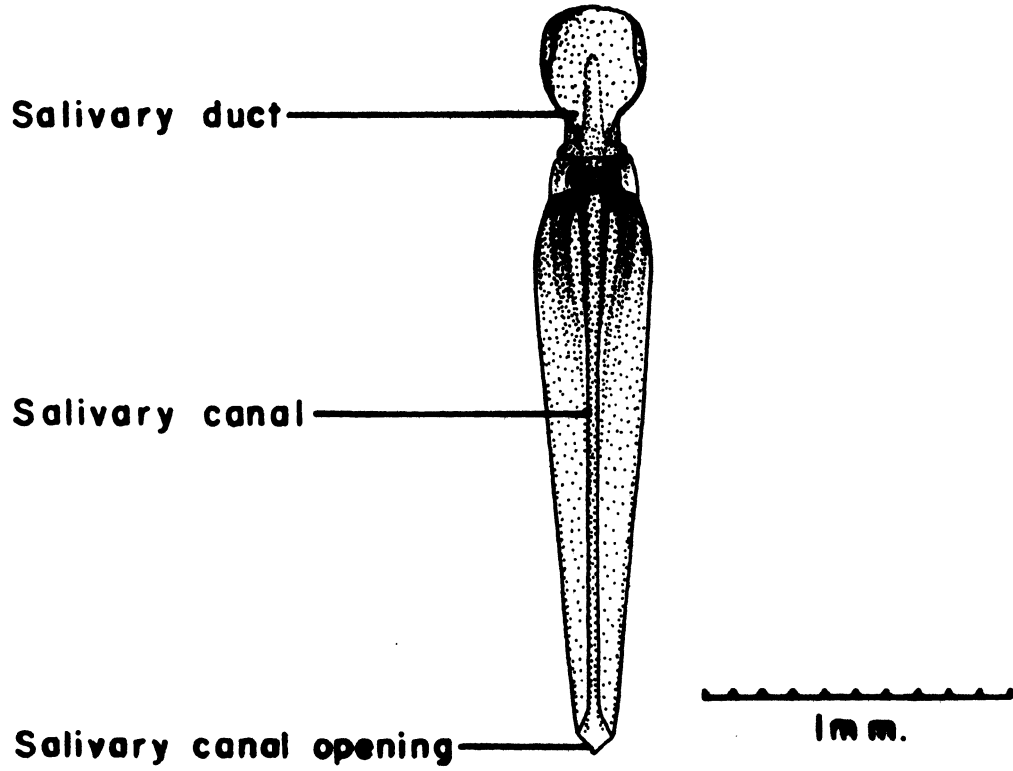


Fig. 22. Hypopharynx of a female Hybomitra lasiophthalma  
(anterior view)

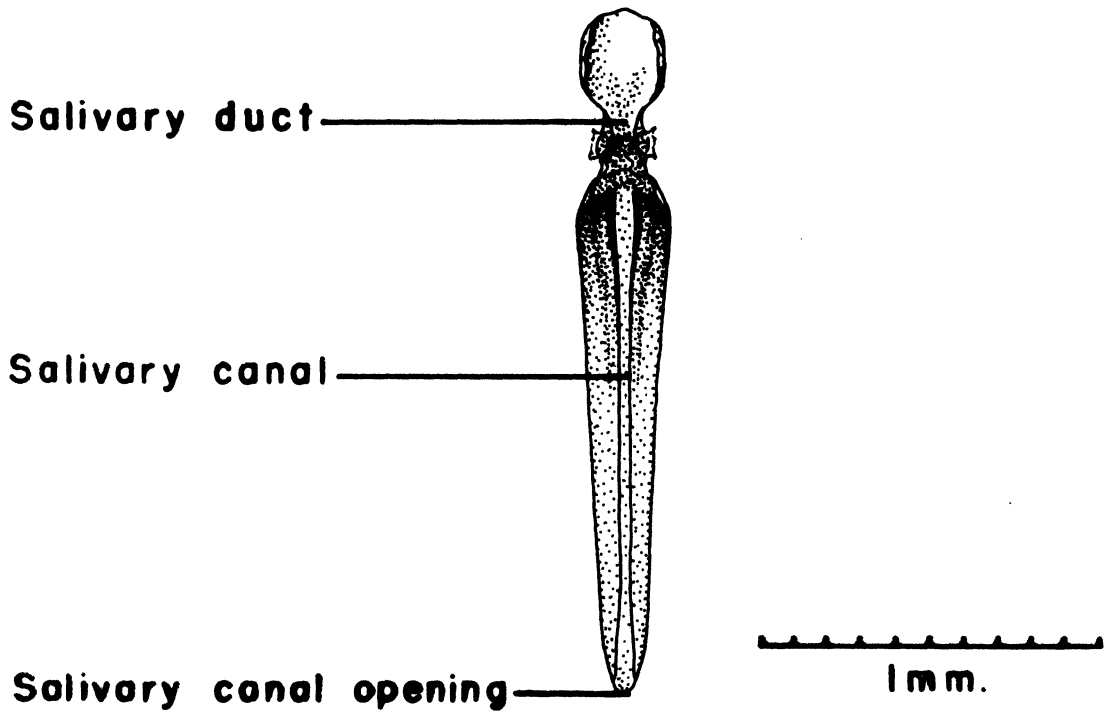


Fig. 23. Anterior view of the labium of a female

Chrysops vittatus

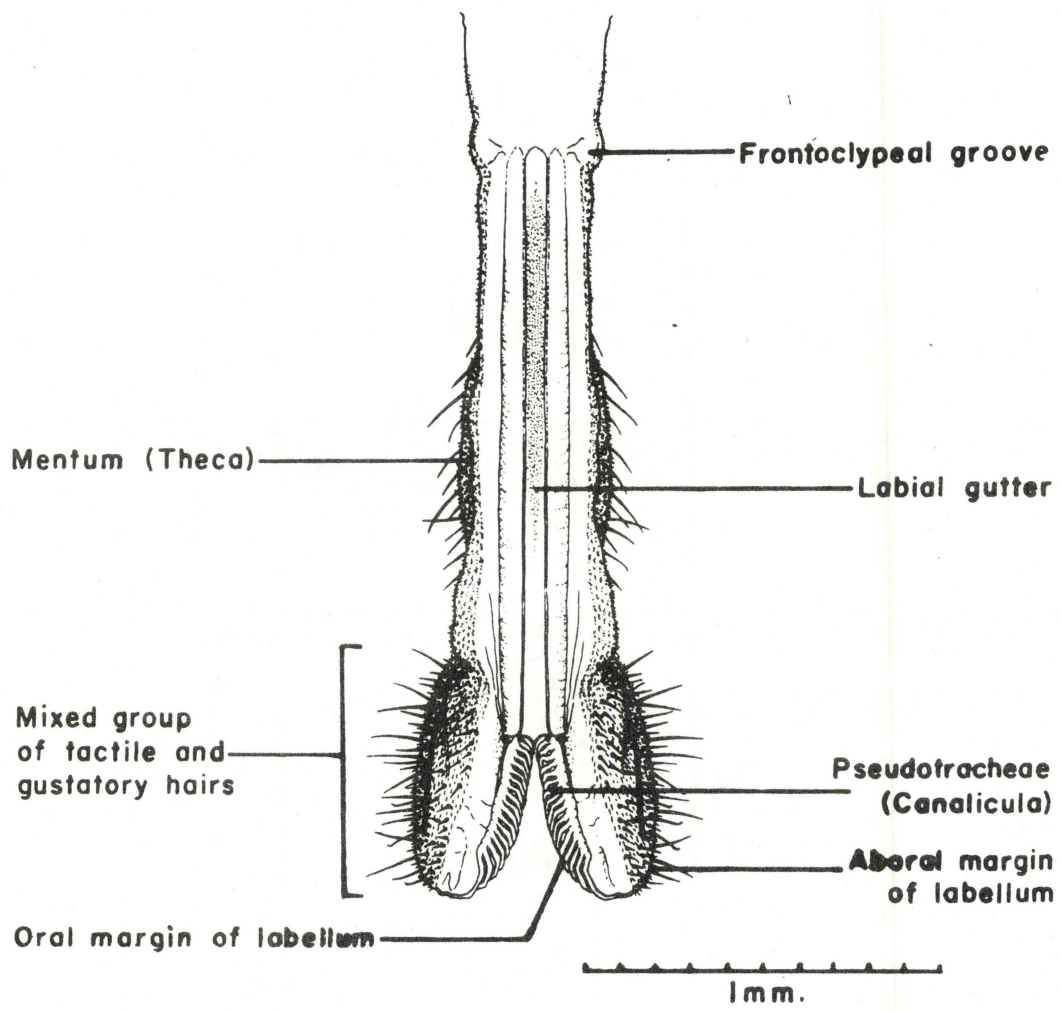




Fig. 24. Anterior view of the labium of a female

Tabanus lineola

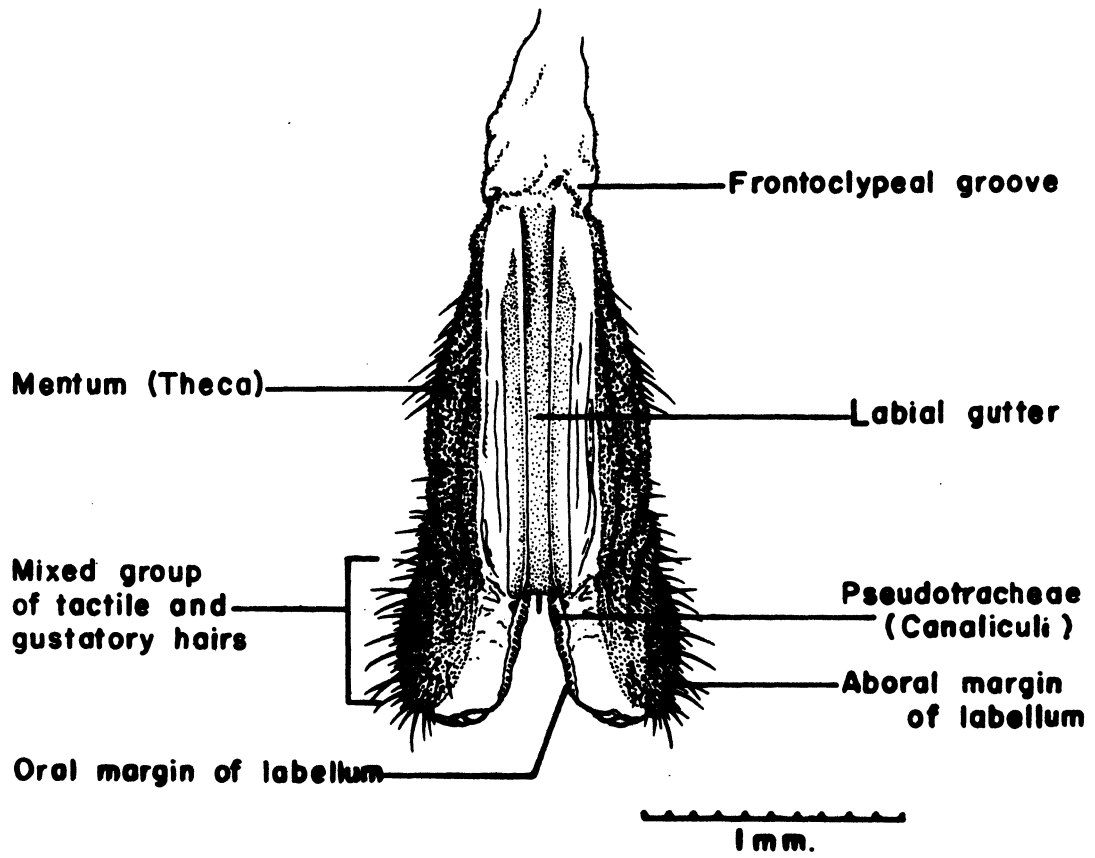


Fig. 25. Anterior view of the labium of a female

Hybomitra lasiophthalma

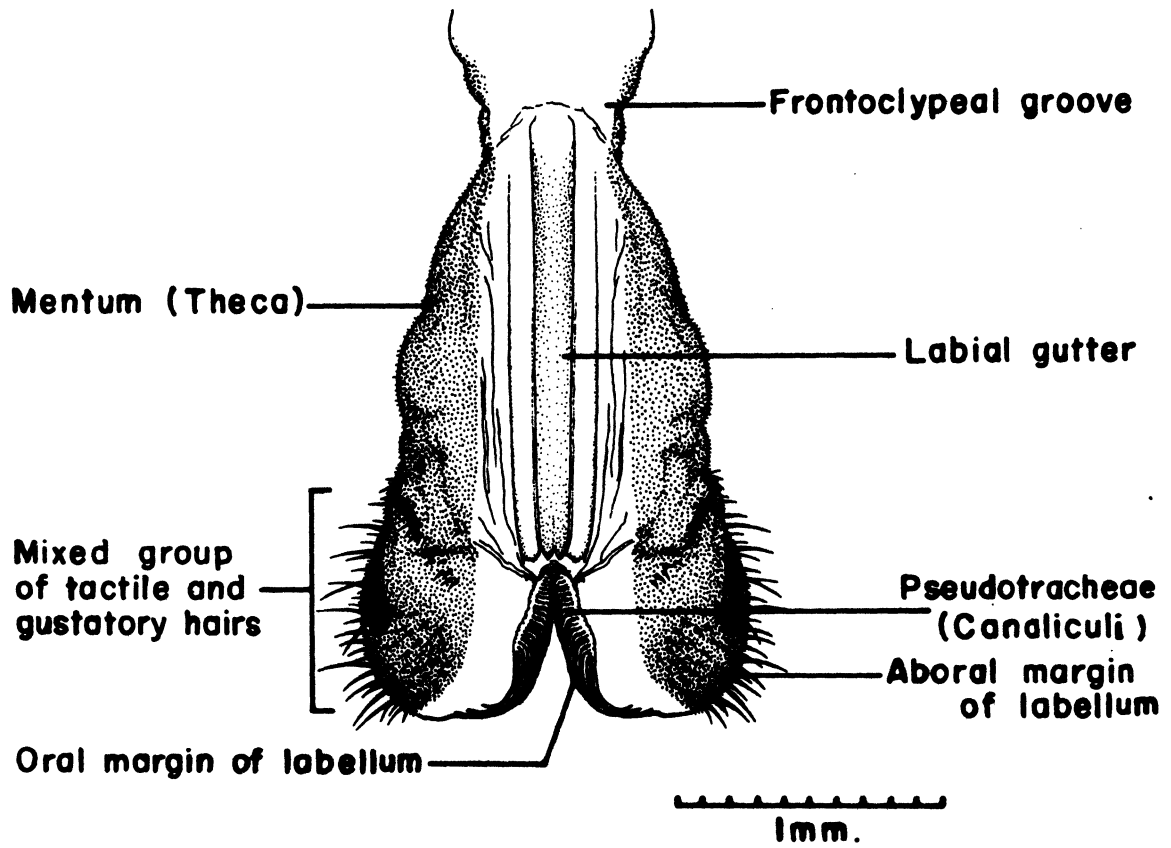


Fig. 26. Posterior view of the labium of a female

Chrysops vittatus

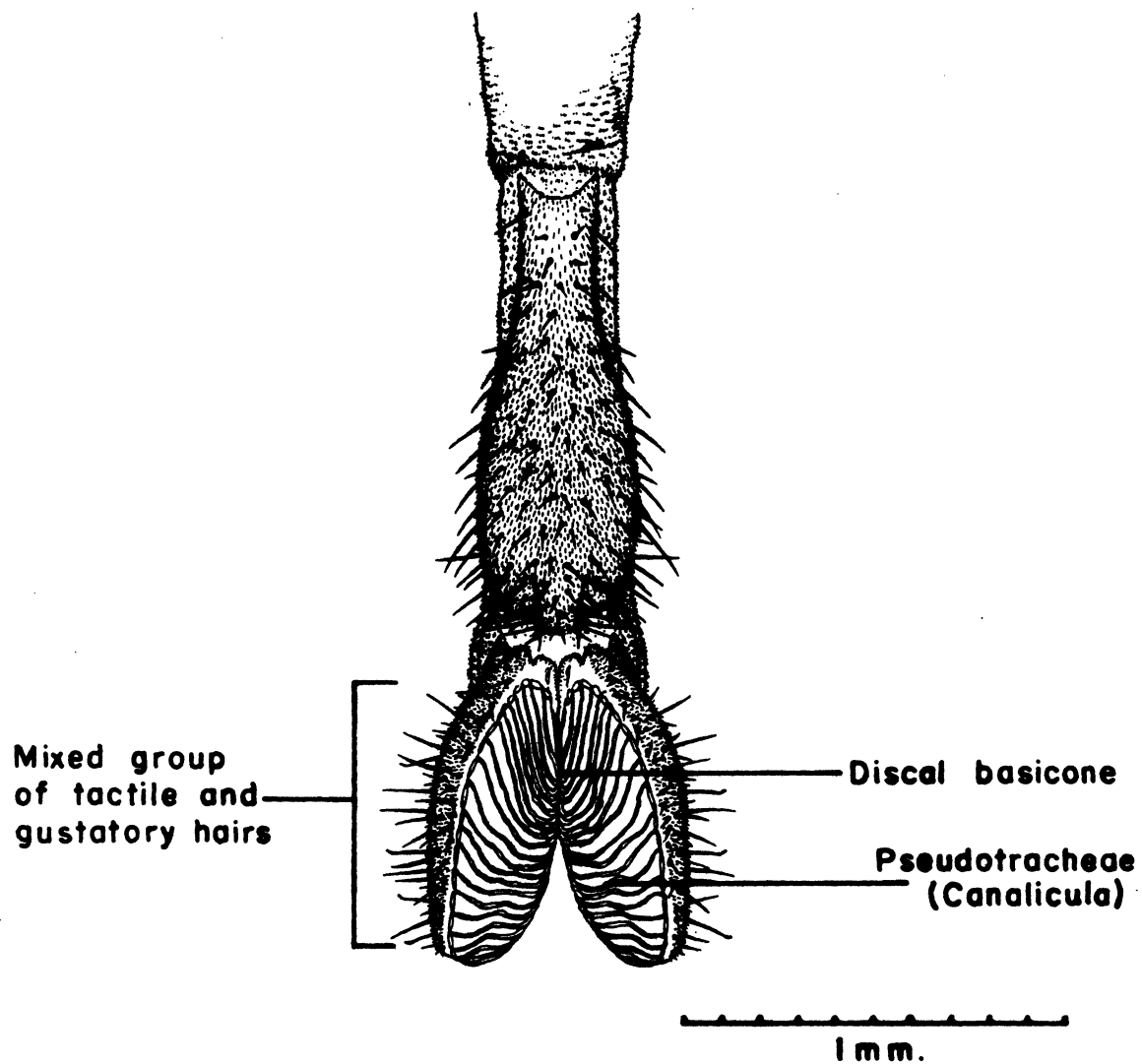


Fig. 27. Posterior view of the labium of a female

Tabanus lineola

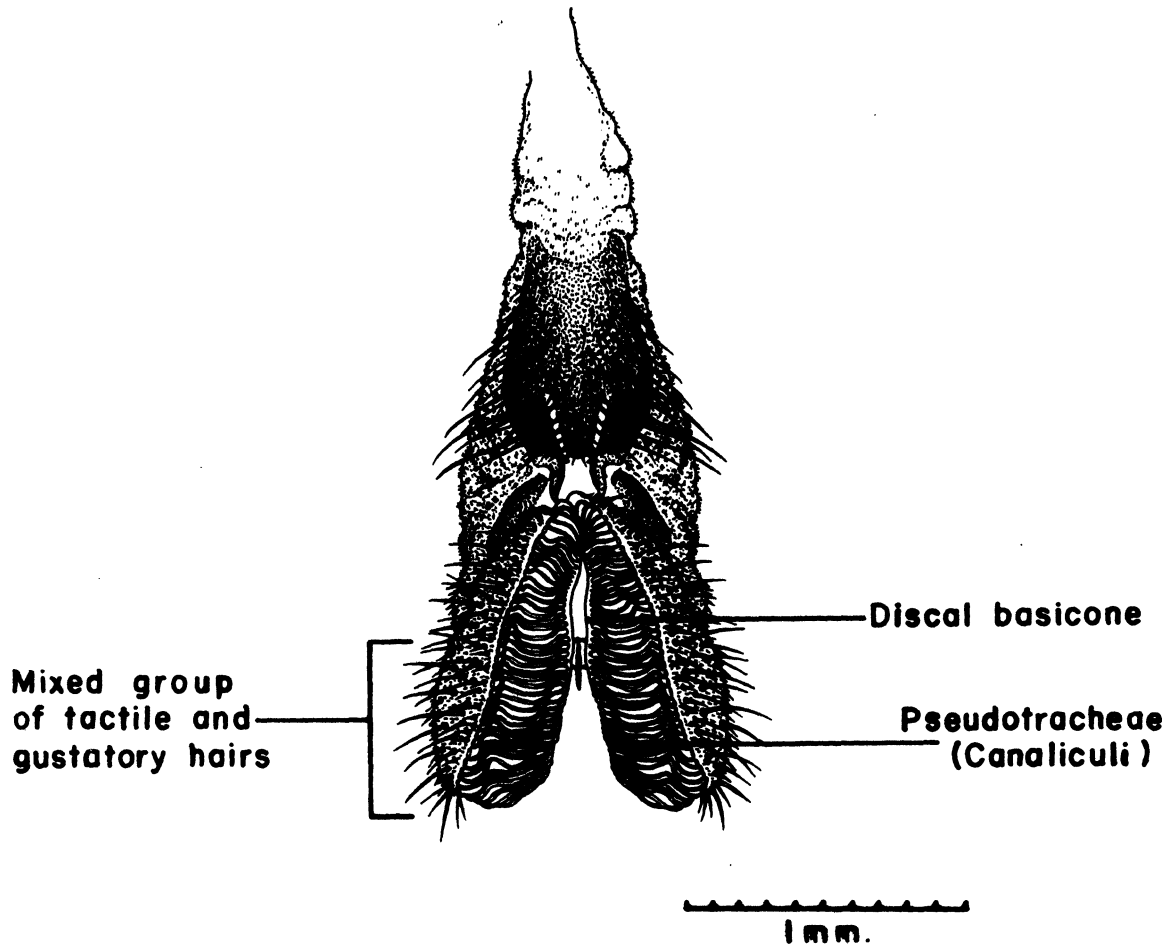
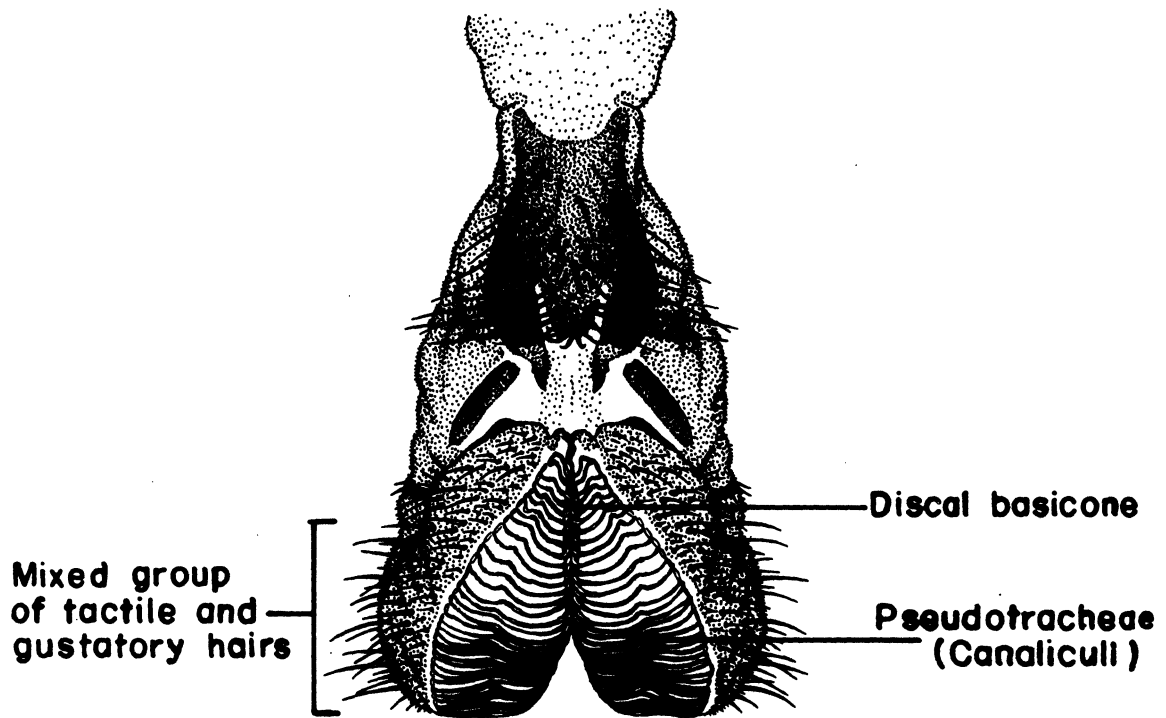




Fig. 28. Posterior view of the labium of a female

Hybomitra lasiophthlana



1 mm.

Fig. 29. Anterior view of the labella of a female  
Chrysops vittatus showing the four principal  
hair types. Types B and C are gustatory.

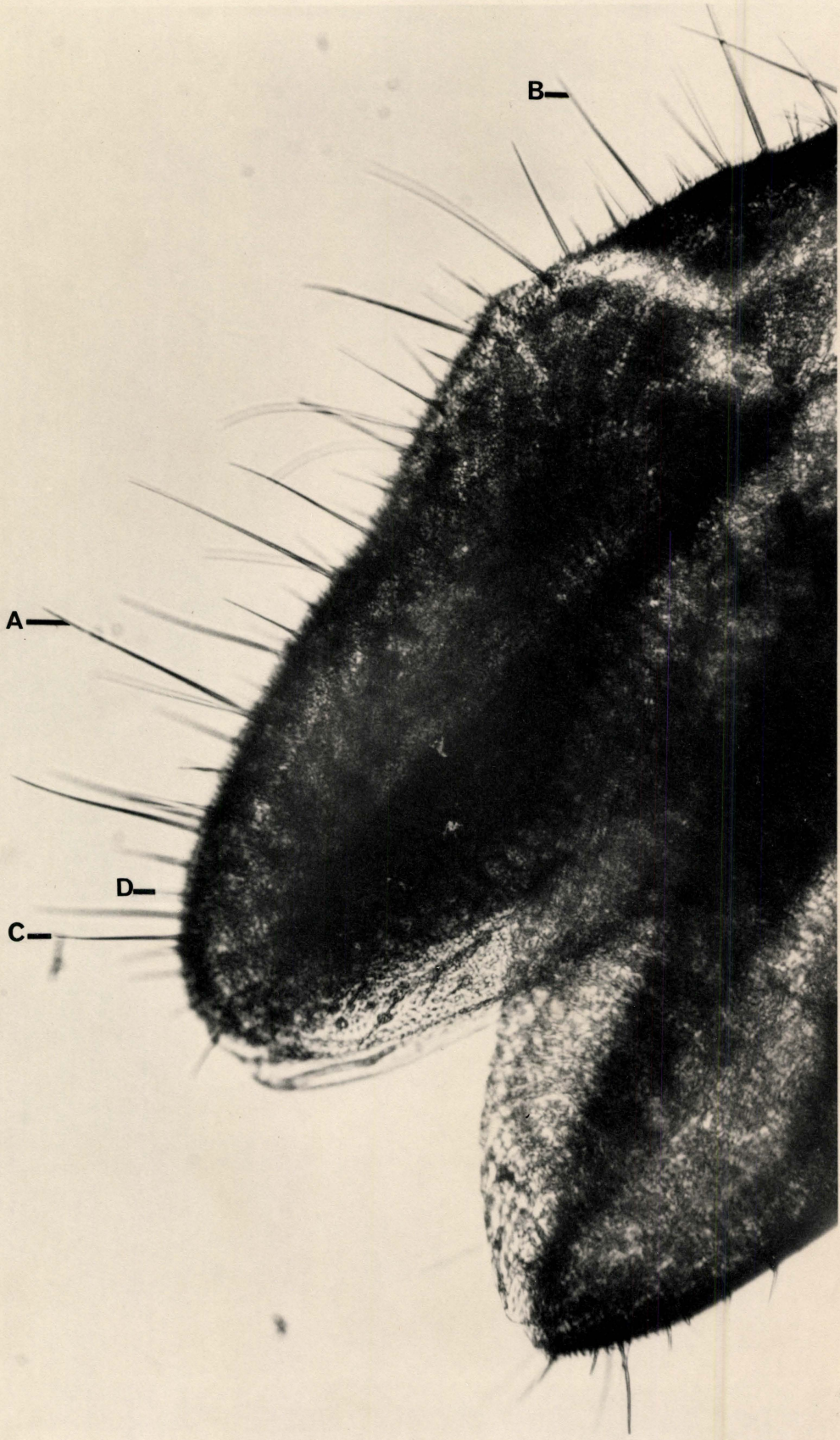
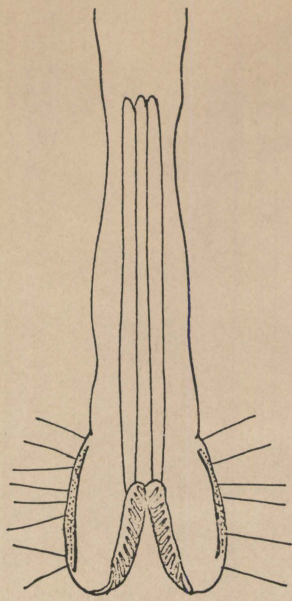
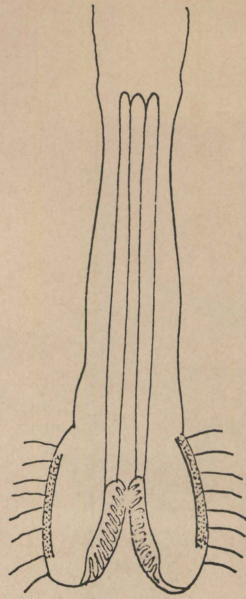


Fig. 30. Labellar sensilla of a female Chrysops vittatus.

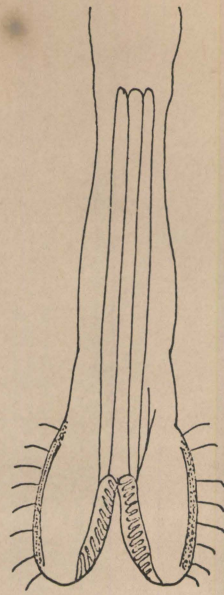
Type B and C sensilla are gustatory.



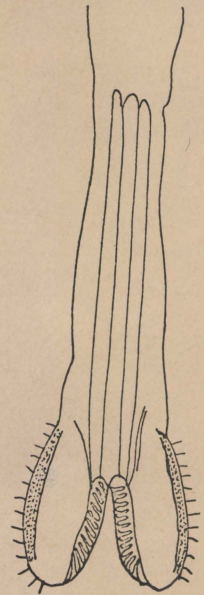
A



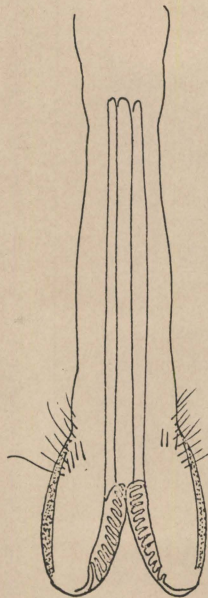
B



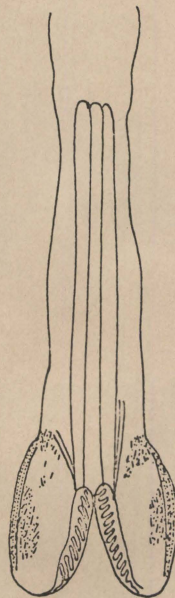
C



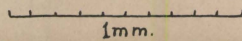
D



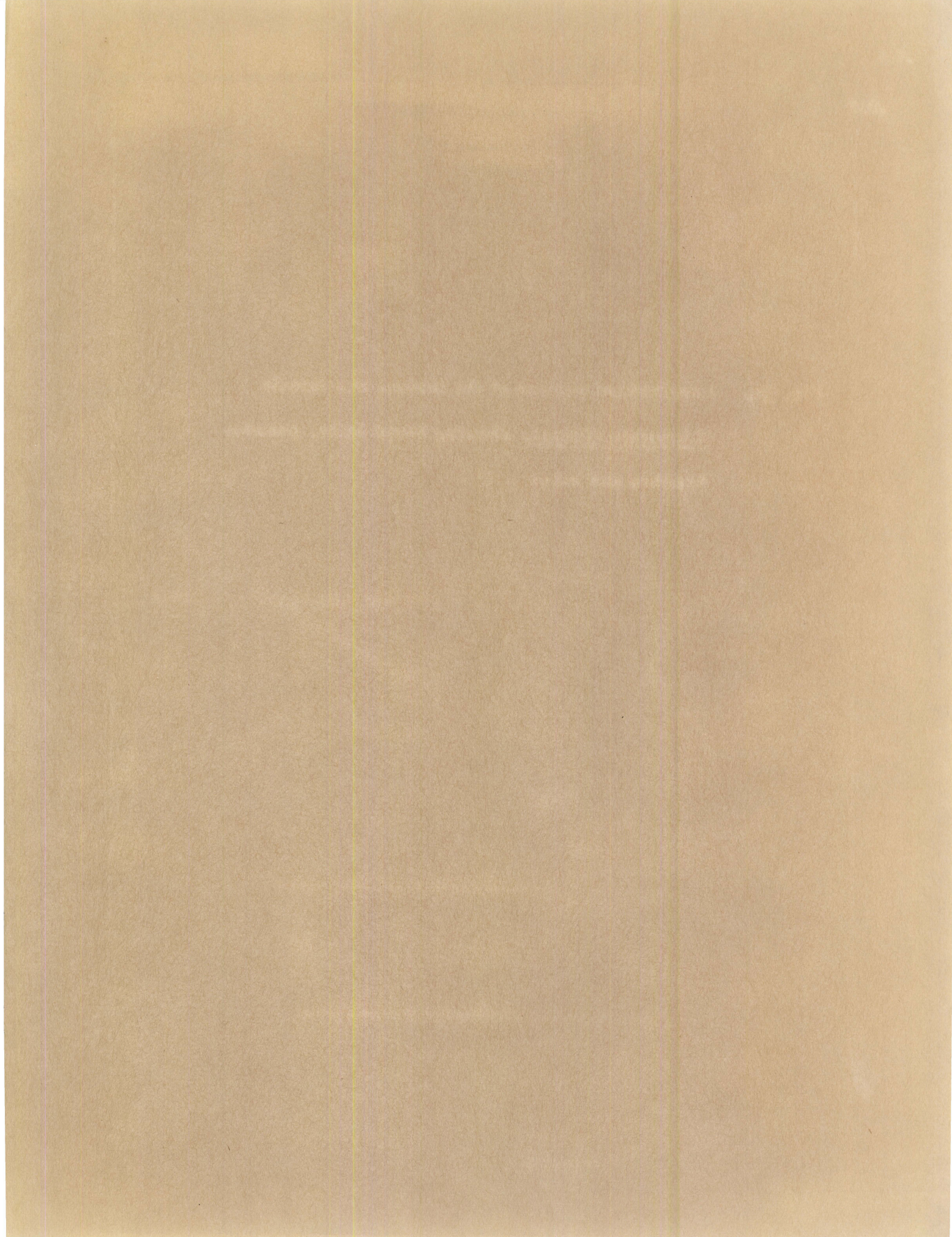
E

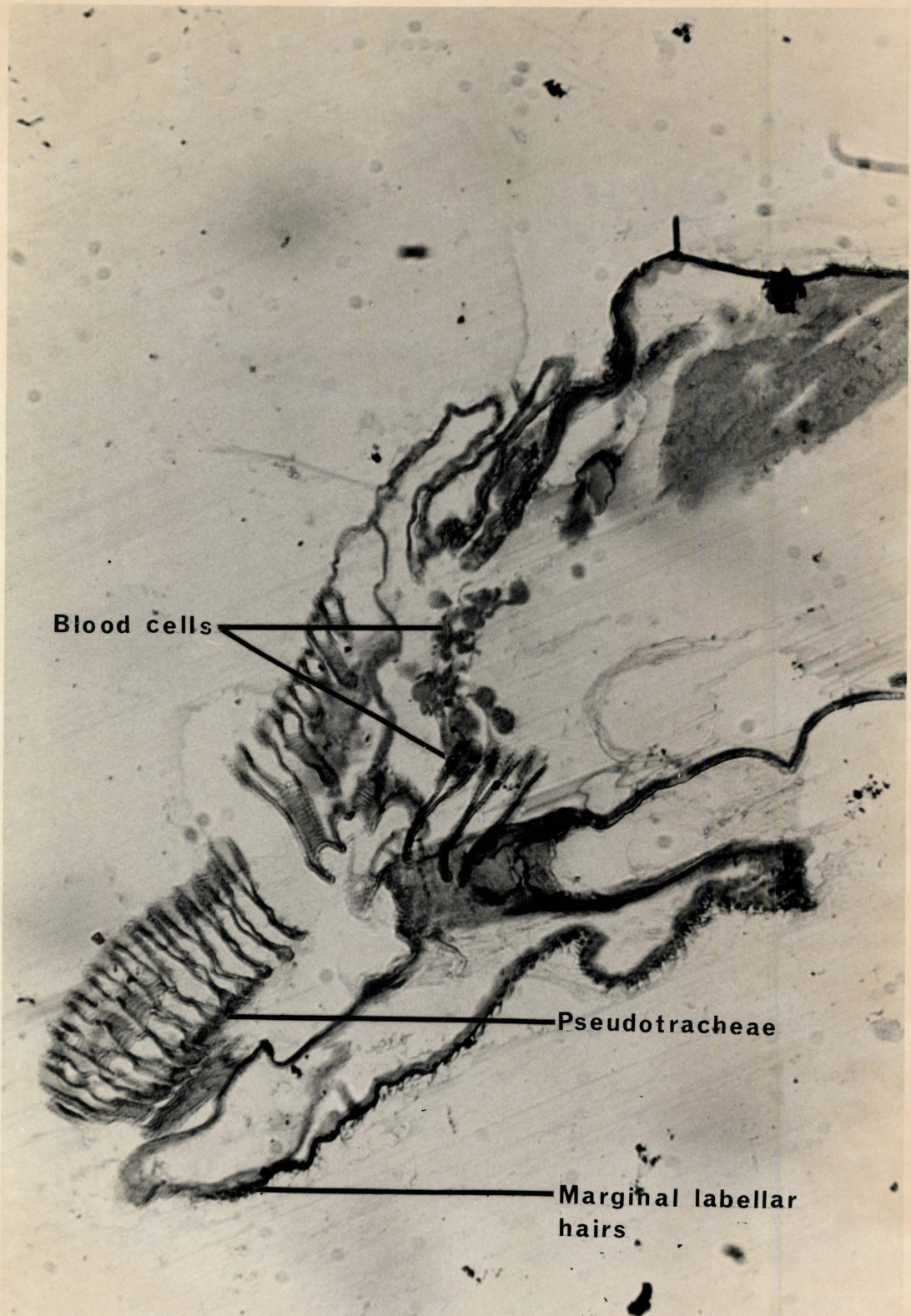


F



1mm.





Blood cells

Pseudotracheae

Marginal labellar  
hairs



Fig. 32. Left foreleg of a female Chrysops vittatus  
showing the distribution of sensilla (lateral view).  
Tarsal segments are numbered 1-5.

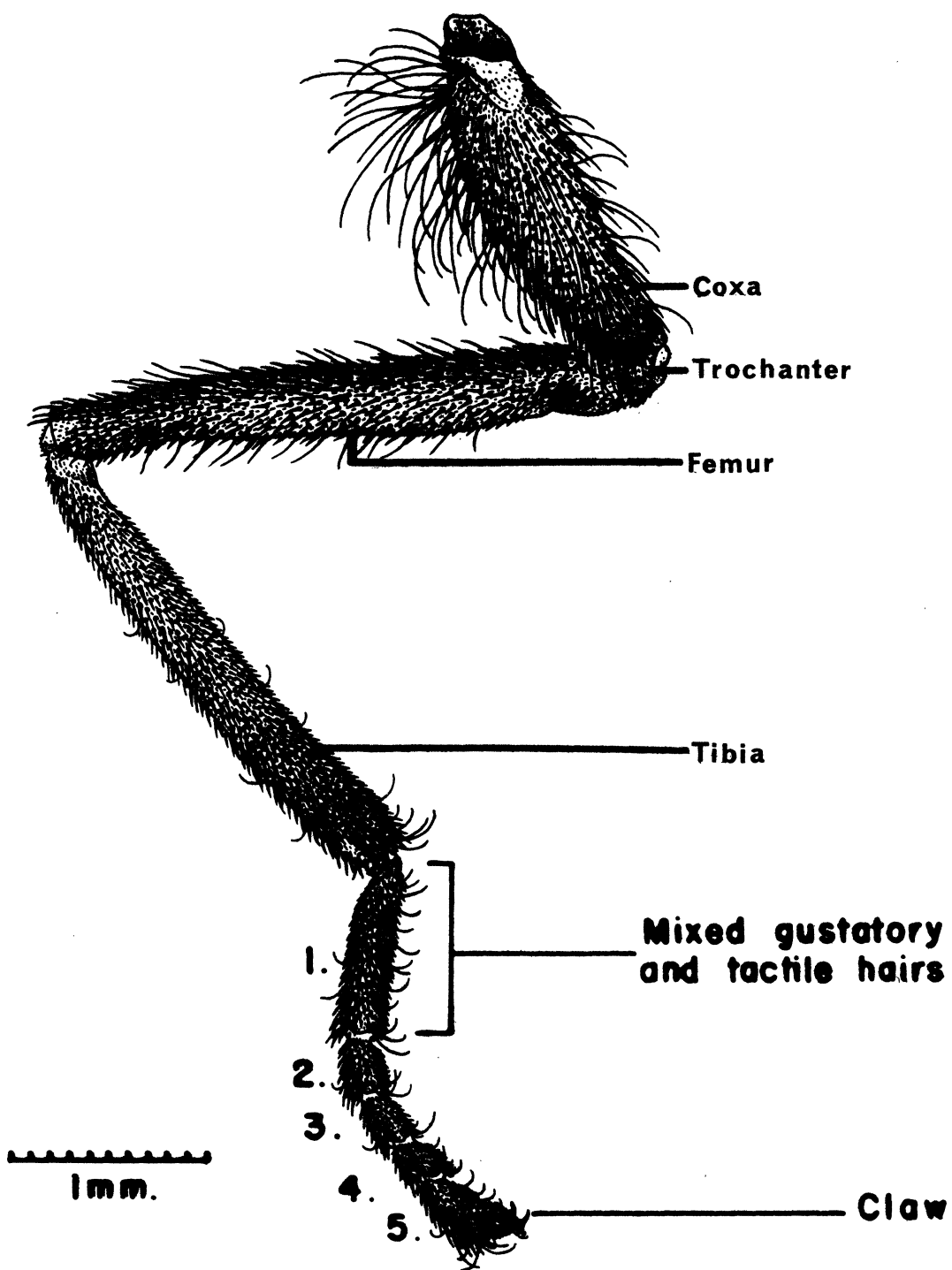


Fig. 33. Left foreleg of a female Tabanus lineola  
showing the distribution of sensilla (lateral view).

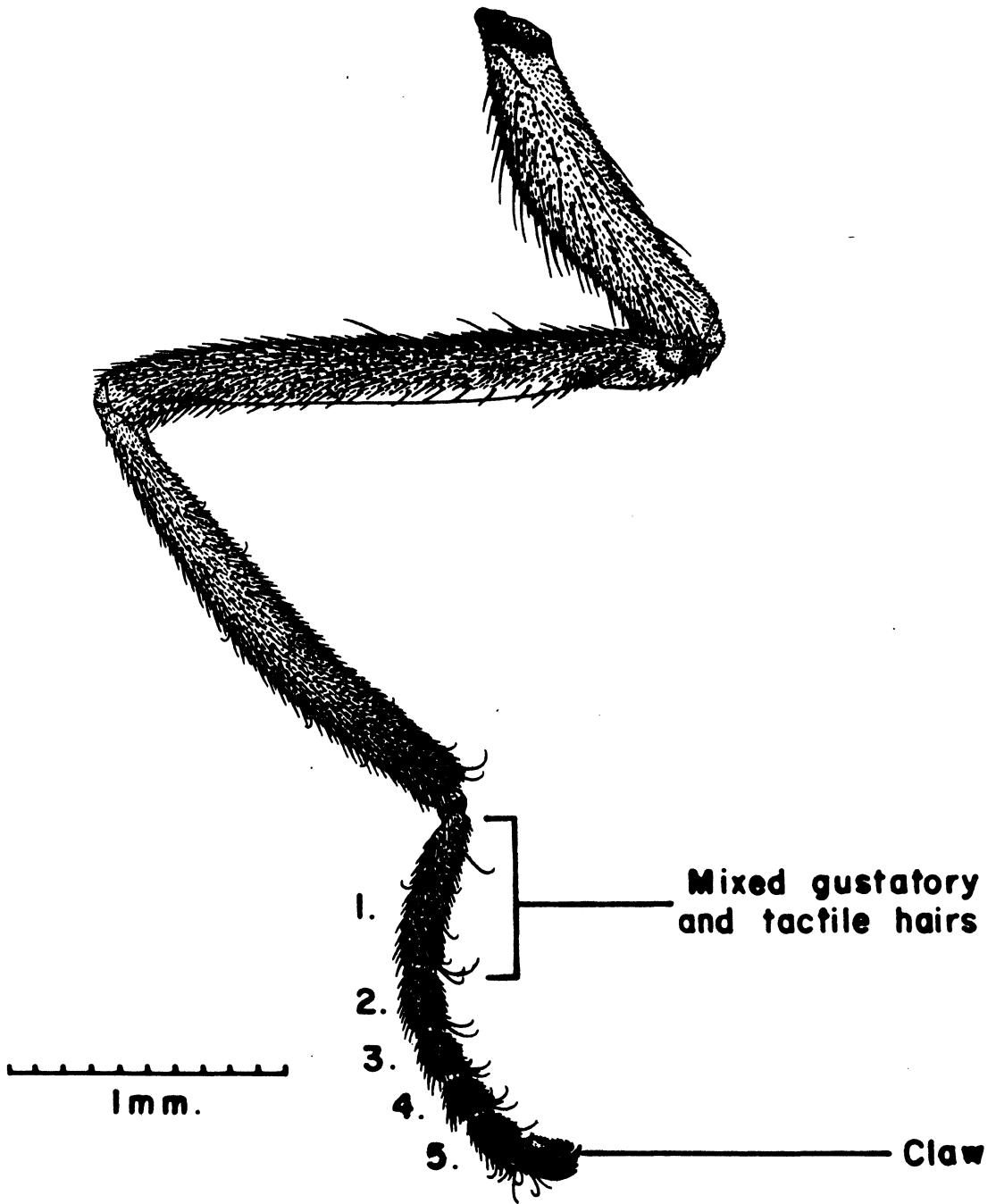


Fig. 34. Left foreleg of a female Hybomitra lasiophthalma  
showing the distribution of sensilla.

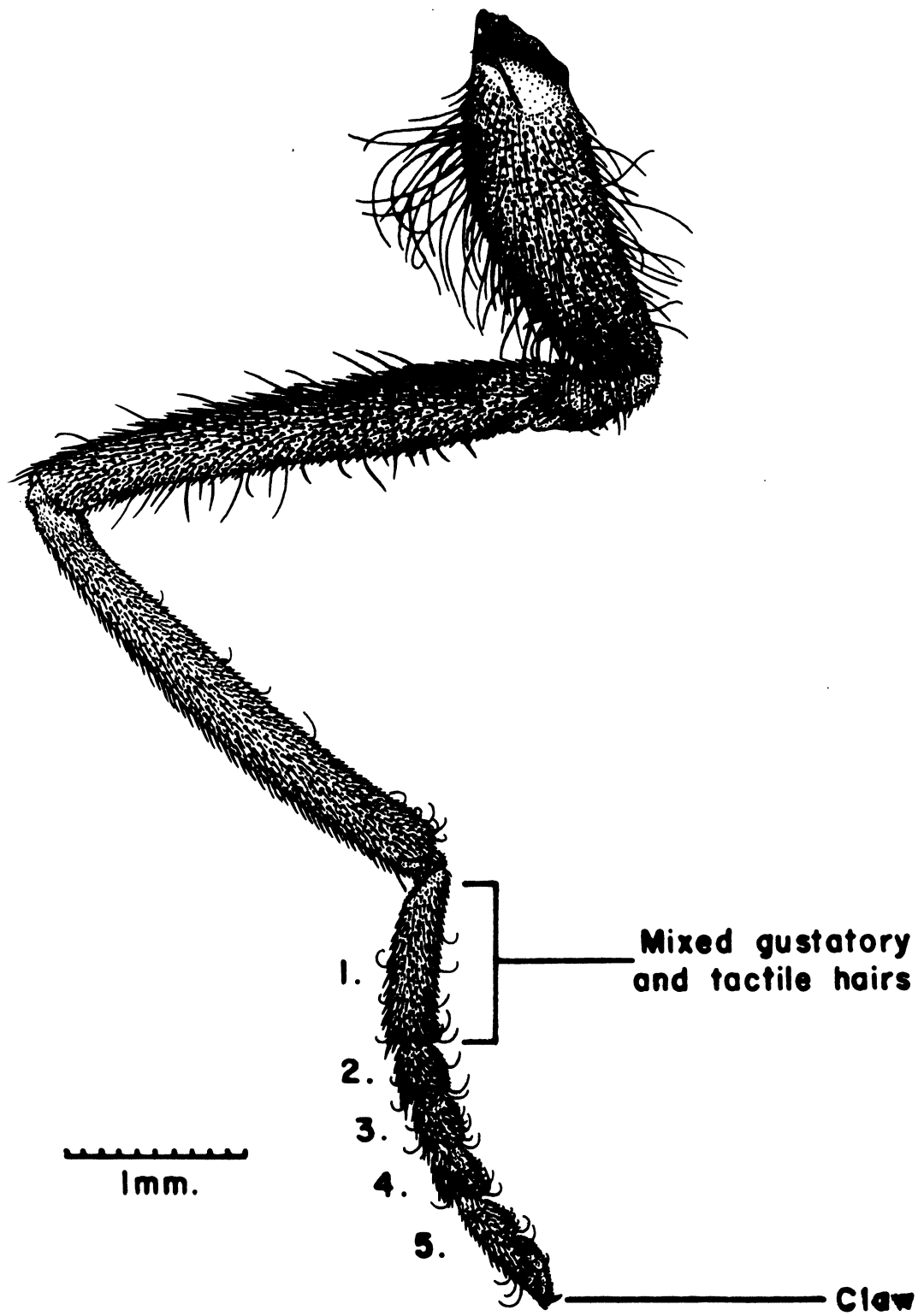
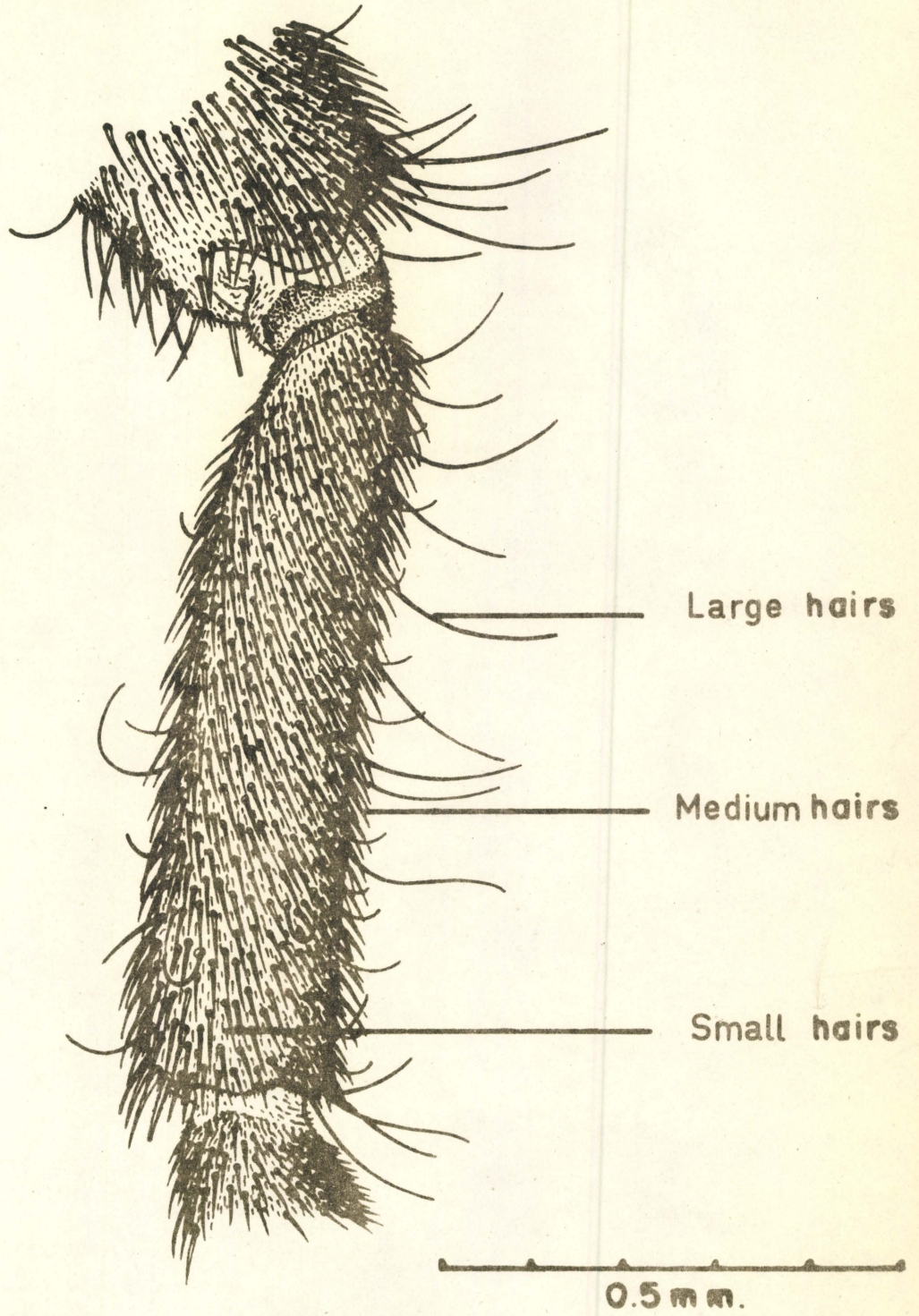


Fig. 35. Enlarged lateral view of the left fore-basitarsus of a female Chrysops vittatus showing the principal types of sensory hairs. Types B and C are gustatory.



Large hairs

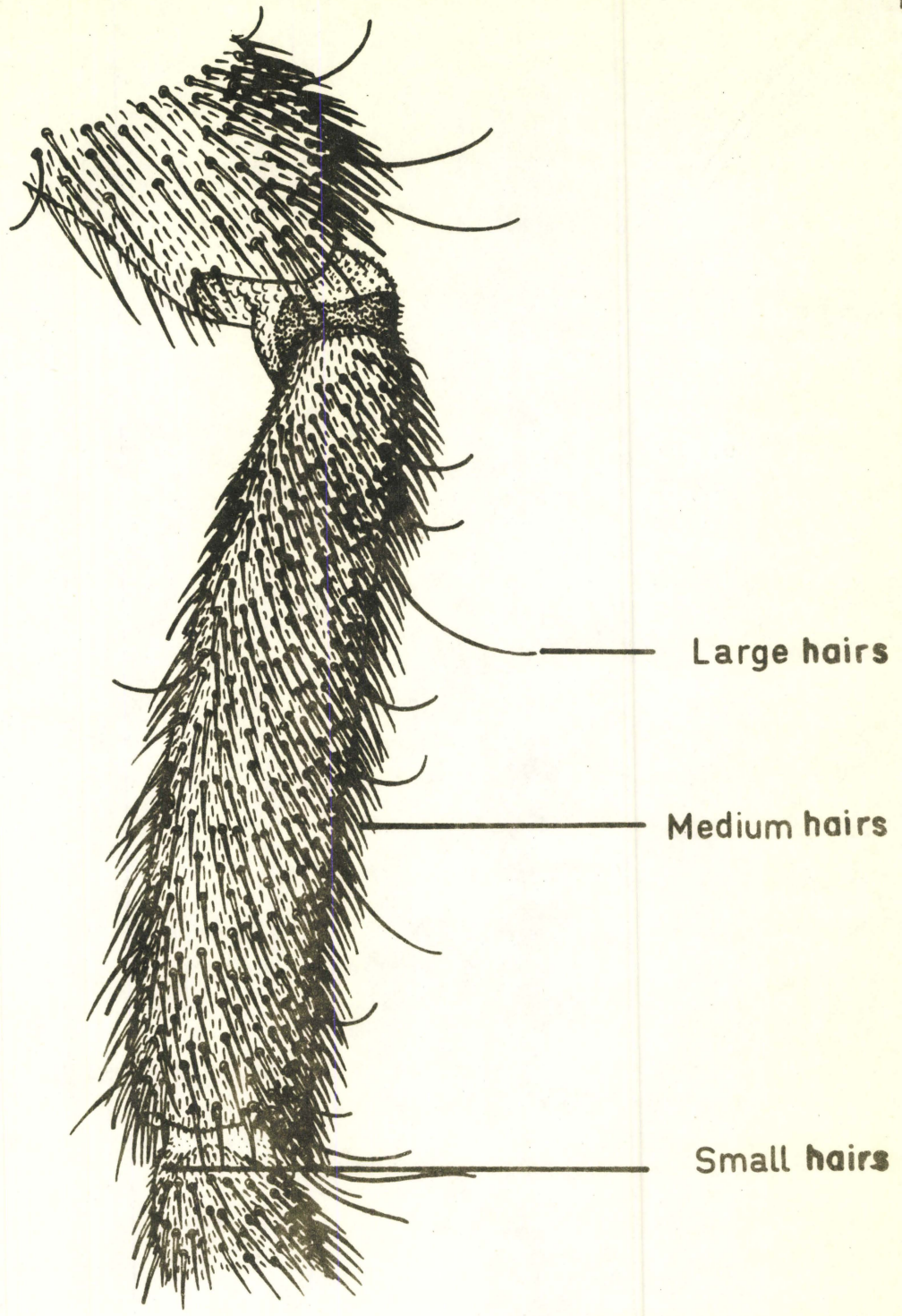
Medium hairs

Small hairs

0.5 mm.



Fig. 36. Enlarged lateral view of the left fore-basitarsus  
of a female Tabanus lineola showing the principal  
types of sensory hairs.



Large hairs

Medium hairs

Small hairs

0.5mm.

Fig. 37. Enlarged lateral view of the left fore-basitarsus of female Hybomitra lasiophthalma showing the principal type of sensory hairs.

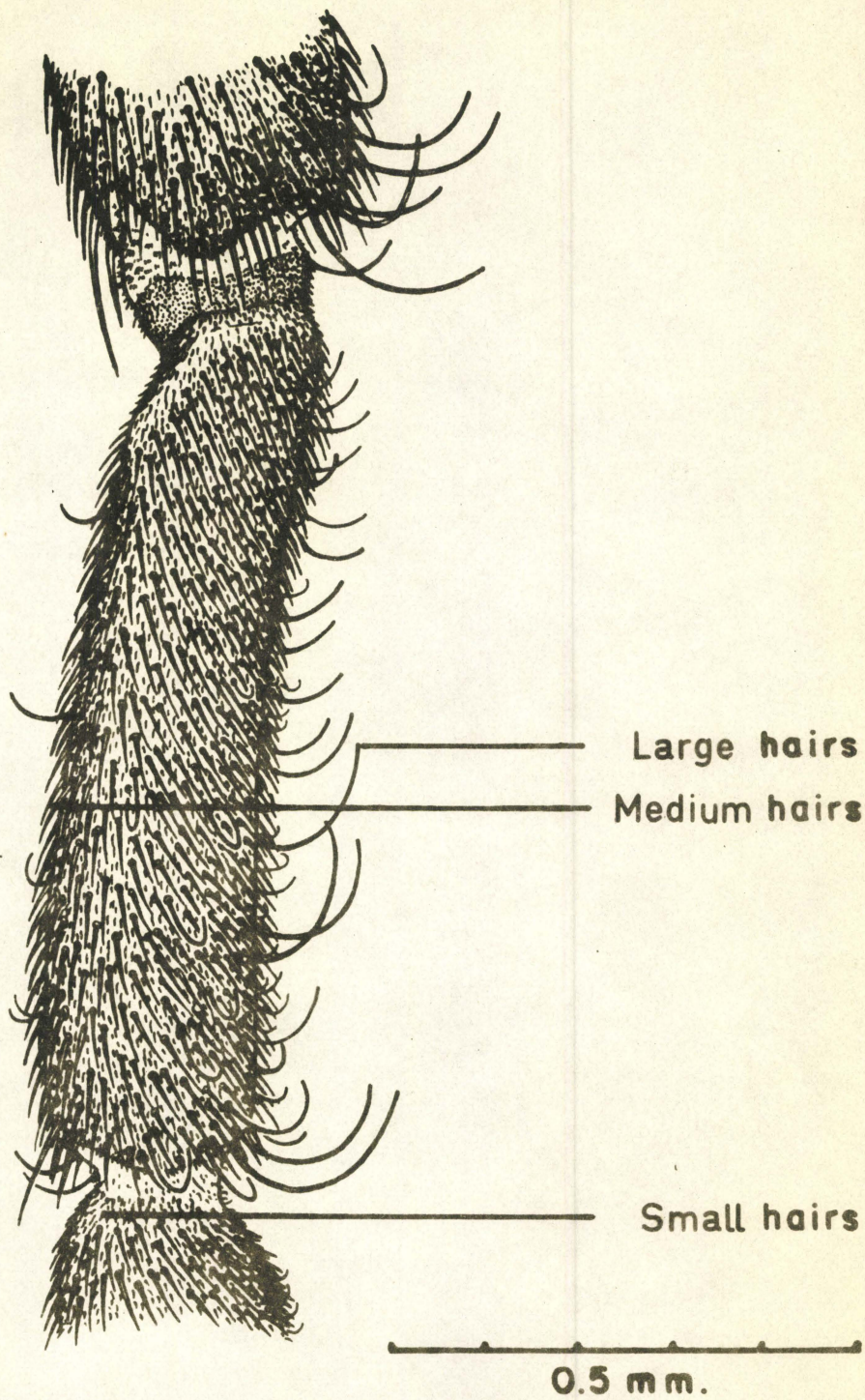


Fig. 38. Mode of sugar feeding in a female Chrysops vittatus

(1-2) Palpation by foretarsi on the substratum.

Note the position of proboscis as carried during the non-feeding period.

(3) Extension of the proboscis on tarsal contact.

(4) Contact of marginal labellar hairs with the substrate, followed by the partial opening of the labellar lobes (see also Fig. 4A).

(5) Application of labella to the acceptable chemical, full opening of lobes and sucking (see also Fig. 5A).

(4A and 5A) Frontal view of the proboscis during the stages in feeding.

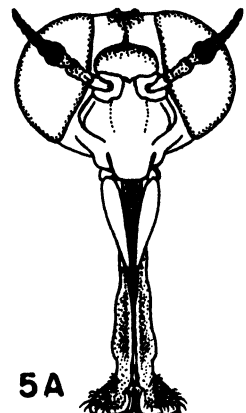
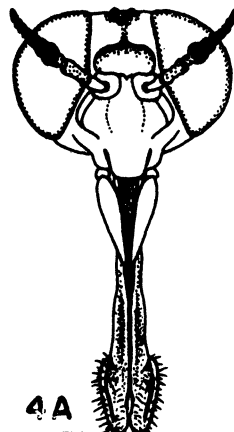
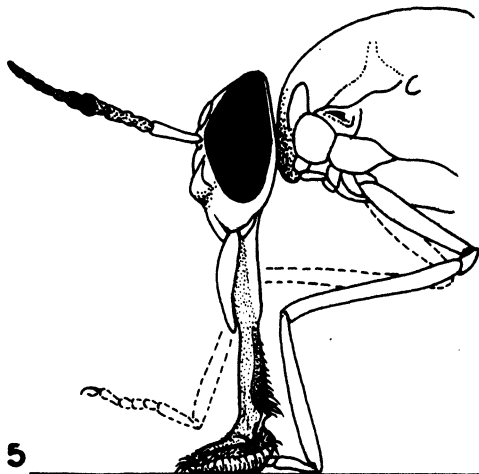
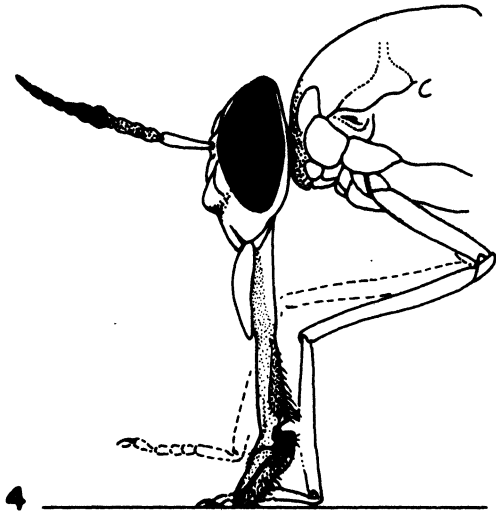
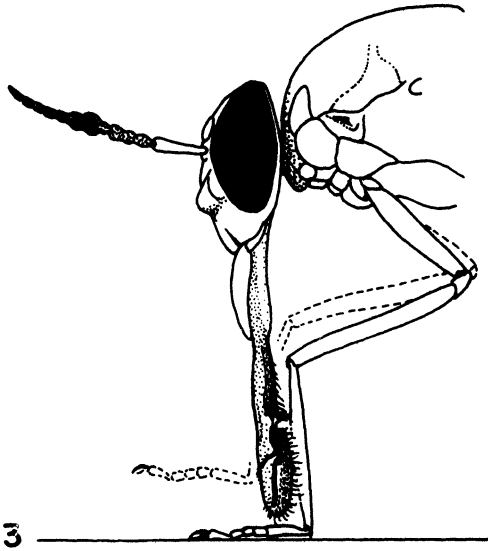
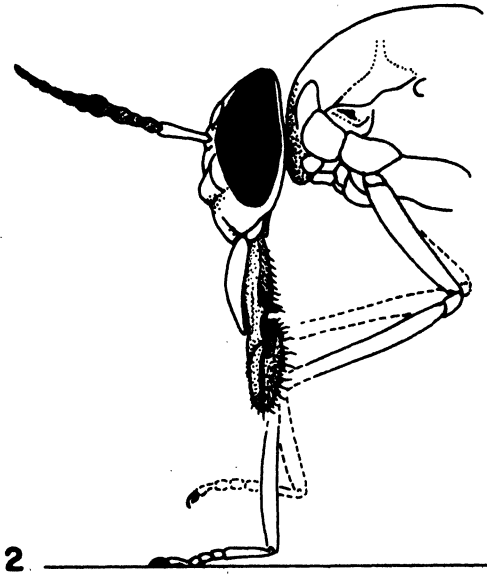
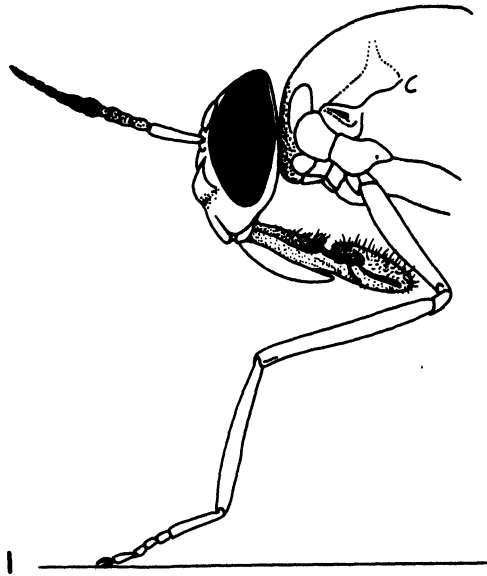


Fig. 39. Mode of sugar feeding in a female Hybomitra  
lasiophthalma.

(1-2) Palpation on the substratum by the foretarsi.

Note in Fig. 1 the position of the proboscis and antenna during non-feeding period.

(3) Tarsal contact with the acceptable chemical triggers extension of the proboscis (see also Fig. 3A) and labellar contact the partial opening of the lobes.

(4) The labella are closely applied to the chemical and sucking is initiated (see also 4A).

(3A and 4A) Frontal view of the stages in Figs. 3 and 4.

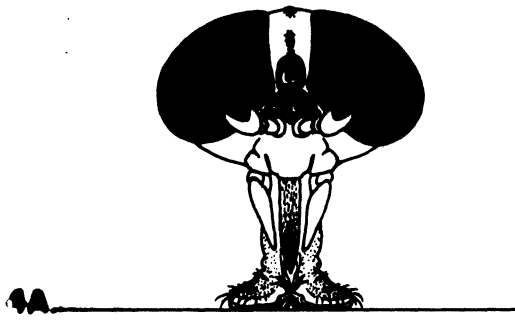
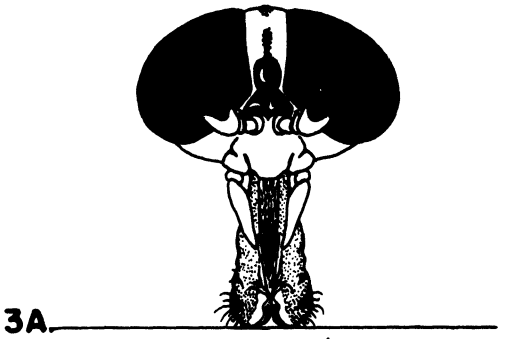
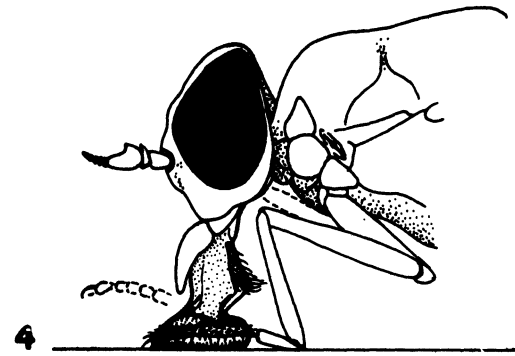
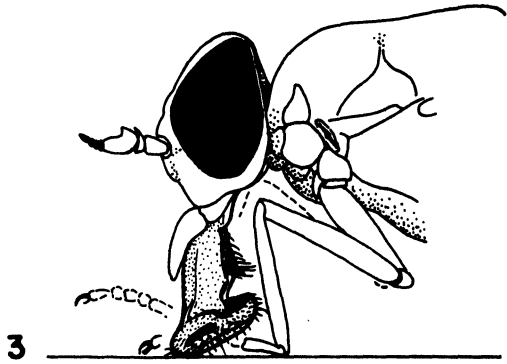
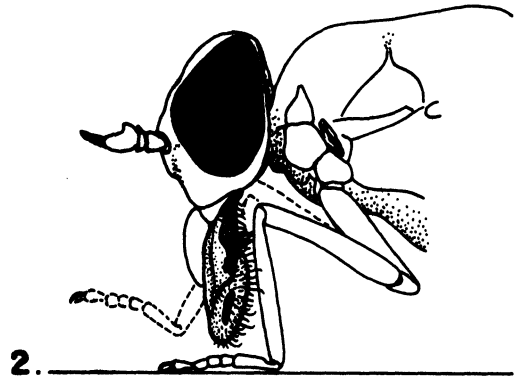
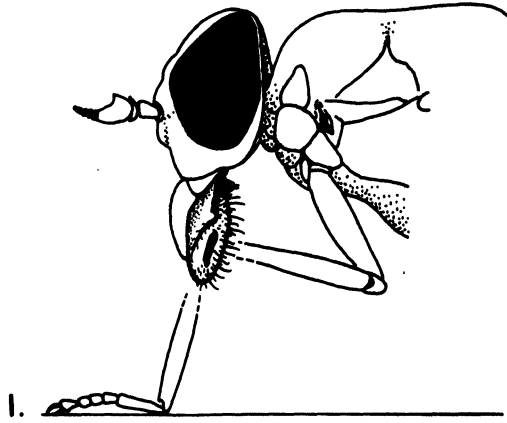
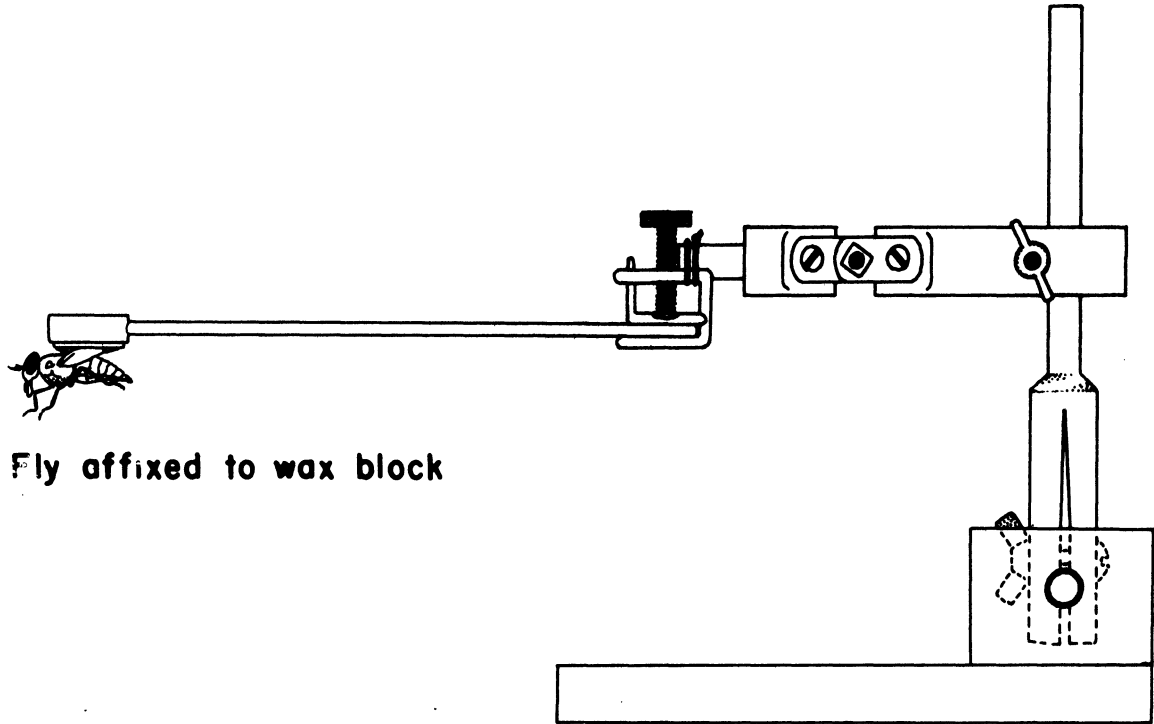




Fig. 40. Apparatus for holding flies during behavioural studies.



**Fly affixed to wax block**

Fig. 41. Tarsal acceptance thresholds of female Chrysops  
vittatus for sugars:

G = glucose; FR = fructose; S = sucrose.

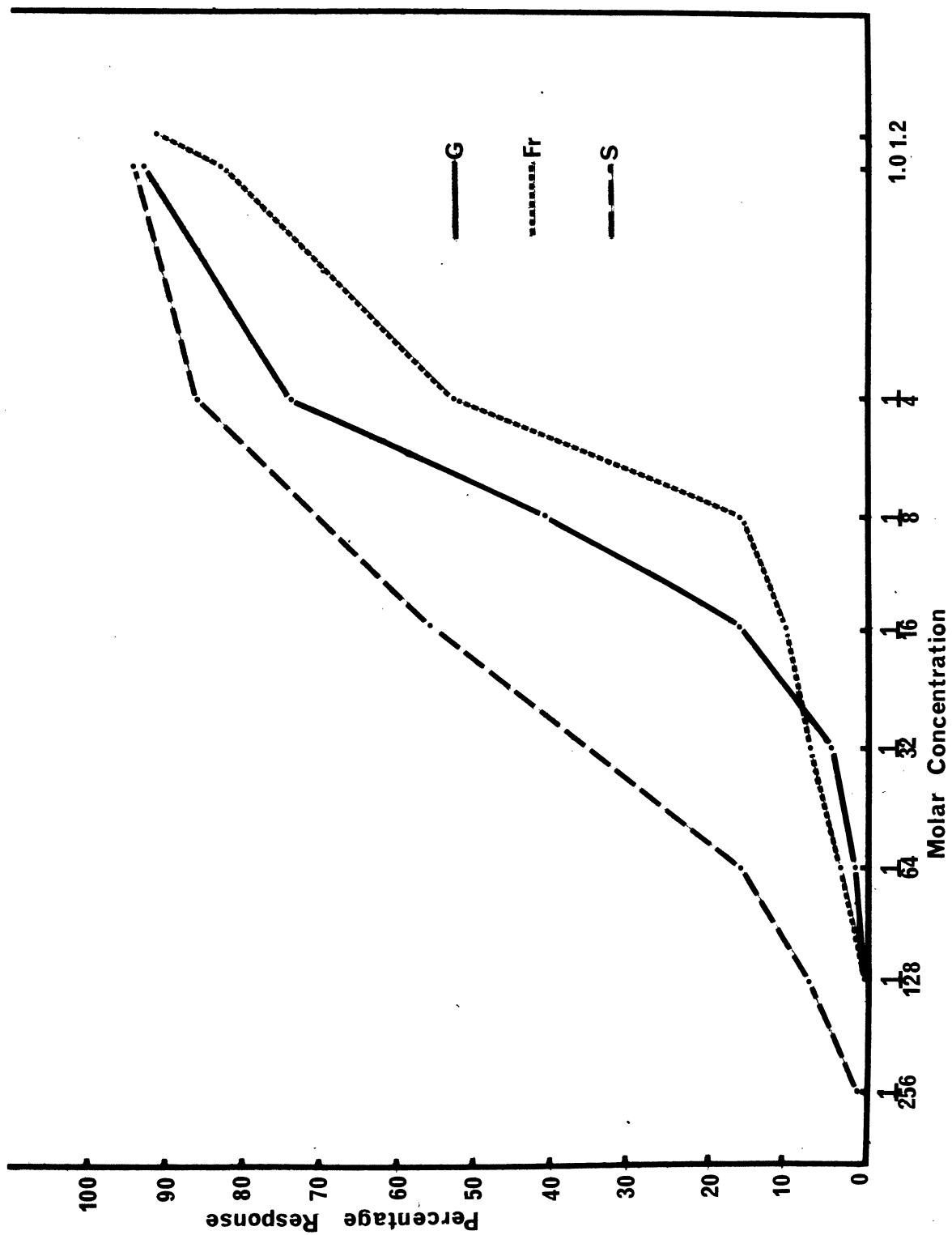


Fig. 42. Labellar acceptance thresholds of female Chrysops  
vittatus for sugars, using 7-9 flies:

S = sucrose

Mz = melezitose

Mb = melibiose

R = raffinose

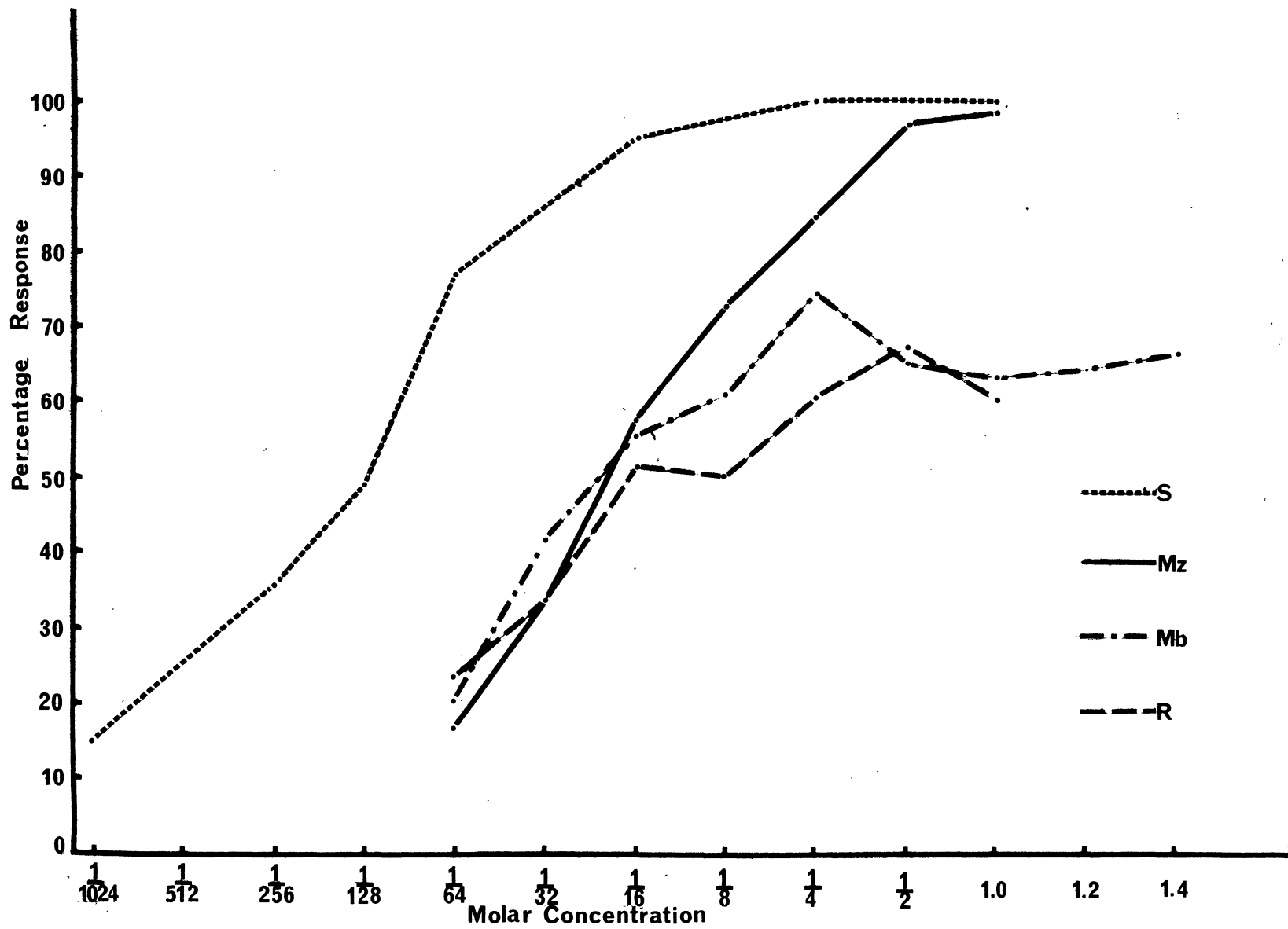


Fig. 43. Labellar acceptance thresholds of female Chrysops

vittatus for sugars:

M = maltose

G = glucose

F = fucose

Fr = fructose

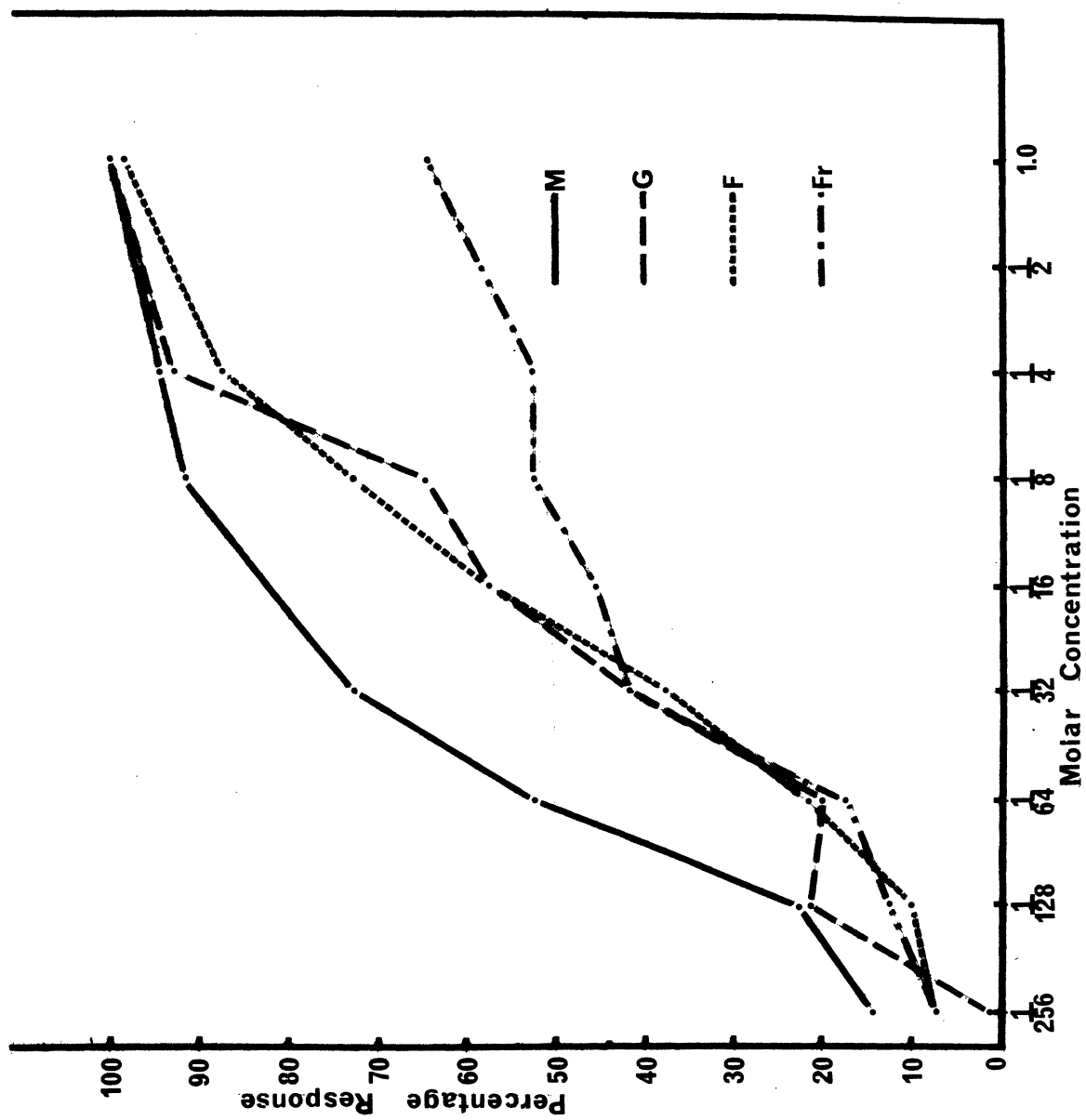




Fig. 44. Paper-partition chromatograph of the pooled crop fluid from 7 Chrysops vittatus females. (Solvent used was n-butanol:acetic acid:water mixture (5:1:4)).

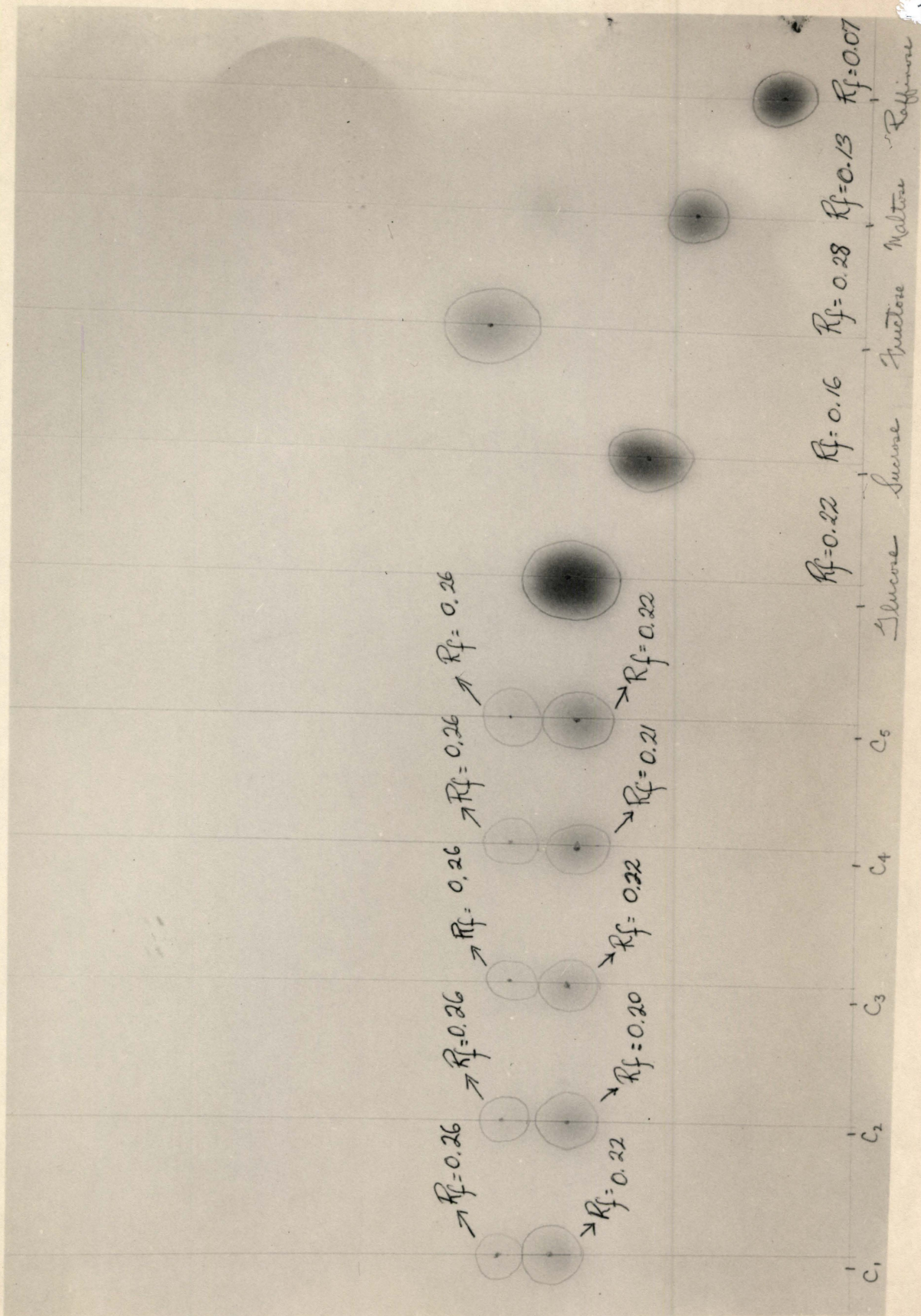


Table I

Measurement studies of the head capsule and mouthparts of female tabanids (in mm.)

	Head		Labrum		Labrum	Mandible		Maxilla				Hypopharynx		Labium with		Labellum	
	L	W	L	W	/Head	L	W	Maxillary palp		Galea		L	W	L	W	L	W
								L	W	L	W						
<i>C.vittatus</i> <sup>*1</sup>	2.1	2.63	1.82	B0.38 T0.05	0.87	1.71	B0.15 T0.01	1.25	B0.1 T0.03	1.8	B0.09 T0.01	1.73	B0.24 T0.01	2.27	B0.24 T0.05	0.86	B0.22
<i>T.lineola</i> <sup>*2</sup>	3.0	4.59	1.75	B0.37 T0.05	0.58	1.74	B0.19 T0.01	1.73	B0.15 T0.03	1.71	B0.15 T0.01	1.59	B0.28 T0.01	2.12	B0.37 T0.05	1.07	B0.25
<i>H.lascophthalma</i> <sup>*3</sup>	3.21	4.93	1.81	B0.39 T0.05	0.57	1.76	B0.22 T0.01	1.85	B0.17 T0.03	1.79	B0.14 T0.01	1.6	B0.26 T0.01	2.23	B0.38 T0.05	1.1	B0.28

Note: L = length

W = width

B = base

T = tip

\* No. of flies used 15

26

36

TABLE IIa

Measurement studies of the labellar trichodes for ten females of *Chrysops vittatus* (in mm.)

Type of trichodes	No. of trichodes		Average					Range				
	Left Labellum	Right Labellum	Length of each hair	Width at the base	Width of the tip	Width of socket	Length of the socket	Length of each hair	Width at the base	Width at the tip	Width of the socket	Length of the socket
A	10-20	10-18	0.154 ± .0180	0.0053 ± .0006	0.00159 ± .0002	0.012 ± .0007	0.0056 ± .0004	0.12 -0.18	0.0042 -0.0062	0.0012 -0.0022	0.010 -0.013	0.0054 -0.0078
B	8-15	9-14	0.0989 ± .0118	0.0046 ± .0004	0.00153 ± .0002	0.011 ± .0007	0.0053 ± .0005	0.084 -0.12	0.0036 -0.0058	0.0012 -0.0018	0.0096 -0.011	0.0048 -0.0072
C	12-18	14-21	0.0668 ± .0117	0.0039 ± .0005	0.00134 ± .0000	0.0095 ± .0008	0.0051 ± .0006	0.048 -0.085	0.0024 -0.0048	0.0010 -0.0014	0.0078 -0.0096	0.0048 -0.0070
D	25-44	18-45	0.0306 ± .0102	0.0028 ± .0005	0.00128 ± .0000	0.0081 ± .0006	0.0050 ± .0006	0.017 -0.048	0.0022 -0.0036	0.0010 -0.0014	0.0070 -0.0090	0.0042 -0.0067
E	9-29	8-27	0.0663 ± .0141	0.0036 ± .0006	0.0003 ± .0000	0.0084 ± .0010	0.0031 ± .0008	0.048 -0.090	0.0026 -0.0054	0.0003	0.0084 -0.012	0.0017 -0.0048
F	100-161	90-169	0.0348 ± .0090	0.0026 ± .0004	0.0003 ± .0000	0.0081 ± .0009	0.0025 ± .0007	0.013 -0.048	0.0024 -0.0036	0.0003	0.0060 -0.0084	0.0018 -0.0048

Six hairs of each type were measured on each of ten flies so that each figure is an average of 60 measurements

Table IIb

Variability in the number of labellar trichodes and pseudotracheae of ten females of Chrysops vittatus

Fly No.	Left labellum						Right labellum						Pseudotracheae	
	Hair type						Hair type						L	R
	A	B	C	D	E	F	A	B	C	D	E	F		
1	10	15	18	25	9	161	10	11	14	18	8	169	21	22
2	13	8	16	30	11	159	12	10	16	39	10	162	24	23
3	14	9	14	25	21	100	13	11	19	21	20	90	20	21
4	11	10	12	44	29	137	11	12	16	45	27	141	22	20
5	12	9	16	72	22	128	12	14	14	34	19	126	21	23
6	12	8	15	34	16	108	12	9	14	35	14	109	24	21
7	14	11	14	29	13	149	14	10	15	26	15	141	23	23
8	20	12	18	27	27	129	18	11	19	33	22	131	23	22
9	10	7	15	32	10	158	11	11	21	26	11	136	21	22
10	12	10	17	37	21	146	12	11	16	36	19	131	20	21

Average =12.8 9.9 15.5 36.5 17.9 134.5 12.5 11.0 15.4 31.3 16.7 133.6 21.9 21.8  
 =13 =10 =16 =37 =18 =135 =13 =11.0 =15 =31 =17 =134 =22 =22

Table II c

Total number of labellar trichodes on the left and the right labellum of ten females of *Chrysops vittatus*

Fly No.	Left labellum	Right labellum
1	238	229
2	237	249
3	183	173
4	243	252
5	259	219
6	193	193
7	230	221
8	233	234
9	232	216
10	243	215
Mean =	229.1	Mean = 220.1

Table III

Sensitivity of the tarsal contact chemoreceptors of female *Chrysops vittatus* to sugars as determined by sample frequency method.

Sugars <sup>a</sup>	Range of molar conc. tested	No. of tests	Range of response	Acceptance threshold (mean)	Standard deviation
Sucrose	0.005-1.0 <sup>1</sup>	490	0.0105-0.175	0.0677	<u>+0.0597</u>
Glucose	0.005-1.0 <sup>2</sup>	560	0.09-0.22	0.150	<u>+0.047</u>
Fructose	0.005-1.2 <sup>3</sup>	630	0.16-0.58	0.298	<u>+0.155</u>

a

order of their abundance in angiospermic flowers

1

7 molar concentration tested on 7 flies in a 10 - trial schedule

2

8 molar concentration tested on 7 flies in a 10 - trial schedule

3

9 molar concentration tested on 7 flies in a 10 - trial schedule

Table IV

Sensitivity of labellar contact chemoreceptors of female *Chrysops vittatus* to sugars  
as determined by sample frequency method

Sugar <sup>a</sup>	Range of molar conc. tested	No. of tests	Range of labellar response	Acceptance threshold (mean)	Standard deviation
Sucrose <sup>b</sup>	0.005-1.0	490* <sup>1</sup>	0.0039-0.0125	0.0067	0.0037
Glucose <sup>c</sup>	0.004-1.0	720* <sup>4</sup>	0.029-0.081	0.046	0.0178
Fructose <sup>b</sup>	0.004-1.0	630* <sup>3</sup>	0.031-0.880	0.234	0.300
Maltose <sup>b</sup>	0.005-1.0	560* <sup>2</sup>	0.0052-0.031	0.0204	0.011
Raffinose <sup>b</sup>	0.01-1.0	490* <sup>1</sup>	0.017-0.746	0.173	0.144
Melibiose <sup>b</sup>	0.01-1.4	630* <sup>3</sup>	0.024-1.32	0.476	0.568
Melezitose <sup>b</sup>	0.01-1.0	490* <sup>1</sup>	0.023-0.331	0.100	0.107
Fucose <sup>bd</sup>	0.004-1.0	560* <sup>2</sup>	0.023-0.104	0.065	0.0086

a order of abundance in angiospermic flowers

b number of flies used 7 (in 10-trial schedule)

c number of flies used 9 (in 10-trial schedule)

d This sugar not present in flower nectar

\* No. of molar concentrations tested

$\sqrt{10}$ ,  $\sqrt{2/8}$ ,  $\sqrt{3/9}$ , and  $\sqrt{4/8}$



Table V

Labellar acceptance threshold of female *Chrysops vittatus* for sugars as determined by the population frequency method.

Name of the sugar	Range of molar conc. tested	No. of tests	Acceptance threshold based on 50% flies responding positively	Standard Deviation.
Glucose <sup>*1</sup>	0.0001-1.0	560 <sup>°a</sup>	0.038	<u>+0.0324</u>
Fructose <sup>*2</sup>	0.0001-1.0	560 <sup>°b</sup>	0.65	<u>+0.608</u>
Maltose <sup>*3</sup>	0.004-1.0	240 <sup>°c</sup>	0.0068	<u>+0.00023</u>
Fucose <sup>*4</sup>	0.002-1.0	150 <sup>°d</sup>	0.048	<u>+0.0042</u>
Melezitose <sup>*5</sup>	0.004-1.0	170 <sup>°e</sup>	0.075	<u>+0.002</u>

\* No. of flies used

° No. of molar concentration tested

<sup>1</sup><sub>40</sub>

a<sub>7</sub> (2 trials)

<sup>2</sup><sub>40</sub>

b<sub>7</sub> (2 trials)

<sup>3</sup><sub>20</sub>

c<sub>12</sub> (1 trial)

<sup>4</sup><sub>15</sub>

d<sub>10</sub> (1 trial)

<sup>5</sup><sub>17</sub>

e<sub>10</sub> (1 trial)

Table VI

Labellar sensitivity of female Chrysops vittatus to amino acids in aqueous solution

Name of the amino acid	Molar conc.	pH at 23°C	Response of the fly			Controls		
			+	+	0	+	+	0
L-Alanine	0.005	5.5	-	2	20	20	2	-
	0.05	5.6	1	-	21	21	1	-
L-Arginine	0.005	5.5	1	-	21	16	6	-
	0.05	5.5	-	2	18	22	-	-
DL-Aspartic acid	0.005	3.4	-	-	22	18	4	-
	0.05	3.3	-	-	22	20	2	-
DL-Glutamic acid	0.005	3.6	2	-	20	21	-	1
	0.05	3.5	-	-	22	19	3	-
L-Cysteine (HCl)	0.005	2.5	-	1	21	20	2	-
	0.05	2.4	-	1	21	18	4	-
Glycine	0.005	5.3	2	2	18	20	2	-
	0.05	5.4	-	-	22	19	2	1
DL-Histidine	0.005	4.3	1	1	20	19	1	2
	0.05	4.2	-	1	21	21	1	-
L-Isoleucine	0.005	5.6	2	-	20	21	1	-
	0.05	5.7	-	-	22	21	1	-
DL-Leucine	0.005	5.6	2	-	20	20	2	-
	0.05	5.7	-	-	22	19	2	1
DL-Methionine	0.005	5.1	1	1	20	18	-	4
	0.05	5.0	-	1	21	18	3	1
L-Proline	0.005	5.6	-	-	22	20	1	1
	0.05	5.7	-	-	22	17	3	2

Table VI (continued)

DL-Threonine	0.005	5.4	-	-	22	19	1	2
	0.05	5.3	-	-	22	19	2	1
L-Valine	0.005	5.4	-	3	19	20	1	1
	0.05	5.3	-	-	22	18	4	-
DL-Lysine	0.005	5.6	1	2	19	18	4	-
	0.05	5.7	1	-	21	20	1	1
DL-Serine	0.005	5.5	1	1	20	19	2	1
	0.05	5.4	-	-	22	13	5	4

Legend    +    = positive response  
           ±    = indefinite response  
           °    = no response

TABLE VII\*

Labellar sensitivity of female Chrysops vittatus to amino acids and nucleotides in phosphate buffer

Name of the amino acid or nucleotide	Molar conc.	Response of the flies			Controls		
		+	±	0	+	±	0
Alanine	0.005	2	4	27	1	5	27
	0.05	1	6	25	1	4	28
Glycine	0.005	2	4	26	3	6	23
	0.05	2	7	22	3	2	27
Glutamic acid	0.005	3	2	27	3	3	26
	0.05	4	5	22	4	3	25
Histidine	0.005	2	7	22	5	3	23
	0.05	9	6	17	2	6	24
Leucine	0.005	7	11	17	4	7	21
	0.05	7	10	15	4	5	23
Lysine	0.005	4	5	23	3	5	24
	0.05	7	9	15	3	4	26
Methionine	0.005	4	2	22	2	7	23
	0.05	8	10	13	1	3	29
ATP	0.05	3	3	11	2	1	14
	0.005	5	2	10	1	4	12
ADP	0.05	0	3	14	3	3	11
	0.005	3	3	11	3	2	12

\* Trials conducted for two days

Legend      += positive response  
               ±= indefinite response  
               0= no response

Table VIII

ATP, ADP and AMP as feeding stimulants for female  
Chrysops vittatus

Chemical	No. of flies tested	No. of flies feeding	Response %
<u>ATP (aqueous)</u>			
0.05 M	20	2	10
0.005 M	20	7	35
Control (water)	20	7	35
<u>ATP (in PO<sub>4</sub> buffer)</u>			
0.05 M	10	2	20
0.005 M	10	4	40
Control (buffer)	10	1	10
<u>ATP (in 0.15 M NaCl)</u>			
0.05 M	10	4	40
0.005 M	10	1	10
Control (0.15 M NaCl)	10	0	0
<u>ADP (aqueous)</u>			
0.05 M	13	0	0
0.005 M	13	3	23
Control (water)	13	3	23
<u>ADP (in 0.15 M NaCl)</u>			
0.05 M	6	0	0
0.005 M	6	0	0
Control (0.15 M NaCl)	6	0	0
<u>AMP (aqueous)</u>			
0.05 M	5	3	60
0.005 M	5	0	0
Control (water)	5	5	100
<u>AMP (in buffer)</u>			
0.05 M	10	1	10
0.005 M	10	3	30
Control (buffer)	10	1	10

Table IX\*

Dispatch of ingested diet by females of *Chrysops vittatus*

Diet	Total Flies	Feeding Flies	% Feeding	Location of the Food		
				Crop	Mid-gut	Both
Whole blood pre-warmed to 37°C	60	18	30	-	18	-
Whole blood + 0.5 M glucose (1:1 v/v)	60	47	78.3	42	-	5
Whole blood + 0.25 M glucose (1:1 v/v)	10	9	90	-	9	-
-- do -- (1:2 v/v)	10	10	100	-	10	-
-- do -- (1:4 v/v)	10	8	80	-	8	-
-- do -- (1:6 v/v)	10	9	90	1	8	-
Sucrose (0.5.M)	50	49	98	49	-	-
Glucose (0.25 M)	10	10	100	8	-	2
Distilled water (Control)	30	7	23.3	7	-	-

\* Flies used were pre-starved for several days, water-satiated.

## APPENDIX I

Effect of starvation on the sensitivity of tarsal and labellar contact chemoreceptors in Hybomitra lasiophthalma (Diptera : Tabanidae)

(Manuscript for publication)

Female tabanids are of considerable medical and veterinary importance owing to their feeding habits and are often incriminated as vectors of parasites (Gordon and Crewe 1953) and pathogens (cf: Anthony 1962). Besides sucking blood both sexes feed on flower nectar (Downes 1958) that supplies energy for many overt behavioural activities such as flight, feeding, mating and oviposition as well as for oögenesis (Hocking 1953). However, owing to the problem of collecting, rearing and identifying the immature forms, research has lagged behind on the role of sensilla and their function in the feeding behaviour of tabanids. Recently I made large scale collection of tabanid larvae and pupae and this material was used to study the effect of starvation on the sensitivity of 3 newly emerged females of Hybomitra lasiophthalma Macq., which possess gustatory sensilla on the foretarsi and aboral margin of the labella, both sensitive to water, sucrose and sodium chloride. (Lall and Davies 1967, 1968).

Larvae and pupae were reared individually in plastic jars and in glass bottles with screen tops at 19 - 21°C. Newly emerged flies were maintained on water only.

The flies were immobilised by chilling and affixed by their wings to waxed ends of applicator sticks. No tests were made for the 1st 24 h. Sucrose being the principal sugar of many angiosperms (Percival 1961), was employed for testing. Prior to



testing, the responsiveness of the flies to water was checked; if their response was positive then they were allowed to imbibe it ad lib. For tarsal tests the appendages were covered by a lens paper "dress" and through a slit the right foretarsus was drawn out. The ascending method of testing as used in the previous studies was employed (for details see Lall and Davies 1967, 1968). The flies were given 5 sec. to respond after which the labella or the tarsi as the case may be were rinsed in distilled water. A period of 10 - 15 min. lapsed between each trial. Throughout the period of testing the flies were maintained on a strict regimen of water diet.

The lowering of the sucrose threshold with time was only slight and after the fourth day the experiments became unreliable as the flies were weak. In flies  $\frac{3}{4}$  - 1 day after emergence, the tarsal threshold was 0.0013 - 0.0050 M and after four days was 0.0013 - 0.0025 M. The labellar thresholds were 0.00063 - 0.0013 M and 0.00031 - 0.00063 M respectively.

The approximate two-fold lowering of the sucrose threshold for both the fore-tarsus and labella of newly emerged tabanid females indicates that they were "sucrose-starved" on emergence. This is substantiated by comparing their thresholds with those of wild-caught females of H. lasiophthalma which usually had already fed on nectar in nature and were then sucrose starved for only one day. The sucrose threshold for the tarsi of wild caught females was 0.0125 - 0.025 M and for the labella 0.015 - 0.031 (Lall and Davies 1957, 1968).

The tarsal thresholds of these flies are 10 times higher than those of newly emerged females of this species and the labellar thresholds are 20 times higher.

Although this is the first report of the effect of starvation on the sensitivity of tabanids to sucrose, comparable results are available for some non-bloodsucking insects, e.g. Pyrameis atalanta L. (Minnich 1922), other butterflies (Anderson 1932) and various insects (see Haslinger 1935). An amazing feature is the magnitude of the decrease in threshold for these insects. A lowering of 10-thousand-fold was reported by Minnich (1929) for Pyrameis, a 100-thousand-fold for butterfly species by Anderson (1932) and a 10-million-fold decrease by Dethier and Rhoades (1954) for Phormia regina (Mg.).

No evidence of autogeny was found in H. lasiophthalma and 4-day old females, when dissected, showed little stored nutrient (fat-body) and the eggs were scarcely developed. Thus newly emerged females of this tabanid species, and probably most others, require a carbohydrate meal (usually flower nectar) within the first 3 or 4 days if they are to survive and engage in their activities.

References

- Anderson, A.L. 1932. The sensitivity of the legs of common butterflies to sugars. *J. Exp. Zool.* 53: 235-59
- Anthony, D.W. 1962. Tabanidae as disease vectors, p. 95-107. In "Biological transmission of disease agents". pp. 93-107, Maramorsch, K. ed. Academic Press, New York.
- Dethier, V.G. & Rhoades, M.V. 1954. Sugar preference: aversion function for the blowflies. *J. Exp. Zool.* 126: 177-203
- Downes, J.A. 1958. The feeding habits of biting flies and their significance in classification. *Ann. Rev. Entomol.* 3: 249-66
- Gordon, R.M. & Crewe, W. 1953. The deposition of infective stages of Loa loa by Chrysops silacea and the early stages of its migration to the deeper tissues of the mammalian host. *Ann. Trop. Med. Parasitol.* 47: 74
- Haslinger, F. 1935. Uber den Geschmacksinn von Calliphora erythrocephala Meigen, and die Verwertung von Zuchern und Zukerkohlen durch diese Fliege. *Z. Vergl. Physiol.* 22: 614-40
- Lall, S.B. & Davies, D.M. 1967. Tarsal sensitivity of female tabanids flies to sucrose and sodium chloride. *Can. J. Zool.* 45: 461-64
- \_\_\_\_\_ 1968. Comparative studies of the labellar sensitivity of female tabanids (Diptera) to sucrose and sodium chloride. *Ann. Entomol. Soc. Amer.* 61: 221-24
- Minnich, D.E. (1922). The chemical sensitivity of the tarsi of the red admiral butterfly, Pyrameis atalanta Linn. *J. Exp. Zool.* 35: 57-81
- \_\_\_\_\_ 1929. The chemical senses of insects. *Quart. Rev. Biol.* 4: 100-112.
- Percival, S.M. (1961). Types of nectar in the angiosperms. *New Phytol.* 60: 35-281.

## APPENDIX II

## TABLE X

Representative angiosperms from Beverley Swamp with type of nectar

Composition

<u>Name of the plant</u>	<u>Nectar type*</u>
<u>Asclepias syriaca</u> L. (Common milkweed)	FG
<u>Potentilla canadensis</u> L. (Rough cinquefoil)	sFG (in <i>P. anserina</i> )
<u>Solidago canadensis</u> L. (Canada goldenrod)	
<u>S. graminifolia</u> (L.) (Narrow-leaved goldenrod)	
<u>Rhus typhina</u> L. (Staghorn sumach)	
<u>Aster ciliolatus</u> Lindl.	
<u>A. simplex</u> Willd.	
<u>A. novae-angliae</u> L.	SFG (balanced)
<u>Erigeron strigosus</u> Muhl	
<u>Cichorium Intybus</u> L.	
<u>Daucus carota</u> L.	
<u>Melilotus alba</u> Desr.	sFG
<u>Echium vulgare</u> L.	sFG
<u>Anemone riparia</u> Fern.	
<u>Cirsium vulgare</u> (Savi) (= <u>lanceolatum</u> (L) Hill.)	SFG

SFG = Sucrose, glucose and fructose

s=small quantity: Balanced=equal quantity

cf:- M. S. Percival (1961). *New Phytol.* 60:235-281.

D. Demianowicz (1964). *Ann. de l'abeille*, 7:289-299.

Shuel (1963: in litt)

APPENDIX II

TABLE XI

A protocol of the method of presenting test molar concentration of sucrose to the labella of female  
Chrysops vittatus and the manner in which response was recorded

MCT	1*	MCT	2	MCT	3	MCT	4	MCT	5	MCT	6	MCT	7	MCT	8	MCT	9	MCT	10
1/1024	0	1.0	+	1/4	+	1/128	0	1/16	+	1/256	+	1/16	+	1/64	0	1/4	+	1/1024	+
1/4	+	1/16	+	1.0	+	1/16	+	1/256	0	1/4	+	1/1024	±	1/256	±	1/128	0	1/16	+
1/128	±	1/64	+	1/1024	0*	1/4	+	1.0	+	1/16	+	1/128	0	1/4	+	1/1024	0	1/128	0
1/16	+	1/1024	0*	1/128	+	1/0	+	1/64	+	1/128	0*	1/0	+	1/1024	0*	1/16	+	1.0	+
1/64	+	1/4	+	1/16	+	1/1024	+	1/4	+	1/0	+	1/64	+	1/16	+	1.0	+	1/256	0
1.0	+	1/256	0	1/64	0	1/64	+	1/1024	+	1/64	+	1/4	+	1/128	0	1/64	+	1/4	+
1/256	+	1/128	+	1/256	0	1/256	±	1/128	0	1/1024	±	1/256	±	1/0	+	1/256	0	1/64	±

MCT = Molar Concentration Tested

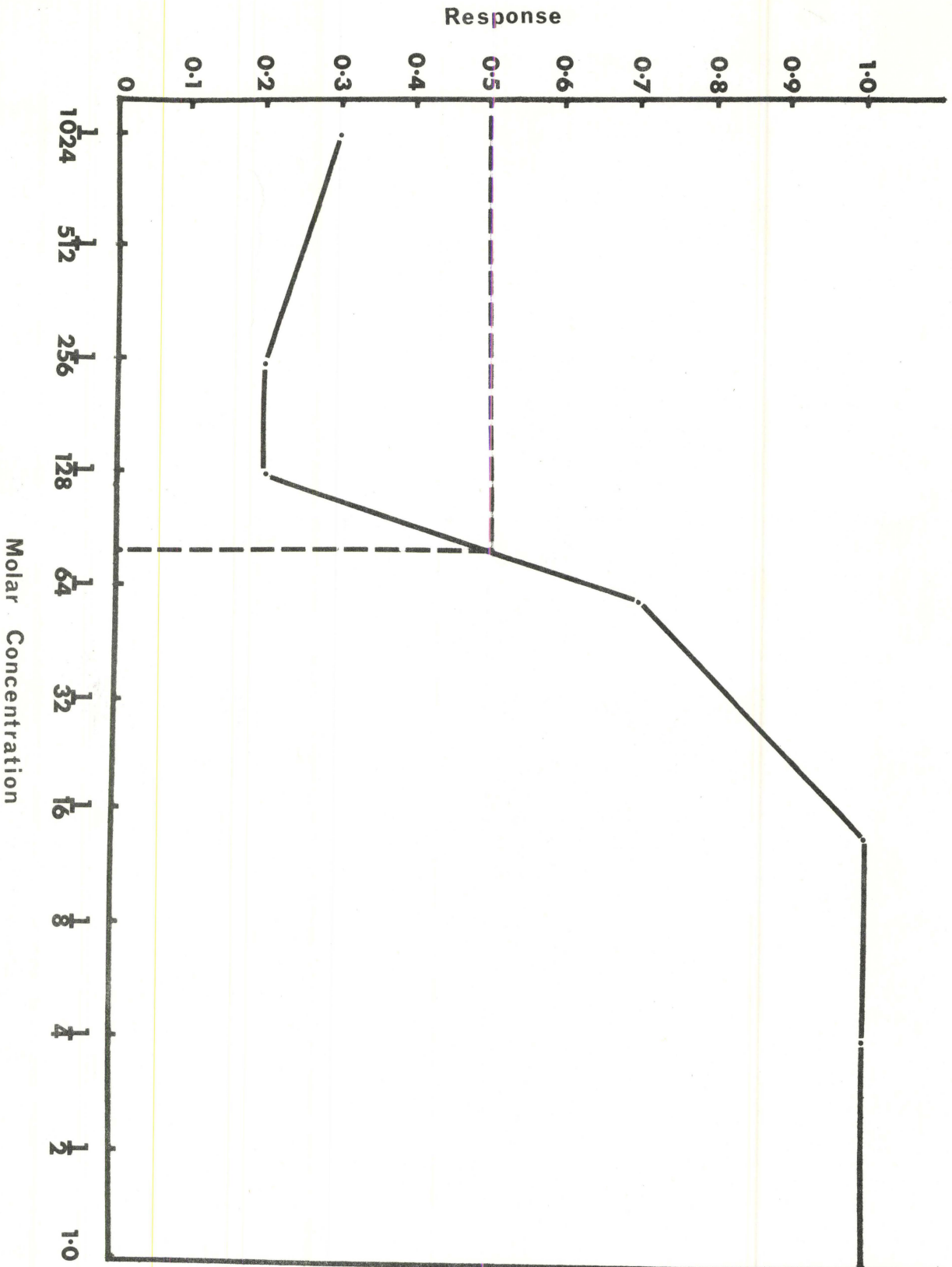
\* = Trial number

+ = Positive Response

± = Indefinite "

0 = No Response

Fig. 45. A protocol of the graph drawn for each fly (7),  
based on averaged data for a total of 10 trials  
at each concentration.  
(data from Table XI)



APPENDIX IITABLE XII

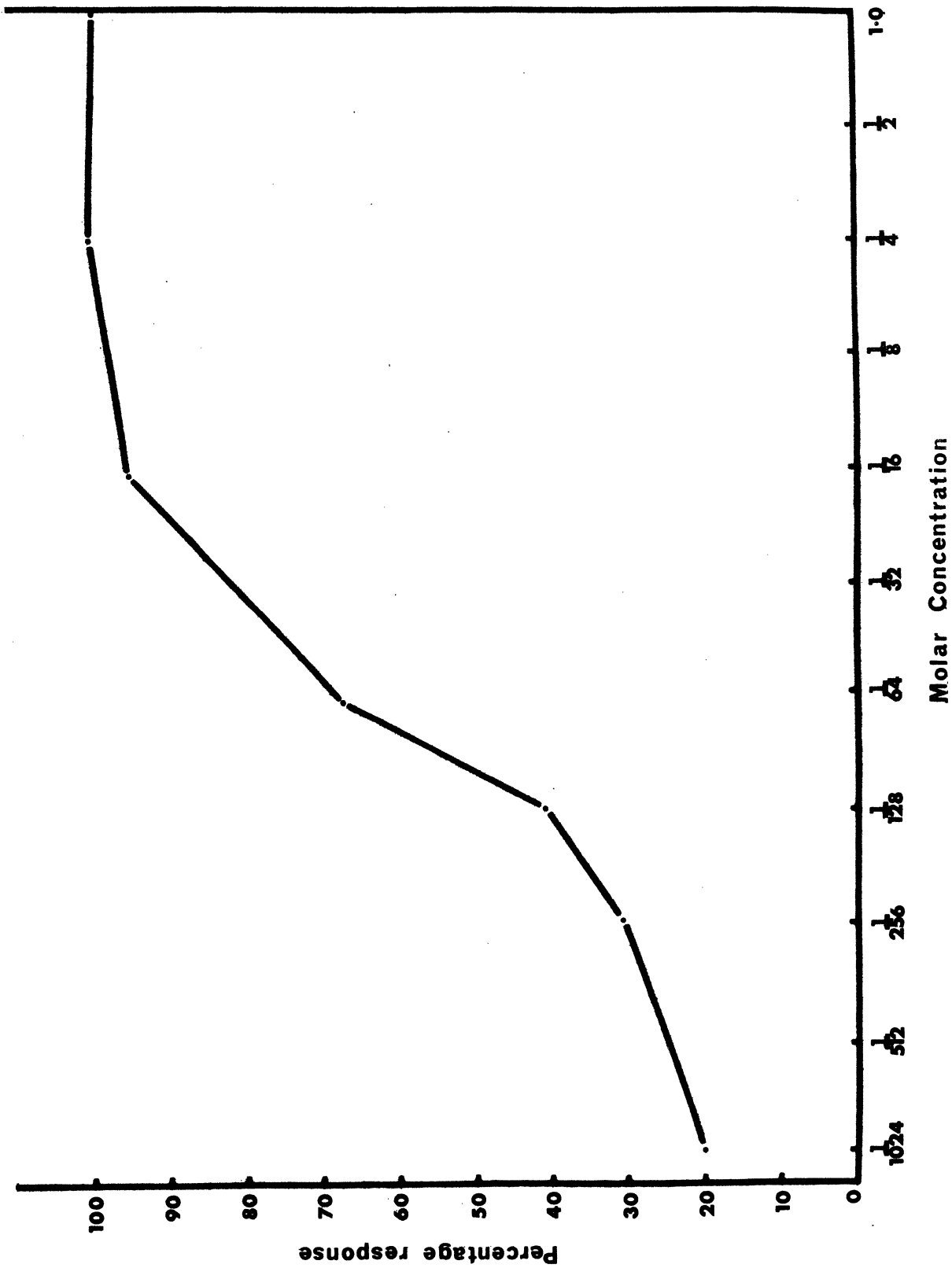
Labellar response of seven females of Chrysops vittatus to sucrose,  
based on averages of ten trials at each concen-  
tration.

Molar concentration	1 <sup>a</sup>	2	3	4	5	6	7	Total	%
1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	6.9	98.5
0.25	1.0	1.0	1.0	1.0	1.0	1.0	1.0	7.0	100.0
0.0625	1.0	1.0	0.8	1.0	1.0	0.9	1.0	6.7	95.7
0.015	0.7	1.0	0.6	0.8	0.7	1.0	0.6	5.4	77.1
0.0075	0.2	0.7	0.3	0.8	0.6	0.1	0.7	3.4	40.8
0.00375	0.2	0.5	0.1	0.5	0.5	0.2	0.5	2.5	30.5
0.0009	0.3	0.3	0.0	0.1	0.3	0.1	0.3	1.4	20.0

a from data in Table XI



Fig. 46. A protocol of the graphs drawn for each sugar,  
based on averaged data for the total number of flies  
tested (7-9).



## APPENDIX II

Table XIII

The order of stimulative effectiveness of sugars for the labellar taste sensilla of females of Chrysops vittatus. A comparison of sample and frequency method for estimating threshold.

Name of the sugar	Range of labella response	Acceptance threshold			
		a Mean	a Rank Order	b	Rank Order
Glucose <sup>1</sup>	0.029-0.081	0.046	2	0.038	2
Fructose <sup>2</sup>	0.031-0.880	0.234	5	0.65	5
Maltose <sup>3</sup>	0.0052-0.031	0.0204	1	0.0068	1
Melezitose <sup>o</sup>	0.023-0.331	0.100	4	0.075	4
Fucose <sup>o</sup>	0.023-0.104	0.065	3	0.048	3

1, 2, 3 Order of abundance in flower nectar

o not present in flower nectar

a sample method

b population method

## APPENDIX II

Table XIV

Longevity of wild-caught females of Chrysops vittatus\* fed on sugars and water.

---

Diet	LD <sub>100</sub>	LD <sub>50</sub>
No water or food (sugar)	1	
Water		16
"Dry" sucrose and water		25
Glucose (I.OM)		19

---

A total of 20 flies were used for each experiment.