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THE UNIVERSITY OF ALBERTA

AUTOGENY AND ANAUTOGENY IN SOME SPECIES OF TABANIDS (DIPTERA: TABANIDAE) IN ALBERTA, CANADA.

BY

ANTHONY WILLIAM THOMAS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled AUTOGENY AND ANAUTOGENY IN SOME SPECIES OF TABANIDS (DIPTERA: TABANIDAE) IN ALBERTA, CANADA submitted by Anthony William Thomas in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The developmental stages of ovarian follicles of tabanid flies were classified according to the systems of Christophers and Mer. Physiological ages of flies were estimated using the method of Polovodova. Ovarian diapause occurred before stage III. Reared females of eight species in five genera did not undergo ovarian diapause when only fed sugar in the laboratory. In five of these species autogeny was confirmed in the field: only parous flies were collected. Nulliparous flies were absent or rare in another seven species, suggesting obligatory autogeny. Reared females of seven species in three genera only developed follicles to stage II when fed sugar in the laboratory. In five of these species, nulliparous flies were common in Manitoba fly traps. In 10 other species nulliparous flies were common. There was evidence for facultative autogeny occurring in *Haematopota americana* Osten Sacken.

Both autogenous and anautogenous species were collected up to and including the start of the third gonotrophic cycle. Anautogenous species had a greater survival between age classes than autogenous species.

Eighty-nine percent of the nulliparous flies caught while seeking a blood meal had stage II follicles. In parous blood-seeking flies the developmental stage of the terminal follicles depended upon whether the species was autogenous, the physiological age of the individual and the time since oviposition.

Autogenous females had greater lipid reserves than anautogenous females (17%-21% vs. 4%-9%); there was no difference between males.

Evolution of blood feeding in tabanids is discussed.

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Part of this research was carried out at the R.B. Miller Biological Station in the Bow River Forest Reserve and I wish to thank its director, Dr. D.A. Boag, Department of Zoology, University of Alberta, for allowing me to use the facilities there.

I wish to thank Dr. R.H. Gooding, his technician and graduate students for their assistance with the work on food reserves.

Dr. L.L. Pechuman, Cornell University, assisted with the identification of specimens.

Drs. W.G. Evans and B. Hocking made available monies without which this work could not have been completed.

AUTOBIOGRAPHICAL SKETCH

I was born on January 10, 1942 in London, England where I received my early education. In 1961 I entered the University of London and graduated in 1964 with a B.Sc. Honours Degree in Zoology. In 1964 I entered McMaster University, Hamilton, Ontario and graduated in 1967 with an M.Sc. degree in Biology. I enrolled in the Ph.D. program of the Department of Entomology of the University of Alberta in September 1967.

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I. DEFINITIONS

The terms autogeny and anautogeny were introduced in 1929 and since then have been used to describe populations of hematophagous and non-hematophagous insects (Downes, 1958; Hocking, 1971; Spielman, 1971). Although these terms have gained universal acceptance the use of the term autogeny by dipterists is somewhat inconsistent. Autogeny was coined by Roubaud (1929) to mean egg maturation without a blood meal or any compensating organic liquid. Weyer (1935) and Callot (1955) used the deposition of yolk in the oocytes by unfed female mosquitoes as their criterion of autogeny. However, most workers have provided their insects with a sugar source which has been shown to be needed as an energy source for flight and normal longevity (Hocking, 1953; Crewe and Beesley, 1963). This sugar-feeding in no way invalidates the concept of autogeny since sugar-feeding will not induce anautogenous females to develop eggs (Lea, 1964). All workers agree that autogeny involves the development of mature oocytes without the adult female having had a previous protein-rich meal. Some, however, suggest or imply that viable eggs must be produced (Chao, 1958; Bellamy and Kardos, 1958; Johnson, 1961; Williams, 1961; Chapman, 1962; Spielman and Weyer, 1965; Saito, 1967; Hudson, 1970). Other workers have used the stage of follicle development, which involved dissection of the females, as the criterion for autogeny (Kardos, 1959; Moore, 1963; Lea, 1964; Spielman, 1964; Harwood, 1966; Linley, 1968; Lea, 1969; Rockel, 1969; Smith and Brust, 1970). Females with follicles in

Christophers' (1911) stages IV or V were considered autogenous. Some of these latter workers did show that similar females were capable of producing viable eggs. Since autogeny has been shown to be a physiological process under direct genetic control (see Spielman, 1971 for a review) any attachment of the requirements of oviposition or egg viability is unwarranted in laboratory studies (Chapman, 1962). These factors are intimately connected with very different stimuli. Because of the erratic oviposition of unfertilized female mosquitoes, Spielman (1957) disregarded egg deposition as a measure of autogeny. Successful mating acted as the stimulus for oviposition, but the presence or absence of mating had no effect on autogenous egg development. The most recent definition of autogeny by Spielman (1971) is a reiteration of his earlier definition (Spielman, 1957): autogeny refers to a situation where there is no specific food-mediated ovarian diapause. No such inconsistency of usage exists with the term anautogeny. It is accepted by all to mean the necessity of a blood meal (in the so-called hematophagous insects) for the development of eggs; or a situation where there is a specific food-mediated ovarian diapause (Spielman, 1971).

In this study, autogeny is used to mean the development of follicles beyond the diapausing stage (stage II of Christophers (1911)) without the female having had a previous blood meal.

II. NOMENCLATURE

Except in three instances, species names used are those given by Philip (1965). *Hybomitra lurida* (Fallen) is used as the senior synonym for *Hybomitra metabola* (McDunnough) (Pechuman and Stone, 1968). *Chrysops nubiapex* Philip is given a species rank, and is used in preference over *Chrysops ater* Macquart as suggested by Pechuman and Burton (1969), since the larvae were identical to those described by Teskey (1969) under *Chrysops nubiapex*. *Atylotus* "*A*" is used for flies reared from larvae which were identical to the description given by Teskey (1969) for this undescribed species. The adults are inseparable from *Atylotus pemeticus* (Johnson).

III. INTRODUCTION

Autogeny is a widespread phenomenon in hematophagous Diptera, having been recorded in all families (Downes, 1958). Reasons for suspecting the occurrence of autogeny in tabanids have been given by some workers. Certain species are restricted to oceanic beaches and these have been thought of as being autogenous because of the lack of suitable hosts in such places, eg. Aegialomyia psammophilus (Osten Sacken) in Florida (Snyder, 1916), Ochrops seurati Surcouf in Tunisia (Surcouf, 1922), and Aegialomyia atlanticus (Johnson), Aegialomyia caribaeorum (Bequaert) and Aegialomyia nervosus (Curran) in the West Indies (Bequaert, 1940). Other species have been thought of as being autogenous because of their abundance and the lack of suitable hosts, e.g. most tabanids in Florida (Snyder, 1916) and Tabanus iyoensis Shiraki in Japan (Saito, 1967). The exact opposite reason, species scarcity and the fact that it is never seen to annoy animals, suggested the occurrence of autogeny in Atylotus bicolor (Wiedemann) and Atylotus ohioensis (Hine) (Snyder, 1916), in the four species of North American Stonemyia and in Goniops chrysocoma (Osten Sacken) (Stone, 1930), and in four species of North American Atylotus (bicolor, pemeticus, thoracicus (Hine), ohioensis) (Fairchild, 1950). Miller (1951) suggested that the majority of tabanids in northern Canada may feed only on nectar. Jones and Anthony (1964) suggested that the females of Merycomyia brunnea Stone are not blood feeders. The mouthparts are weakly developed and the females were never seen on livestock. Olsufiev (1966) stated that the North American Aegialomyia living on sea shores do not feed on blood. Seventeen species in seven genera in

the Ethiopian region, one species in Brazil, and two species in the Oriental region lack mandibles and thus, by inference, must be autogenous (Oldroyd, 1954; 1957). Six of these species are restricted to oceanic beaches. Actual proof of autogeny has been shown in four Canadian species, *Chrysops aestuans* Wulp, *Chrysops fulvaster* Osten Sacken, *Chrysops mitis* Osten Sacken and *Tabanus reinwardtii* Wiedemann (Cameron, 1926); one European species, *Haematopota pluvialis* (Linnaeus) (Cameron, 1934); one Japanese species, *Tabanus iyoensis* (Saito, 1967); and one American species, *Chrysops fuliginosus* Wiedemann (Rockel, 1969).

IV. OBJECTIVES

The objectives of this study were:

1. to determine how widespread the phenomenon of autogeny was in the tabanids of one area (Alberta, Canada). Because evidence of autogeny in tabanids is meagre, an intensive study of a few species was not attempted but an attempt was made at an extensive study involving as many species as could be obtained.
2. to determine the number of gonotrophic cycles and the survival between age classes in the field.
3. to determine whether there was a relationship between the stage of follicle development and blood seeking.
4. to compare food reserves, lipid and soluble protein, within and between autogenous and anautogenous adults.
5. to attempt an explanation of the evolution of blood feeding in tabanids.

V. MATERIALS AND METHODS

1. LOCALITIES

During this study, tabanids were collected in 12 localities in Alberta.

In 1968, adults, larvae and pupae were collected at George Lake (53°57' N, 114°06' W; 2,000 feet) 50 miles northwest of Edmonton. This locality is in the Canadian Zone of the Boreal Region in ecological area 10, poplar, (Strickland, 1938). The area was originally forested with balsam poplar, aspen and willow with stands of spruce and pine (Strickland, 1938). The George Lake locality is a one square mile area of forest and lake surrounded by cleared land. Four habitats in this locality were sampled from early June until late July when tabanids became scarce. Two Manitoba fly traps (Thorsteinson, Bracken and Hanec; 1965) were placed in a *Carex* meadow bordered on the west by a forest of *Populus tremuloides* Michx. and *Populus balsamifera* L. and on the east by a floating *Typha* mat adjacent to an eutrophic lake. One Manitoba fly trap and a Malaise trap (Townes, 1962) were in an open *Carex* and *Typha* habitat alongside an intermittent stream flowing out of the lake. Another Manitoba fly trap was alongside this stream, but in the forest. Two Manitoba fly traps were in a bog consisting of *Sphagnum* moss covered with a dense growth of *Ledum groenlandicum* Oeder. This bog was surrounded by a forest of *Populus* and *Picea glauca* (Moench). Larvae and pupae were collected along the lakeshore and stream edges.

In 1968, adults were collected from July 31 to August 10 in the vicinity of Nordegg (52°28' N, 116°04' W; 4,500 feet) 60 miles

west of Rocky Mountain House. This locality is in the Mountain Zone of the Boreal Region in ecological area 20, north central rocky-mountain, (Strickland, 1938). The entire area is heavily wooded with lodgepole pine (Pinus contorta latifolia Engelm.) and white spruce (Picea glauca (Moench.)) as the dominant tree species. Adults were collected with a Manitoba fly trap in a muskeg alongside Shunda Creek, and also by netting females attracted to horses in a nearby meadow. Larvae and pupae were collected in the aforementioned muskeg in 1970.

During the 1969 season, adults, larvae and pupae were collected in the Bow River Forest Reserve in the vicinity of the R.B. Miller Biological Station on the Sheep River at Gorge Creek ($50^{\circ}38'$ N, $114^{\circ}39'$ W; 5,000 feet). This locality is in the Foothill Zone of the Boreal Region in ecological area 17 (Strickland, 1938). The dominant tree species are aspen, spruce, lodgepole pine and willow. One Manitoba fly trap was erected in an open area in a white spruce forest alongside Macabee Creek at 4,500 feet. Two Manitoba fly traps were in a partially flooded meadow at the edge of a beaver pond surrounded by a spruce and aspen forest at 5,000 feet. One Manitoba fly trap was in a Sphagnum bog bordered on the west by a forest of lodgepole pine and on the other sides by wet meadow with willow, at 5,200 feet. Two other Manitoba fly traps were in a muskeg and Carex marsh surrounded by lodgepole pine at 5,200 feet. One Manitoba fly trap was in a dry meadow adjacent to a horse corral on the banks of the Sheep River gorge at 5,000 feet. Also, all flies entering the buildings of the Biological Station were collected. Larvae and pupae were collected in all the above mentioned habitats except the dry

meadow and the Biological Station.

A few flies were collected in the central and northern rocky mountain ecological areas at Banff and Jasper in 1970.

In 1969, larvae and pupae were collected in the Transition Zone of the Boreal Region in ecological area 6, southern extension of northern prairie, (Strickland, 1938). Tabanids were collected from sandy shores of man-made lakes at Turner Valley and Hartell.

Larvae and pupae of *Chrysops discalis* Williston and adults of *Hybomitra pediontis* (McAlpine) were collected at McGregor Lake near Milo. This lake is in ecological area 3, southern prairie, of the Transition Zone of the Boreal Region (Strickland, 1938).

During 1970, larvae and pupae were collected in the vicinity of Edmonton: from a muskeg at Winterburn on the western outskirts of the city; from sand at the edge of Lake Wabamun 30 miles west of the city and from a clear-cut in a spruce forest near Lake Wabamun; from a roadside ditch at Leduc 15 miles south of the city; and from a drying slough at Devon 20 miles southwest of the city. All these localities are in ecological area 10, poplar, of Strickland's (1938) classification.

2. MANITOBA FLY TRAPS

The Manitoba fly traps were modified slightly from the original design of Thorsteinson *et al*. (1965). Whereas the holding cage at the top of their trap was relatively small and charged with cyanide to kill the flies as quickly as possible, in my traps it was made 18 inches in diameter, 12 inches high and covered with fly screen. This large cage prevented damage to the flies and kept them alive.

3. TREATMENT OF ADULTS

The traps were emptied daily, through a sleeve at the side of the cage, in the early evening when tabanid activity had ceased. Flies were transported to the laboratory in one-pint plastic food containers packed in ice. Flies were either killed with cyanide and dissected immediately or kept alive and given water to drink, placed in sealed containers and kept at -12 C for later dissection. Some of these flies were dissected six months after capture and the condition of their ovaries was indistinguishable from those of recently killed flies.

4. COLLECTION AND REARING OF LARVAE

In 1968 and 1969, larvae were collected using the methods described by Teskey (1962). In 1970, a portable field separator was developed to separate tabanid larvae from moss. The adults from larvae which pupated within six weeks of the collection date were used for ovary development studies and chemical analyses. Larvae which had not pupated within six weeks of the collection date were killed and preserved. This was done to try and reduce any favourable or unfavourable bias in the amount of food stored which may occur in laboratory reared material. Usually, mature larvae were collected and these pupated within about three weeks of the collection date. Predaceous species were offered housefly maggots; _Chrysops_ species were usually not fed although _Chrysops frigidus_ Osten Sacken did eat first instar housefly maggots. Larvae were maintained individually in plastic test tubes and kept at 21 C. Pupae were kept

at, and adults allowed to emerge and harden their wings at, 13 C. Females were then either maintained on sugar at 21 C and examined for ovary development, or, along with males, fed water and used for chemical analyses after 12 - 24 hr at 21 C.

5. REMOVAL AND EXAMINATION OF OVARIES

Recently killed or frozen females were held in the left hand and the terminal abdominal segments were exposed by pulling them out with #5 iris forceps. The two terminal segments were cut off with fine scissors and discarded. The tips of the fine forceps were then placed into the abdomen through this hole and all the internal organs were extracted. The organs were placed in a drop of Ringer's solution and the ovaries removed and placed in a fresh drop of Ringer's on a slide. Great care was not required. If the ovaries were missed the first time, further groping with the forceps eventually proved successful. In every fly, except when the oocytes were mature or nearly so, the ovaries were intact. This was almost certainly due to the presence of an ovary sheath. With practice, ovaries were ready for examination within 30 seconds of picking up the female. This method has the advantage that the specimens can be pinned and kept in a collection. So little damage is done that close inspection is needed to distinguish between intact and dissected flies.

The ovaries were dissected, using two pairs of #5 forceps, beneath a dissecting microscope. The ovary sheath was torn off and the ovarioles teased apart. The developmental stage of the terminal follicle was recorded and the ovariole was examined for dilations (yellow bodies). Dissection and examination were performed under a

magnification of X25 or X50 using reflected light on a black background. Various combinations of gentian violet stain and sodium chloride solutions were tested (Giglioli, 1963) but no increase in definition or ease of examination was gained. If anything, the sodium chloride solution rendered the ovariole sheath and tunica opaque thus making the oocyte more difficult to see. For age determination it is important that complete ovarioles be examined, i.e. the pedicel must be attached to the calyx.

The gut was examined, visually, for any traces of ingested blood.

6. OVARY STRUCTURE AND FOLLICLE DEVELOPMENT

The ovaries of tabanids occupy a varying volume of the female's abdomen depending on their stage of development. Each ovary consists of numerous ovarioles and is enclosed within an ovarian sheath which gives it a somewhat firm structure. As in all Diptera the ovarioles are of the polytrophic type; the oocyte and nurse cells are within the same follicle. The terminal oocytes in each ovariole develop simultaneously and are usually laid together as a batch of eggs.

Hine (1906) noted that females of Tabanus sulcifrons Macquart had follicles in many stages of development. In 1911 Christophers first described the visible changes that occur in the development of the follicles of a blood sucking insect (mosquito). He divided the developmental changes into five stages, emphasizing that these were purely artificial as the developmental process was continuous. His method remains the basis for almost every subsequent description of follicle development in blood sucking insects.

Mer (1936) subdivided two of Christophers' stages, i.e. stage I into stage N and stage I, stage II into stage I-II and stage II. His greatest contribution, however, was to recognize that follicle development was not as continuous a process as Christophers envisaged. Thus although recognizing seven morphological stages, he recognized two physiological ones. The first of these was follicle development up to stage II (in _Anopheles sacharovi_ Favr) which was achieved at the expense of reserves accumulated during larval life or by the female obtaining a carbohydrate meal. Development up to this stage was continuous and the morphological stages up to this stage, i.e. N, I, I-II, are somewhat arbitrary. Development beyond stage II did not occur until the female had a blood meal and then development was again continuous. The morphological stages beyond stage II, i.e. III, IV, V, are again arbitrary.

Although Christophers' stages and Mer's refinements of them were developed to describe mosquito follicles they have been applied, sometimes with modifications, to most other blood feeding flies, e.g. Simuliidae (Lewis, 1957), Muscidae and Hippoboscidae (Detinova, 1962), Ceratopogonidae (Linley, 1965) and Tabanidae. Many authors have commented on ovary development in tabanids. Cameron (1934) referred to a female of _Haematopota pluvialis_ as having half-developed ovaries one day after emergence and another as having fully-developed ovaries seven days after emergence. Gordon, Chwatt and Jones (1948) used Christophers' (1911) classification to record follicle development in _Chrysops silacea_ Austen and _Chrysops dimidiata_ Wulp. Bonhag (1959) recorded the ovaries of _Tabanus sulcifrons_ as being immature. Davey and O'Rourke (1951) recorded the

ovaries of *Chrysops silacea* and *C. dimidiata* as being developed or undeveloped. Duke, Crewe and Beesley (1956) fed wild caught *Chrysops silacea* on blood and dissected them from four to seven days later. They classified the ovaries as being undeveloped, partially developed or fully developed depending on the size of the eggs and the volume the ovaries occupied within the abdomen. Crewe (1961) recorded changes in ovary development of *Chrysops silacea* by the volume of the ovaries. Again in reference to *Chrysops silacea*, Crewe and Beesley (1963) referred to wild caught females as having completely undeveloped ovaries. Corbet and Haddow (1962) used Macan's (1950) classification to record the developmental stage of the ovaries of *Tabanus thoracinus* Palisot de Beauvois. Corbet (1964b) used a modification of Christophers' (1911) and Macan's (1950) methods to record the ovarian stages in *Tabanus par* Walker, *Tabanus taeniola* Palisot de Beauvois, *Tabanus thoracinus*, *Chrysops centurionis* Austen and *Chrysops funebris* Austen. He divided stage II into early II, II, and late II depending upon the magnification required to see yolk in the oocyte (Corbet, 1964a). Raybould (1967) used Christophers' (1911) stages to record development in *Chrysops bicolor* Cordier. Rockel (1969) used Christophers' (1911) and Mer's (1950) classifications for his study of *Chrysops fuliginosus*. Davies and Lall (1970) recorded four-day old *Hybomitra lasiophthalma* (Macquart) as having scarcely-developed eggs.

a. Follicle developmental stages

In this study the developmental stage of the terminal follicle was classified using the stages recognized by Christophers (1911) and

Mer (1936). For convenience these stages are redefined here.

Stage N : Follicle consists of eight undifferentiated cells. Follicles varied from 64 microns to 106 microns long and from 40 microns to 100 microns wide.

Stage I : Oocyte becomes differentiated from the nurse cells. No yolk granules observed when viewed at X50 magnification. Follicles approximately 240 microns long by 180 microns wide.

Stage I-II: Yolk granules visible around the nucleus but they do not reach the posterior wall of the oocyte. Follicle approximately 300 microns long by 250 microns wide.

Stage II : Yolk extends to posterior wall of oocyte and usually present throughout oocyte. Oocyte occupies from one-third to one-half of the follicle. Follicle approximately 350 microns long by 300 microns wide.

Stage III : Oocyte occupies more than one-half of the follicle. The length:width ratio of the follicle is less than 2:1. Follicles approximately 600 microns long by 400 microns wide.

Stage IV : Follicles elongate so that length:width ratio is greater than 2:1. Nurse cells still visible at anterior pole of egg. Follicles approximately 1200 microns long by 550 microns wide.

Stage V : Follicles take on shape of mature egg. Length:width ratio is greater than 5:1. Nurse cells no longer visible. Follicle approximately 1300 microns long by 250 microns wide.

b. Physiological age classing

The physiological age of hematophagous insects is expressed in terms of the number of gonotrophic cycles a female has completed. Each gonotrophic cycle normally consists of three phases, the search for a host and the feeding on blood, the digestion of the blood and the maturation of the oocytes, and oviposition (Detinova, 1962). A fly that has not completed its first gonotrophic cycle is said to be nulliparous or a nullipar, and one that has completed one or more cycles is said to be parous. When the number of gonotrophic cycles completed is known the terms uniparous, biparous, etc. are used (Bertram,1962). There is no simple relationship between physiological age and chronological age although in any one locality a parous fly is likely to be older than a nulliparous one. Since knowledge of the physiological age composition of a population of hematophagous insects is a useful tool from the veterinary and medical aspects of disease transmission there has been much research on methods of aging these insects. An excellent review is to be found in Detinova (1968). Since this review, Hitchcock (1968) reported on his study of the age composition of _Anopheles quadrimaculatus_ Say in the U.S.A.

c. Age classing methods for tabanids.

The most obvious indication that a fly is parous is by the finding of retained mature oocytes in the reproductive system. Gordon _et al_. (1948) found that nine out of 492 _Chrysops silacea_ and _C. dimidiata_ had retained at least one mature oocyte. This

method is of little use as the percentage of flies retaining mature oocytes is low. Lewis (1960) found retained mature oocytes in 14% of parous _Chrysops bicolor_, while Raybould (1967) recorded 11% in the same species. Gordon _et al_. (1948) realized that oocyte retention only occurred in some flies as many flies had remnants of an old blood meal in their guts and others were infected with _Loa loa_. Both these features were regarded as evidence of parousness.

Corbet (1959) used the method of Colless (1958) (see below) to separate nulliparous and parous _Haematopota_ and _Tabanus_. Lewis (1960) examined the crop, malpighian tubules, fat body, accessory glands, the gut for parasites, time of biting, and ovary structure in an attempt to distinguish between parous and nulliparous _Chrysops bicolor_. Only ovary structure could separate the two age classes with certainty. The method used by Colless (1958), Lewis (1960), Duke (1960), Lutta (1964), Corbet (1964b), Raybould (1967) and Rockel (1969) was the one first demonstrated by Polovodova in Russia (see Detinova, 1962) with anopheline mosquitoes. The method consists of counting the number of follicular relics in an ovariole. After each oviposition, the follicular epithelium forms a small yellow resin-like globule which remains in the ovariole forming a dilation in the tunica. The number of these yellow bodies in an ovariole indicates the number of gonotrophic cycles the female has undergone. However, some difficulty has been experienced with _Anopheles melas_ Theobald (Giglioli, 1965). In this species only one dilation forms in each ovariole regardless of the number of ovulations that have occurred. Thus, ideally, before Polovodova's method is used to determine physiological age it should be checked against individuals of known reproductive history.

Polovodova's method of physiological aging was used in this present study. Because the method could not be checked with individuals of known age a slight change in nomenclature was used. A fly that showed evidence of completing only one gonotrophic cycle was recorded as being minimally uniparous, and one that showed evidence of completing two as minimally biparous. I include the word "minimally" in the designation of physiological age since it is not known if a dilation was retained after each oviposition. The presence of two dilations in some species suggests that this was in fact the case. However, the possibility exists that dilations may break down or otherwise be expelled from the ovariole.

The ovarioles of nulliparous tabanids have clear follicular tubes with no dilations or granules. Immediately after oviposition the ovariole is sac-like and about the size of the recently shed mature oocyte. Over a period of about two days this sac contracts and the cellular debris within it becomes obvious as a follicular relic. Up to and including this stage the fly was recorded as being in the sac stage. Further contraction of this sac-like dilation continues until a discrete oval or spherical dilation remains. This dilation contains a yellow or orange follicular relic but to avoid confusion with the uncoloured relic it is here referred to as a "yellow body". These yellow bodies form at the anterior (nearest germarium) end of the ovariole just posterior to the next developing follicle. When two yellow bodies were present in one follicular tube the fly was recorded as minimally biparous. When a follicular tube contained a yellow body and a sac-like dilation <u>anterior</u> to it the fly was recorded as being minimally biparous and in the sac stage.

The major objective of studying the physiological age composition of an adult population was to confirm the presence of autogeny. In a species that can be shown to be autogenous in the laboratory, by its developing mature oocytes without having had a blood meal, it is important to determine if this potential is realized in the field. One way of approaching this problem is to age-grade females attempting to obtain a blood meal. If only parous flies are collected and there is no evidence of them having had a blood meal, then this is good evidence that the species does realize its autogenous potential. Whether this reasoning can be used in reverse, i.e. if only parous flies are collected throughout a season then this species must be autogenous, is as yet a moot point. It would seem to depend, in a large part, on the number of individuals involved. Although the absence of nulliparous flies in host-simulating traps may confirm or suggest that a species is obligatory autogenous for the first gonotrophic cycle, the converse is not necessarily true. The presence of nulliparous flies does not mean that a species is obligatory anautogenous. If only nulliparous flies were collected this would be strong evidence for obligatory anautogeny. However, if both nulliparous and parous flies were collected interpretation would have to be more cautious. The parous flies would have to be examined to determine whether they had had a blood meal. This could probably have been achieved by examining the gut tracheation as has been done in *Anopheles* mosquitoes (Detinova, 1962; p. 76-77). This method has yet to be shown to be applicable to tabanids.

Subsidiary objectives of age-grading were to obtain information on the number of gonotrophic cycles and the relative survival between

age classes. When coupled with a study of the developmental stage of the terminal follicle, any relationship between time of blood seeking and follicle development would be apparent. The number of gonotrophic cycles completed was determined by counting the dilations in an ovariole. The relative survival of an age class was calculated as:

$$\frac{\text{number of females in a given age class}}{\text{number of females in previous age class}} \times 100.$$

7. FOOD RESERVES

a. Introduction

As autogenous hematophagous insects do not feed on blood during the first gonotrophic cycle, the nutrient for this batch of eggs must be obtained during the larval feeding stage. Although egg development is under neurohormonal control (Englemann, 1968; Spielman, 1971) the question remains as to whether anautogenous species have enough nutrient reserves to develop eggs. Thus some authors have compared the food reserves of autogenous and anautogenous mosquitoes. Roubaud and Toumanoff (1930) estimated that autogenous _Culex molestus_ stored greater quantities of fat during larval life than did anautogenous _Culex pipiens_. Clements (1956) estimated that the size of the fat body in mature larvae of _Culex pipiens_ was less extensive than that in _C. molestus_. This size difference was more pronounced in the adults although no differences (histochemically) in the nature or extent of the reserves within the fat body cells were found. He concluded that the anautogenous _C. pipiens_ contained sufficient reserves to be able to develop some eggs without the need of a blood meal. The first quantitative approach to the problem was

made by Twohy and Rozeboom (1957). They made a comparison of nitrogen, carbohydrates and fat content in female pupae and adults of autogenous *C. molestus* and anautogenous *C. pipiens* and showed that *C. molestus* contained more lipids, glycogen and nitrogen than did *C. pipiens* but that *C. pipiens* had a greater proportion (% dry weight) of nitrogen than did *C. molestus*. They also nullified Clements' (1956) suggestion that *C. pipiens* had enough food reserves for some egg development by showing that by the time unfed *C. molestus* deposited their autogenous eggs *C. pipiens* females had used up all their lipid and glycogen reserves. Males of *C. molestus* also contain more food reserves than males of *C. pipiens* (Rozeboom and Twohy, 1958). Lang (1963) also compared the food reserves of pupae and adults of *C. pipiens* and *C. molestus*. Rearing temperature affected the amount of lipid stored by the larvae. Briegel (1969) found that autogenous females of *Culex pipiens pipiens* were significantly heavier, had a distinctly higher total nitrogen content and contained twice as much protein as anautogenous *Culex pipiens fatigans*. No work has been done on the food reserves of tabanids although Rockel (1969) attempted to record the amount of fat body present in recently emerged and field collected specimens of *Chrysops fuliginosus*.

The objective of this part of the study was to compare wet, dry, lipid and soluble protein weights within and between species using reared males, reared females and field collected females.

b. Materials and methods

Field collected flies were kept alive for 24 hr and reared flies for 24-36 hr after emergence. Both groups were given water

ad lib. Each fly was placed in a 50 x 15 mm screw-capped vial and cooled in ice-water. When immobile, the wings were removed and the fly weighed to the nearest 100 micrograms. This weight was recorded as the wet weight. Wings were measured, from the origin of the humeral plate to the wing apex, to the nearest 0.17 mm in all species except *Chrysops frigidus* in which they were measured to the nearest 0.08 mm. The flies were replaced in the vials and stored at -20 C for up to six months. Subsequently, each fly was pre-cooled in dry-ice and acetone and then lyophilized for 72 hr in a Virtis freeze drying apparatus.having a temperature of -40 C and a system vacuum of 0.2 mm Hg. and then weighed. This weight was recorded as the freeze-dry weight.

c. Determination of total lipid

Preliminary tests showed that not all the water was always extracted in the freeze drying apparatus. Thus each fly was placed in a 60 x 20 mm vial which was 2/3 filled with anhydrous calcium sulphate (Drierite) and heated in an oven at 75 - 80 C for 24 hr. These vials were then stoppered and allowed to cool. Flies were then weighed. This procedure was repeated until a constant weight was achieved. This weight was recorded as the oven-dry weight. Intact insects, less wings, were then extracted with diethyl ether in a Soxhlet apparatus for 72 hr and again dried to a constant weight using the method outlined above. This weight was recorded as the residual dry weight and the difference between it and the oven-dry weight was recorded as the amount of lipid.

d. Determination of soluble protein

Reared lyophilized flies were individually homogenized in 2.5 ml to 10 ml (depending on the size of the fly) of ice-cold 10^{-4} M phosphate buffer (pH 7.4) using a 2 ml hand homogenizer. The volume was chosen so that a concentration of protein in the colorimeter tube of between 20 and 140 micrograms was obtained when 0.1 ml of the supernatant was used in a total volume of 5 ml; a range which fell within the straight part of the standard curve. The homogenate was centrifuged at 12,000 g for 30 min at 2 C in a Sorvall RC2-B refrigerated centrifuge. The supernatant was decanted and 0.1 ml of it was used for soluble protein determination. The method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard, was used. Colour development was allowed to proceed for 30 min in the dark and was measured at 500 nm using a Bausch & Lomb Spectronic '20' colorimeter. Each assay was replicated three times. Field collected flies were not assayed for soluble protein as these flies had invariably fed on sugar which interferes with colour development (Gerhardt and Beevers, 1968). Reared flies were not fed any carbohydrate.

e. Dry weight readings

Although oven dried flies usually weighed less than freeze dried flies the difference was negligible. No significant difference in dry weight could be found between oven dried flies (used for lipid determination) and lyophilized flies (used for soluble protein determination) of the same sex and species. Thus for the results and analyses the dry weights of both groups were not kept separate.

f. Adult size

Direct comparisons of the weights of food reserves are difficult to interpret when the individuals being compared are of different weights and volumes. Thus, in comparison of food reserves an attempt was made to only compare flies of the same size. There has been much work on the size of insects, especially in the study of growth. Size has been measured in terms of linear measurement of sclerotized parts (e.g. Dyar's law) and in weight (e.g. Przibram's rule) (Wigglesworth, 1965). Such "laws" are not applicable to all insects and in fact may not even be applicable to different parts of the same insect. The concept of allometric growth has been reviewed by Teissier (1960).

There is probably no one measurement that expresses the differences in all aspects of size between two species. Spielman (1957) made the general statement that wing length was commonly used as a criterion of size and used it in his work on autogeny in mosquitoes. Stanley (1935) showed that wing length in Drosophila decreased with increased rearing temperature. He made no comment on any other measurement. Jackson (1946) used the length of a wing vein as a measure of size in tsetse flies and this measurement of size was used for many years by subsequent workers. Hosoi (1954) showed that there was a close correlation between wing length and dry weight in Culex pipiens pallens over normal rearing temperatures. However, at low rearing temperatures there was a disproportionate elongation of the wings and at high rearing temperatures there was an excess shortening when compared with body weight which varied little. Bursell (1960) was dissatisfied with wing length as a measure of

size in tsetse flies. He considered the residual dry weight (= dry weight less fat) as an adequate measure of size because it remained constant throughout adult life. It was, however, of no use once flies had had a blood meal; neither was it a particularly convenient measurement. Thus he attempted to find a more satisfactory measure of size. This was performed by correlating various linear measurements with residual dry weight. The measurement giving the closest correlation being regarded as the best measure of size. He discarded wing length because it had significantly less correlation than dorsal thoracic surface, even though it fulfilled precisely his criteria for a satisfactory measure of size (i.e. convenient to measure, constant throughout life). He decided upon dorsal thoracic surface even though this measurement was affected by the temperature at which the pupal stage was maintained. Christophers (1960, p. 406-415) went into a detailed discussion of the most desirable criterion of size in *Aedes aegypti*. He concluded that wing length was the most suitable for the purpose. Van den Heuvel (1963) determined the effect of rearing temperature on the lengths of body parts in *Aedes aegypti*. His result pertinent to the present study was that a temperature change during pupation affected wing length, a high temperature at the moment of emergence caused shortening of the wings and a low temperature caused lengthening. Wing length is used as a measure of adult size in the Ceratopogonidae (Linley 1968; Linley, Evans and Evans, 1970; Whitsel and Schoeppner, 1970).

There has been no work on the criteria for size in tabanids. In this study, wing length was used as the measure of size. Reared flies were collected as mature larvae or pupae and thus the rearing

temperature was unknown. But it is well known that insects have preferred temperatures (Uvarov, 1931; p. 54). For tabanid larvae the preferred temperature has been shown to be about 22 C (Khan, 1953; Shamsuddin, 1966). Wing length has been shown to deviate from the expected only at extreme (for the species) high and low temperatures (Hosoi, 1954; van den Heuvel, 1963), i.e. at temperatures away from the preferendum, which in Aedes aegypti is about 30 C (Omardeen, 1957; Thomas, 1967). Presumably, field collected tabanid larvae developed at their preferred temperature thus eliminating any disproportionate lengthening or shortening of the adults' wings. Since pupae were maintained at a constant temperature (13 C) any effect of this temperature on the emerging adult should be the same for all species. Thus wing length is considered to be a convenient and valid measurement of adult size in this study.

8. STATISTICAL ANALYSES

a. Tests of independence

To determine whether there was any association between the developmental stage of the terminal follicles, the physiological character of the species, the physiological age of individuals, and when a fly sought a blood meal, two-way tests of independence were carried out. Since the marginal totals in the two-way tables were not fixed but varied and reflected population parameters, these experiments were considered to be of a Model I design and thus a G-test was used for the test of independence (Sokal and Rohlf, 1969; Chapt. 16.4). When total sample size was less than 200, Yate's correction for continuity was applied. The calculated G statistic

was compared with a tabled chi-square value for 1 df (3.84 at 5% level, 6.64 at 1% level). Various null hypotheses were tested (see Table XV); e.g. the presence of yolk in the terminal follicles of autogenous uniparous flies is independent of the time since the last oviposition. In Table XV this is presented as: stage vs. age of autogenous unipars. If the calculated G statistic was greater than the tabled chi-square value then the null hypothesis was rejected; if less it was accepted. The probability of obtaining the calculated G statistic by chance was recorded.

b. t-tests

In the intraspecific comparisons of wing length, wet weight, dry weight, lipid weight and soluble protein weight, means were compared by a t-test. Variances were checked for equality (homoscedasticity) by means of a two-tailed F test and the appropriate two-tailed t-test was then used. When variances were unequal an approximate t-value was calculated (Steel and Torrie, 1960; p. 81). The null hypothesis tested by each t-test was that there was no difference between the means of the two samples. The significance level gives the probability of the calculated t-value being obtained by chance. When this probability was less than 5% ($P < 0.05$) the difference between the two means was regarded as significant. Both absolute weights, e.g. mg of lipid, and proportional amounts, e.g. lipids as percentage of dry weight, were compared between samples.

c. Regression analyses

The intercept and slope of a Model II regression equation

were calculated. Wing length was chosen as the independent variable and the various weight measurements, i.e. wet, dry, lipid, soluble protein, were regressed upon it. Because wing length could not be measured without error and a single straight line that best fitted the data was required, the regression lines were computed using Bartlett's three-group method (Simpson, Roe and Lewontin, 1960, p. 230; Sokal and Rohlf, 1969, p. 483).

d. Correlation coefficients

Correlation coefficients between wing length and the various weight measurements were computed and compared with the tabled critical values (Rohlf and Sokal, 1969; Table Y) to test the null hypothesis that the correlation coefficient of the population from which the sample was taken was zero. When the calculated value exceeded the tabled value (for n-2 df) at the 5% level, the null hypothesis was rejected.

VI. RESULTS

1. FOLLICLE DEVELOPMENT IN REARED FEMALES

Eight species, Atylotus "A", Haematopota americana Osten Sacken, Tabanus reinwardtii Wiedemann, Chrysops frigidus Osten Sacken, Chrysops mitis Osten Sacken, Chrysops nubiapex Philip, Hybomitra frontalis (Walker), Hybomitra itasca (Philip), developed follicles beyond the diapausing stage (stage II) when fed sugar in the laboratory (Table I). These species are considered autogenous.

Seven species, Haematopota americana, Chrysops discalis Williston, Chrysops excitans Walker, Chrysops furcatus Walker, Hybomitra epistates (Osten Sacken), Hybomitra illota (Osten Sacken), Hybomitra typhus (Whitney) did not develop follicles beyond stage II (Table II). These species are considered anautogenous.

In Haematopota americana the two females from George Lake were anautogenous whereas those from the other localities were autogenous. This species probably exhibits facultative autogeny, depending upon amount of food reserves. More data are needed.

a. Discussion

Two of the autogenous species, Chrysops mitis and Tabanus reinwardtii, were shown to be autogenous by Cameron (1926). The others are all new records.

That Atylotus "A" was collected is interesting. The adults of this species are inseparable from Atylotus pemeticus (Teskey, 1969) and yet A. pemeticus had never been collected west of Minnesota (Philip, 1965). I found the larvae of Atylotus "A" to be common, being

Table I. Ovary development of reared female tabanids fed on sugar. Autogenous species.

Species	Number of Females	Developmental Stage of Terminal Follicle	Locality
Atylotus "A"	3	V	Winterburn
H. americana	1	V	Nordegg
	1	III	Hartell
	1	IV	Hartell
T. reinwardtii	1	IV	Hartell
C. frigidus	1	IV	Hartell
	2	IV and V	Winterburn
C. mitis	9	V	Wabamun
	5	V	Turner Valley
	1	V	Hartell
C. nubiapex	5	V	Hartell
	3	V	Turner Valley
	1	III	Turner Valley
H. frontalis	1	IV	Devon
	2	V	Devon
H. itasca	1	V	Bow River Forest

Table II. Ovary development of reared female tabanids fed on sugar. Anautogenous species.

Species	Number of Females	Developmental Stage of Terminal Follicle	Locality
H. americana	2	II	George Lake
C. discalis	10	I and II	McGregor Lake
C. excitans	2	I and II	Nordegg
C. furcatus	18	II	George Lake, Nordegg, Hartell
H. epistates	1	II	George Lake
H. illota	1	I	Devon
H. typhus	3	II	Bow River Forest

collected in every muskeg searched. Two males were also collected. The abundance of the larvae and the absence of adult records suggests that this species has peculiar feeding habits. The developmental stage of the follicles next to the mature oocytes was Stage II. This was unique, as in other species having mature oocytes development had only reached Stage 1. The possibility exists that this species is autogenous for all its gonotrophic cycles.

Tabanus reinwardtii may be autogenous for two or more gonotrophic cycles. In this study the larvae were amongst the most abundant, although rearing proved difficult. No adults were collected. Other authors have commented on the abundance of the larvae and the rareness of the adult (Cameron, 1926; Philip, 1928; Stone, 1930; Tashiro, 1950; Roberts, 1956; Pechuman, 1957), but none of them postulated why the adults were so rarely encountered.

2. PHYSIOLOGICAL AGE STUDIES

a. Confirmation of autogeny

The physiological ages of field-caught females in five of the species, *Chrysops frigidus*, *Chrysops mitis*, *Chrysops nubiapex*, *Hybomitra frontalis*, *Hybomitra itasca*, whose reared members developed mature oocytes in the laboratory without a blood meal is shown in Table III. Because no nulliparous flies were collected this is taken as confirmation that these species are autogenous for the first gonotrophic cycle. However, in each of three species the sample size is so small that considerable doubt exists as to the extent of autogeny.

b. Evidence for obligatory autogeny

In seven species, Atylotus incisuralis, Glaucops fratellus, Hybomitra astuta, Hybomitra lanifera, Hybomitra liorhina, Hybomitra rhombica osburni, Hybomitra rupestris, nulliparous flies were never, or rarely, collected (Table IV). Thus these species are suspected of being autogenous for the first gonotrophic cycle. However, no reared females were examined and thus proof of autogeny is lacking. The low sample sizes in three of the species does not eliminate the possibility that the absence of nulliparous flies was due to chance non-capture. The collected nulliparous flies are explained as chance captures. Such an explanation has a precedent in that 30 male tabanids were collected in Manitoba fly traps during this study (Thomas, 1970).

c. Evidence for facultative autogeny

In Haematopota americana, all 26 females collected at Nordegg between July 31 and August 10 1968 were parous as were four females collected in the Bow River Forest between July 8 and August 9 1969. However, at George Lake, one nulliparous female was collected with ingested blood in its gut and with stage IV follicles. The blood fed nulliparous fly suggests anautogeny whereas the parous individuals suggest autogeny.

d. Evidence for anautogeny

The physiological ages of field caught females in five of the species, Chrysops excitans, Chrysops furcatus, Hybomitra epistates, Hybomitra illota, Hybomitra typhus, whose reared females did not

develop terminal follicles beyond stage II when fed sugar is shown in Table V. Because of the common appearance of the nulliparous flies these species are considered to be anautogenous. Whether this anautogeny is obligatory of facultative cannot be determined from the data.

In 10 other species, Hybomitra affinis, Hybomitra arpadi, Hybomitra lasiophthalma, Hybomitra lurida, Hybomitra melanorhina, Hybomitra nuda, Hybomitra opaca, Hybomitra pediontis, Hybomitra tetrica hirtula, Hybomitra zonalis, the presence of nulliparous individuals (Table VI) suggests that these species are anautogenous. However, no reared females were examined.

e. Number of gonotrophic cycles

Six out of the 12 autogenous species had individuals which had completed two gonotrophic cycles (Tables III and IV). In the anautogenous species, nine out of 15 species had individuals which had completed two gonotrophic cycles (Tables V and VI).

f. Survival between age classes

The estimation of survival of flies from one age class to another was only applicable to the Bow River Forest data since it was only in this locality that a full season's collection was examined for parity.

In the autogenous species, survival of nullipars cannot be estimated since no idea of the population size of the nullipars was obtained. In the anautogenous species, estimates of survival ranged from 0% to 71.4% (Tables V and VI).

Table III. Physiological ages and survival of field collected female tabanids. Species which were autogenous when reared in the laboratory.

Species	Number of Females			Survival of Unipars*	Locality and Date
	Nullipars	Unipars	Bipars		
C. frigidus	0	1	0		George Lake, 24.VII
	0	5	0		Nordegg, 31.VII - 10.VIII
	0	6	0		Gorge Creek, 17.VI - 9.VIII
	0	1	0		Winterburn, 12.VI
C. mitis	0	8	0		Nordegg, 31.VII - 10.VIII
	0	3	0		Gorge Creek, 16.VI - 27.VII
C. nubiapex	0	2	0		Nordegg, 31.VII
	0	4	0		Gorge Creek, 16.VI - 18.VI
H. frontalis	0	34	0		George Lake, 9.VII - 25.VII
	0	95	0		Nordegg, 31.VII - 10.VIII
	0	112	14	12.5%	Gorge Creek, 17.VI - 21.VIII
	0	1	0		Winterburn, 12.VI
	0	0	2		Wabamun, 28.VII
H. itasca	0	31	1	3.2%	Gorge Creek, 22.VII - 9.VIII

* (number of females in a given age class/number in previous age class) x 100

Table IV. Physiological ages and survival of field collected female tabanids. Species which may be autogenous for the first gonotrophic cycle.

Species	Number of Females			Survival of Unipars*	Locality and Date
	Nullipars	Unipars	Bipars		
A. incisuralis	0	2	0		Nordegg, 31.VII - 1.VIII
	0	34	0		Gorge Creek, 23.VII - 21.VIII
G. fratellus	0	6	1	16.7%	Gorge Creek, 7.VIII - 9.VIII
	0	1	0		Banff, 3.VIII
H. astuta	0	4	0		Gorge Creek, 9.VIII - 11.VIII
H. lanifera	0	2	0		Banff, 1.VIII
	0	6	2		Jasper, 21.VIII
H. liorhina	0	1	0		Nordegg, 1.VIII
	1	90	0		Gorge Creek, 22.VII - 21.VIII
H. rhombica osburni	0	13	0		Nordegg, 1.VIII - 10.VIII
	1	267	2	<1%	Gorge Creek, 15.VI - 21.VIII
H. rupestris	1	13	0		Nordegg, 31.VII - 10.VIII 1968
	1	73	6	8.2%	Gorge Creek, 2.VII - 21.VIII
	0	1	0		Nordegg, 24.VI 1970

* (number of females in a given age class/number in previous age class) x 100

Table V. Physiological ages and survival of field collected female tabanids. Species whose reared females did not develop follicles beyond stage II when fed sugar; probably anautogenous for all gonotrophic cycles.

Species	Number of Females			Survival of Nullipars*	Survival of Unipars*	Locality and Date
	Nullipars	Unipars	Bipars			
C. excitans	5	6	0			Nordegg, 31.VII - 2.VIII
	1	0	0			Gorge Creek, 18.VI
C. furcatus	7	0	0			George Lake, 10.VII - 25.VII
	29	37	0			Nordegg, 31.VII - 10.VIII
	7	5	1	71.4%	20%	Gorge Creek, 18.VI - 7.VIII
H. epistates	6	0	0			George Lake, 9.VII - 12.VII
	5	1	1	20.0%		Gorge Creek, 18.VI - 24.VII
H. illota	224	48	0			George Lake, 3.VII - 25.VII
	0	3	0			Nordegg, 31.VII - 10.VIII
	26	7	1	26.9%	14.3%	Gorge Creek, 9.VI - 28.VII
H. typhus	95	32	0			Nordegg, 31.VII - 10.VIII
	26	11	5	42.3%	45.5%	Gorge Creek, 16.VI - 9.VIII

* (number of females in a given age class/number in previous age class) x 100

Table VI. Physiological ages and survival of field collected female tabanids. Species which are probably anautogenous for all gonotrophic cycles; no reared females examined.

Species	Number of Females			Survival of Nullipars*	Survival of Unipars*	Locality and Date
	Nullipars	Unipars	Bipars			
<u>H.</u> <u>affinis</u>	14	36	3			Nordegg, 31.VII - 10.VIII
	126	49	31	38.9%	63.3%	Gorge Creek, 9.VI - 10.VIII
<u>H.</u> <u>arpadi</u>	1	6	0			Nordegg, 31.VII - 10.VIII
	70	13	0	18.6%		Gorge Creek, 15.VI - 27.VII
<u>H.</u> <u>lasiophthalma</u>	3	3	0			George Lake, 8.VII - 11.VII
	10	53	3			Nordegg, 31.VII - 10.VIII
	88	45	14	51.1%	31.1%	Gorge Creek, 9.VI - 10.VIII
<u>H.</u> <u>lurida</u>	0	2	1			Nordegg, 31.VII 1968
	29	33	24		72.7%	Gorge Creek, 9.VI - 9.VII
	2	1	0			Nordegg, 3.VI 1970
<u>H.</u> <u>melanorhina</u>	0	3	0			Nordegg, 1.VIII - 10.VIII
	2	1	0			Gorge Creek, 9.VII - 28.VII
<u>H.</u> <u>nuda</u>	2	0	0			George Lake, 11.VI - 26.VI
	20	30	22		73.3%	Gorge Creek, 9.VI - 28.VII
<u>H.</u> <u>opaca</u>	1	0	0			Nordegg, 1.VIII
	1	1	0			Gorge Creek, 23.VII - 28.VII
<u>H.</u> <u>pediontis</u>	11	0	0			McGregor Lake, 11.VII
<u>H.</u> <u>tetrica</u> <u>hirtula</u>	1	0	0			Nordegg, 2.VIII
	14	5	0	35.7%		Gorge Creek, 17.VI - 26.VII
<u>H.</u> <u>zonalis</u>	2	1	0			Nordegg, 2.VIII - 10.VIII
	5	3	1	60.0%	33.3%	Gorge Creek, 15.VI - 27.VII

* (number of females in a given age class/number in previous age class) x 100

Survival of unipars in autogenous species was estimated at being from 0% to 16.7% (Tables III and IV); whereas in the anautogenous species it was estimated at being from 0% to 73.3% (Tables V and VI).

g. Discussion

Perhaps some discussion as to the validity of the inference, that if only parous flies are captured the species is autogenous, is warranted. The question arises as to why nulliparous flies were never collected. At least three explanations are possible. Firstly, nullipars were not collected because they did not seek a blood meal; and they did not seek a blood meal because they had enough food reserves to develop a batch of eggs without it. A second possibility is that they did seek a blood meal but the population was so low that none were captured in Manitoba fly traps. The third possibility is that nullipars may not fly far from the larval habitat and thus if traps are not in the larval habitat the adults will not be collected. This possibility can be discounted for seven species as larvae were found in the immediate vicinity of the traps.

Linley _et al_. (1970) showed that reared female _Culicoides furens_ matured eggs in the laboratory without having had a blood meal and thus concluded that this species is autogenous. They supported their argument with the evidence that all the females attacking them in the field were parous.

Because parous flies of an autogenous species are collected in Manitoba fly traps it is evident that autogeny is restricted to the first gonotrophic cycle. The species is anautogenous for subsequent cycles. This parallels autogeny in mosquitoes (Spielman, 1971).

A type of age class distribution that was suggestive of limited autogeny occurring was observed in Hybomitra lurida and Hybomitra nuda. In both these species nulliparous females were common but were outnumbered by uniparous females (Table VI). However, parous females were collected on the first day the traps were operational (June 9) (Figs. 1 and 2). The most probable explanation is that these species emerged exceptionally early, in late May, and the peak emergence of nullipars was missed and mostly parous flies were collected. In other parts of Canada these two species are amongst the first to appear (Miller, 1951; Davies, 1959; Hanec and Bracken, 1964; Smith, Davies and Golini, 1970).

The technique of age classing females attracted to a trap to determine the number of gonotrophic cycles has the disadvantage that it may not indicate the maximum number of cycles the species completes. One can only determine the number of cycles completed after a female has begun (by seeking a blood meal) the next cycle. Thus, females completing their second gonotrophic cycle may not live to begin their third and the maximum age of the species will be recorded as uniparous. This problem will only be solved by collecting and aging females. immediately after they have oviposited in the field. In this study ovipositing females were never seen in the field. It is probable that some members of each species completed their last gonotrophic cycle and thus an additional cycle can be added to the maximum number recorded in this study. Thus, the 12 species: *Atylotus incisuralis*, *Haematopota americana*, *Chrysops excitans*, *C. frigidus*, *C. mitis*, *C. nubiapex*, *Hybomitra arpadi*, *H. astuta*, *H. liorhina*, *H. melanorhina*, *H. opaca* and *H. tetrica hirtula*, which were recorded as being uniparous and

HYBOMITRA LURIDA BOW RIVER 1969

NULLIPAROUS

NUMBER OF FEMALES

10
5
0

9 14 19 24 29 4 9

MINIMALLY UNIPAROUS

NUMBER OF FEMALES

10
5
0

9 14 19 24 29 4 9

MINIMALLY BIPAROUS

NUMBER OF FEMALES

10
5
0

9 14 19 24 29 4 9

JUNE JULY

Fig. 1 Age composition of female Hybomitra lurida (Fallen) collected in the Bow River Forest, Alberta, in 1969.

HYBOMITRA NUDA BOW RIVER 1969

NULLIPAROUS

OF FEMALES

5

0

9 14 19 24 29 4 9 14 19 24 29

MINIMALLY UNIPAROUS

NUMBER OF FEMALES

15

10

5

0

9 14 19 24 29 4 9 14 19 24 29

MINIMALLY BIPAROUS

OF FEMALES

5

0

9 14 19 24 29 4 9 14 19 24 29

JUNE JULY

Fig. 2 Age composition of female *Hybomitra nuda* (McDunnough) collected in the Bow River Forest, Alberta, in 1969.

beginning their second gonotrophic cycle probably completed it. The 15 species: *Glaucops fratellus*, *Chrysops furcatus*, *Hybomitra affinis* *Hybomitra epistates*, *H. frontalis*, *H. illota*, *H. itasca*, *H. lanifera*, *H. lasiophthalma*, *H. lurida*, *H. nuda*, *H. rhombica osburni*, *H. rupestris*, *H. typhus*, and *H. zonalis* which were recorded as beginning a third gonotrophic cycle probably completed it. In Russia, most species are characterised by completing two gonotrophic cycles (Lutta, 1964; 1965; 1967), although Olsufiev (1940) recorded five cycles in *Tabanus autumnalis*.

It is interesting to compare the number of gonotrophic cycles completed by autogenous species with the number completed by anautogenous species. Thus in the group that probably completed two cycles, seven out of twelve (58%) were autogenous. In the group completing three cycles, six out of fifteen (40%) were autogenous. The implication is that autogenous species have fewer gonotrophic cycles than anautogenous species.

Comparison between autogenous and anautogenous unipars indicates that the anautogenous species had a greater survival between gonotrophic cycles than did the autogenous species.

The survival rate of *Chrysops silacea* in the field was shown to be of the same order as that in the laboratory (Kershaw *et al.*, 1957). They suggested that half of a group of flies survived the first five days. Mortality rate thus appeared to increase with chronological age (Kershaw, Chalmers and Duke, 1954). Development and oviposition of eggs in *Chrysops silacea* requires at least six days (Duke, Crewe and Beesley, 1956). Thus the survival rate of nullipars would be in the order of 50%, and the survival of unipars would be in the order of 20%.

In my study, 71% of nulliparous *Chrysops furcatus* survived to become unipars and 20% of the unipars survived to become bipars; suggesting that the mortality rate increases with physiological age. These figures, however, are only based on 13 flies. The survival of *Hybomitra* species was based on larger samples. In *H. affinis* there was a greater percent survival between unipars and bipars (63%) than between nullipars and unipars (39%). In *H. typhus* survival was equal. In *H. lasiophthalma* 51% of the nullipars survived to become unipars and 31% of the unipars survived to become bipars. The problem posed by Kershaw *et al.*(1957), as to whether mortality rate in tabanids in natural conditions is constant or increases with age, is still unsolved.

3. OVARIAN STAGE AND BLOOD SEEKING

a. Nulliparous flies

There was a marked consistency within and between species with regard to the developmental stage of the terminal follicles and blood seeking activity (Table VII). The majority of nulliparous females had stage II follicles, with only 9.4% having terminal follicles lacking yolk (stage I) and only 1.4% having follicles developed beyond stage II.

b. Minimally uniparous flies

These flies were separated into recently oviposited females, i.e. in the sac stage, and older females, i.e. with yellow bodies.

In the autogenous species in the sac stage there were two distinct groups based upon the development of the terminal follicles (Table VIII). In one group, *Hybomitra astuta*, *Hybomitra itasca*,

Hybomitra liorhina, almost all the flies had undifferentiated follicles; while in the other group of 10 species the majority of females had terminal follicles differentiated but 80% of them lacked yolk. No females had follicles developed beyond stage II.

In the anautogenous species in the sac stage half the females (53%) had differentiated oocytes lacking yolk (Table IX).

In the autogenous species with yellow bodies most females (67%) had terminal follicles in stage II of development (Table X).

In the anautogenous species with yellow bodies most females (83%) had terminal follicles in stage II of development (Table XI).

c. Minimally biparous flies

These flies were also divided into recently oviposited females and older females.

The few autogenous flies in the sac stage had oocytes lacking yolk (Table XII).

In the anautogenous species in the sac stage, 87% of the females had terminal oocytes lacking yolk (Table XII).

In the autogenous species having two yellow bodies, 70% of the females had deposited yolk in the terminal oocyte (Table XIII).

In the anautogenous species having two yellow bodies, 52% of the females had oocytes lacking yolk (Table XIII).

d. Evidence for anautogeny

In 1968, some flies attacking man in the field were permitted to feed on his blood and were then collected. They were kept in the laboratory in the hope they would produce viable eggs. However, no

Table VII. Developmental stages of terminal follicles in blood seeking tabanids. Nulliparous flies.

Species	Number of Females							Total
	Developmental Stages							Examined
	N	I	I-II	II	III	IV	V	
C. excitans		3		3				12
C. furcatus		6	1	30			2	82
H. affinis		20	13	107				259
H. arpadi		16	17	37			1	90
H. epistates		3		8				13
H. illota		5	2	195			3	242
H. lasiophthalma		10		89	1	1		219
H. liorhina							1	92
H. lurida		1	3	27				92
H. melanorhina				2				6
H. nuda		1	2	18			1	72
H. opaca		1		1				3
H. pediontis				11				11
H. rhombica osburni						1		270
H. rupestris				2				95
H. tetrica hirtula				15				20
H. typhus		8	1	112				169
H. zonalis				7				12
Totals		74	39	664	1	2	8	

Table VIII. Developmental stages of terminal follicles in blood seeking tabanids. Minimally uniparous flies in the sac stage; autogenous species.

Species	N	I	I-II	II	III	IV	V	Total Examined
	Number of Females							
	Developmental Stages							
A. incisuralis		20		2				36
H. americana		3						31
G. fratellus		2		1				8
C. frigidus		6						13
C. mitis		5						11
C. nubiapex		3		1				6
H. astuta	4							4
H. frontalis		64	2	5				255
H. itasca	30	1						32
H. lanifera		1						10
H. liorhina	80	10						92
H. r. osburni	2	153	4	63				270
H. rupestris		58		5				95
Totals	116	326	6	77				

Table IX. Developmental stages of terminal follicles in blood seeking tabanids. Minimally uniparous flies in the sac stage; anautogenous species.

Species	Number of Females Developmental Stages							Total Examined
	N	I	I-II	II	III	IV	V	
C. excitans		1		3				12
C. furcatus		13						82
H. affinis		38	3	30				259
H. arpadi		11	1	5				90
H. epistates				1				13
H. illota		1	1	3				242
H. lasiophthalma		36	3	20				219
H. lurida		21	2	9				92
H. melanorhina		2		1				6
H. nuda		9	2	17				72
H. opaca				1				3
H. tetrica hirtula		2		1				20
H. typhus	3	10		19				169
H. zonalis				4				12
Totals	3	144	12	114				

Table X. Developmental stages of terminal follicles in blood seeking tabanids. Minimally uniparous flies with yellow bodies; autogenous species.

Species	Number of Females							Total
	Developmental Stages							Examined
	N	I	I-II	II	III	IV	V	
A. incisuralis		4		9	1			36
H. americana		10		18				31
G. fratellus				4				8
C. frigidus		1		6				13
C. mitis		4		2				11
C. nubiapex				2				6
H. frontalis		61		108				255
H. lanifera				5	2			10
H. liorhina		1						92
H. r. osburni		9		35	1			270
H. rupestris		6		18				95
Totals		96		207	4			

Table XI. Developmental stages of terminal follicles in blood seeking tabanids. Minimally uniparous flies with yellow bodies; anautogenous species.

Species	Number of Females Developmental Stages							Total Examined
	N	I	I-II	II	III	IV	V	
C. excitans		1		1				12
C. furcatus		10		19				82
H. affinis		4		10				259
H. arpadi		2						90
H. illota		1		30				242
H. lasiophthalma		4		38				219
H. lurida		1		2		1		92
H. melanorhina				1				6
H. nuda				2				72
H. tetrica hirtula				2				20
H. typhus				11				169
Totals		23		116		1		

Table XII. Developmental stages of terminal follicles in blood seeking tabanids. Minimally biparous flies, sac stage plus yellow body; autogenous and anautogenous species.

Species	Number of Females							Total
	Developmental Stages							Examined
	N	I	I-II	II	III	IV	V	
1/ Autogenous species:								
G. fratellus		1						8
H. frontalis		8						255
H. itasca	1							32
H. lanifera			1					10
H. r. osburni		2						270
H. rupestris		4						95
Totals	1	15	1					
2/ Anautogenous species:								
H. affinis		31		2				259
H. epistates				1				13
H. illota		1						242
H. lasiophthalma		11		2				219
H. lurida		16		4				92
H. nuda		9	2					72
H. typhus		4						169
H. zonalis		1						12
Totals		73	2	9				

Table XIII. Developmental stages of terminal follicles in blood seeking tabanids. Minimally biparous flies, two yellow bodies; autogenous and anautogenous species.

Species	Number of Females Developmental Stages N	I	I-II	II	III	IV	V	Total Examined
1/ Autogenous species:								
H. frontalis		2		5				255
H. lanifera				1				10
H. rupestris		1		1				95
Totals		3		7				
2/ Anautogenous species:								
C. furcatus		1						82
H. affinis				1				259
H. lasiophthalma		1		3				219
H. lurida		3		2				92
H. nuda		6		3				72
H. typhus				1				169
Totals		11		10				

eggs were laid. When these flies died or were killed their ovaries were examined for follicle development. Most of the flies had mature or almost-mature oocytes (Table XIV) but in some, development had not passed beyond the resting stage (stage II). Field collected, non-blood-fed females never developed follicles beyond stage II. Whether this means that some individuals require more than one full blood meal to develop eggs or whether some flies were unsuccessful in obtaining a full blood meal is unknown.

e. Discussion

The stage of follicular development at which nulliparous and parous flies come to bite is often characteristic of the species (Duke, Crewe and Beesley, 1956; Corbet, 1964a). Since very few nulliparous flies were collected with terminal follicles developed beyond stage II any stage prior to and including stage II can be considered to be when ovarian diapause occurs. Presumably, these females carry enough food reserves over from the larval stage to enable them to deposit some yolk in the oocytes but require a blood meal for the maturation of the oocytes.

Whether or not yolk is present in the terminal oocytes immediately after oviposition depends upon whether the species is autogenous or anautogenous and whether the female is uniparous or biparous (Table XV). In sac stage flies, significantly more anautogenous unipars than autogenous unipars had yolk present in the terminal follicles (G = 52.6). However, there was no significant difference between sac stage biparous flies (G = 2.586). By the time a yellow body had formed in the uniparous flies (2-3 days after

Table XIV. Follicle development in blood fed tabanids.

N	Species	Fed	Died	Age	Follicle Development
1	H. frontalis	24.VII	6.VIII	1-par	stage IV
1	" "	24.VII	7.VIII	?	stage V
1	" "	25.VII	18.VIII	1-par	stage II
1	" "	25.VII	19.VIII	1-par	stage II
6	" "	3.VIII	13-15.VIII	?	stage V
1	" "	3.VIII	13.VIII	1-par	stage II
1	H. illota	25.VII	5.VIII	?	stage IV
1	" "	25.VII	6.VIII	?	stage V
1	H. lasiophthalma	3.VIII	13.VIII	?	stage V
1	H. r. osburni	3.VIII	13.VIII	1-par	stage III
1	H. typhus	3.VIII	13.VIII	?	stage V
1	" "	3.VIII	15.VIII	1-par	stage III
2	C. furcatus	3.VIII	15.VIII	?	stage V
1	H. americana	3.VIII	13.VIII	?	stage V
1	" "	3.VIII	13.VIII	1-par	stage II

oviposition) most females had deposited yolk in their terminal oocytes. However, there were still significantly more anautogenous flies with yolk than autogenous flies with yolk (G = 11.78). There was no significant difference between autogenous and anautogenous biparous flies (G = 0.784). Yolk deposition was dependent upon the time since the last oviposition in both autogenous and anautogenous species regardless of whether the fly was uniparous or biparous (G = 171 for autogenous unipars, G = 55.3 for anautogenous unipars, G = 9.49 for autogenous bipars, G = 9.045 for anautogenous bipars). The longer the time, the more probable was yolk to be present.

Thus, in general, anautogenous flies begin depositing yolk (in the next oocyte) before or immediately after the first oviposition whereas autogenous flies do not deposit it until a day or so after their first oviposition. Whether this delay in the deposition of yolk reflects the unavailability of precursors or simply a delay in their mobilization is debatable. In biparous flies there is a similar delay in yolk deposition in both autogenous and anautogenous species.

Thus the developmental stage of the terminal follicle when tabanids come to bite depends upon the physiological character, physiological age and how soon after oviposition (if the fly is parous) a blood meal is sought.

Table XV. Tests of independence between developmental stage of terminal follicles, physiological character of the species, and physiological ages of the individuals.

Data were analysed as Model I designs of two-way tests of independence using the G - statistic. Yate's correction was used when sample size was less than 200.

Hypothesis tested	DF	G	P
Stage vs. physiological character of young unipars	1	52.6	$P < 0.005$
Stage vs. physiological character of old unipars	1	11.78	$P < 0.005$
Stage vs. age of autogenous unipars	1	171	$P < 0.005$
Stage vs. age of anautogenous unipars	1	55.3	$P < 0.005$
Stage vs. physiological character of young bipars	1	2.586	$0.5 > P > 0.1$
Stage vs. physiological character of old bipars	1	0.784	$0.5 > P > 0.1$
Stage vs. age of autogenous bipars	1	9.49	$P < 0.005$
Stage vs. age of anautogenous bipars	1	9.054	$P < 0.005$

Where: physiological character = autogenous or anautogenous

young = flies in sac stage

old = flies with yellow bodies

4. FOOD RESERVES

a. Intraspecific comparisons

The statistics referring to wet weights of reared autogenous species are shown in Table XVI, of reared anautogenous species in Table XVII, of collected autogenous and anautogenous species in Table XXIII.

The statistics referring to dry weights of reared autogenous species are shown in Table XVIII, of reared anautogenous species in Table XIX, of collected autogenous and anautogenous species in Table XXIV.

The statistics referring to lipid weights of reared autogenous species are shown in Table XX, of reared anautogenous species in Table XXI, of collected autogenous and anautogenous species in Table XXV.

The statistics referring to soluble protein weights of reared autogenous and anautogenous species are shown in Table XXII.

The proportions of water, lipid and soluble protein in autogenous species are shown in Table XXVI and of anautogenous species in Table XXVII.

The probabilities of the observed differences between mean wing lengths, mean wet weights, mean dry weights, mean lipid weights, and mean soluble protein weights of reared male, reared female and collected female tabanids occurring by chance are shown in Table XXVIII.

The probabilities of the observed differences between the mean proportions of water, lipid and soluble protein of reared male, reared female and collected female tabanids occurring by chance are shown in Table XXIX.

b. Interspecific comparisons

The interspecific comparisons are presented as a series of figures where the various weight measurements are plotted against wing length. Lines of best fit (Model II regression) and polygons indicating the range of individuals' weights are shown. Individual weights are shown when too few individuals were measured for the fitting of a regression line.

Wet weights of reared males are shown in Fig. 3, dry weights in Fig. 4, lipid weights in Fig. 5, and soluble protein weights in Fig. 6.

Wet weights of reared females are shown in Fig. 7, dry weights in Fig. 8, lipid weights in Fig. 9, and soluble protein weights in Fig. 10.

Wet weights of collected females are shown in Fig. 11, dry weights in Fig. 12, and lipid weights in Fig. 13.

Table XVI. Mean wet weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared autogenous tabanids.

N	Sex	Species	$\overline{X} \pm$ S.D.	$\overline{Y} \pm$ S.D.	a	b	r	Significance Level
4	M	*Atylotus "A"*	9.18±0.417	53.95±11.17	-182.75	25.78	.96	$0.05 > P > 0.01$
6	F	" "	8.61±0.477	54.03± 5.45	- 41.39	11.08	.90	$0.05 > P > 0.01$
1	M	*T. reinwardtii*	11.91	107.4				
3	F	" "	13.00	176.20				
9	M	*C. frigidus*	5.88±0.333	11.87± 2.17	- 8.89	3.53	.64	$P > 0.05$
9	F	" "	6.43±0.597	17.76± 6.02	- 43.83	9.58	.98	$P < 0.01$
2	M	*C. mitis*	8.50	34.05				
2	F	" "	9.44	61.60				
2	F	*C. nubiapex*	8.25	37.65				
28	M	*H. frontalis*	11.61±0.590	97.20±16.98	-166.84	22.73	.75	$P < 0.01$
24	F	" "	11.59±0.584	134.08±26.87	-351.95	41.94	.92	$P < 0.01$
2	F	*H. itasca*	9.69	74.45				

Table XVII. Mean wet weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared anautogenous tabanids.

N	Sex	Species	$\overline{X}\pm$S.D.	$\overline{Y}\pm$S.D.	a	b	r	Significance Level
19	M	*C. excitans*	9.50±0.247	44.86±4.81	- 56.59	10.68	.54	0.05 > P > 0.01
31	F	" "	10.07±0.232	51.16±4.98	-133.25	18.62	.59	P < 0.01
1	F	*C. furcatus*	7.65	17.4				
1	M	*H. epistates*	11.73	88.3				
6	F	" "	13.06±0.250	103.03±8.65	-203.02	23.43	.50	P > 0.05
24	M	*H. illota*	10.38±0.400	69.67±10.90	-149.50	21.12	.79	P < 0.01
24	F	" "	10.82±0.357	76.09±11.27	-217.31	27.11	.78	P < 0.01
2	F	*H. lurida*	11.15	95.60				
1	F	*H. typhus*	9.35	45.7				

Table XVIII. Mean dry weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared autogenous tabanids.

N	Sex	Species	$\overline{X}\pm$S.D.	$\overline{Y}\pm$S.D.	a	b	r	Significance Level
3	F	T. reinwardtii	13.00	53.90				
1	F	H. americana	8.50	13.3				
11	M	C. frigidus	5.87±0.338	2.82±0.578	- 2.15	0.85	.59	$P > 0.05$
10	F	" "	6.46±0.571	5.05±1.78	- 13.44	2.86	.97	$P < 0.01$
2	M	C. mitis	8.50	9.45				
2	F	" "	9.44	18.25				
2	F	C. nubiapex	8.25	11.30				
27	M	H. frontalis	11.64±0.582	28.47±6.55	- 87.31	9.95	.83	$P < 0.01$
22	F	" "	11.61±0.576	40.81±9.19	-127.92	14.53	.89	$P < 0.01$
2	F	H. itasca	9.69	23.65				

Table XIX. Mean dry weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared anautogenous tabanids.

N	Sex	Species	$\overline{X} \pm$ S.D.	$\overline{Y} \pm$ S.D.	a	b	r	Significance Level
19	M	C. excitans	9.50±0.247	11.31±1.15	- 15.24	2.79	.59	$P < 0.01$
31	F	" "	10.07±0.232	13.05±1.43	- 39.24	5.19	.54	$P < 0.01$
1	F	C. furcatus	7.65	4.0				
1	M	H. epistates	11.73	27.7				
6	F	" "	13.06±0.250	27.30±2.70	- 98.20	9.61	.76	$P > 0.05$
23	M	H. illota	10.38±0.408	18.44±3.31	- 47.69	6.37	.81	$P < 0.01$
23	F	" "	10.81±0.362	20.50±3.07	- 56.92	7.16	.75	$P < 0.01$
2	F	H. lurida	11.15	25.35				
1	F	H. typhus	9.35	11.7				

Table XX. Mean lipid weight ($\bar{Y}$, mg.) and mean wing length ($\bar{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared autogenous tabanids.

N	Sex	Species	$\bar{X} \pm$ S.D.	$\bar{Y} \pm$ S.D.	a	b	r	Significance Level
1	F	*H. americana*	8.50	2.2				
3	F	*T. reinwardtii*	13.00	10.47				
3	M	*C. frigidus*	6.03±0.545	0.40±0.163				
5	F	" "	6.42±0.701	0.84±0.428	- 2.17	0.47	.96	$P < 0.01$
2	M	*C. mitis*	8.50	1.00				
2	F	" "	9.44	3.75				
2	F	*C. nubiapex*	8.25	2.05				
15	M	*H. frontalis*	11.67±0.694	4.82±1.99	- 26.39	2.67	.84	$P < 0.01$
12	F	" "	11.53±0.593	6.91±0.273	- 44.79	4.48	.90	$P < 0.01$
2	F	*H. itasca*	9.69	3.60				

Table XXI. Mean lipid weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared anautogenous tabanids.

N	Sex	Species	$\overline{X}\pm$S.D.	$\overline{Y}\pm$S.D.	a	b	r	Significance Level
10	M	*C. excitans*	9.44±0.269	0.79±0.251	- 4.76	0.59	.66	0.05 > P > 0.01
15	F	" "	10.10±0.279	0.89±0.423	- 9.67	1.05	.50	P < 0.05
1	F	*C. furcatus*	7.65	0.2				
1	M	*H. epistates*	11.73	5.4				
3	F	" "	12.92	1.33				
10	M	*H. illota*	10.39±0.464	2.31±0.924	- 10.87	1.27	.63	P = 0.05
12	F	" "	10.88±0.441	2.03±1.27	- 18.05	1.84	.46	P > 0.05
2	F	*H. lurida*	11.15	2.20				
1	F	*H. typhus*	9.35	0.5				

Table XXII. Mean soluble protein weight ($\bar{Y}$, mg.) and mean wing length ($\bar{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of reared autogenous and anautogenous tabanids.

N	Sex	Species	$\bar{X} \pm$ S.D.	$\bar{Y} \pm$ S.D.	a	b	r	Significance Level
1/ Autogenous species:								
8	M	*C. frigidus*	5.81±0.253	0.62±0.23	3.04	-0.42	-.06	$P > 0.05$
5	F	" "	6.50±0.488	1.43±0.47	- 4.77	0.95	.96	$P < 0.01$
12	M	*H. frontalis*	11.60±0.430	5.28±0.89	- 14.84	1.73	.68	$0.05 > P > 0.01$
10	F	" "	11.71±0.570	7.88±1.59	- 16.98	2.12	.81	$P < 0.01$
2/ Anautogenous species:								
9	M	*C. excitans*	9.58±0.209	2.88±0.25	- 6.78	1.01	.40	$P > 0.05$
16	F	" "	10.04±0.181	3.71±0.56	- 10.25	1.39	.21	$P > 0.05$
3	F	*H. epistates*	13.20	8.20				
13	M	*H. illota*	10.37±0.380	4.43±0.67	- 8.64	1.26	.79	$P < 0.01$
11	F	" "	10.74±0.250	5.04±1.01	- 20.24	2.35	.46	$P > 0.05$

Table XXIII. Mean wet weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of collected female autogenous and anautogenous tabanids.

N	Species	$\overline{X}\pm$S.D.	$\overline{Y}\pm$S.D.	a	b	r	Significance Level
1/ Autogenous species:							
3	*H. americana*	8.33	22.93				
15	*C. frigidus*	7.09±0.375	14.44±2.52	- 26.82	5.82	.72	$P < 0.01$
8	*C. mitis*	9.44±0.407	35.36±9.59	-176.73	22.48	.80	$0.05 > P > 0.01$
6	*C. nubiapex*	8.02±0.474	18.92±1.62	23.24	-0.54	.03	$P > 0.05$
60	*H. frontalis*	12.06±0.571	71.01±13.27	-156.02	18.83	.71	$P < 0.01$
2/ Anautogenous species:							
2	*C. excitans*	10.46	53.30				
15	*C. furcatus*	8.43±0.632	24.76±5.45	- 44.06	8.16	.92	$P < 0.01$
1	*H. epistates*	11.39	60.4				
52	*H. illota*	11.06±0.471	63.54±9.50	-129.84	17.48	.76	$P < 0.01$
3	*H. lurida*	11.67	64.50				
6	*H. typhus*	10.26±0.677	56.82±9.51	- 75.56	12.91	.98	$P < 0.01$

Table XXIV. Mean dry weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of collected female autogenous and anautogenous tabanids.

N	Species	$\overline{X}\pm$S.D.	$\overline{Y}\pm$S.D.	a	b	r	Significance Level
1/ Autogenous species:							
3	*H. americana*	8.33	6.00				
15	*C. frigidus*	7.09±0.375	3.87±0.73	- 9.16	1.84	.85	$P < 0.01$
8	*C. mitis*	9.44±0.407	9.90±2.99	- 57.10	7.10	.74	$0.05 > P > 0.01$
6	*C. nubiapex*	8.02±0.474	5.07±0.641	2.71	0.29	.36	$P > 0.05$
12	*H. frontalis*	12.61±0.569	20.28±5.72	- 71.93	7.59	.60	$0.05 > P > 0.01$
2/ Anautogenous species:							
15	*C. furcatus*	8.43±0.632	7.13±1.77	- 14.33	2.54	.85	$P < 0.01$
1	*H. epistates*	11.39	17.9				
12	*H. illota*	11.15±0.444	18.41±3.33	- 43.60	5.56	.73	$P < 0.01$
6	*H. typhus*	10.26±0.677	16.08±2.59	- 20.47	3.56	.98	$P < 0.01$

Table XXV. Mean lipid weight ($\overline{Y}$, mg.) and mean wing length ($\overline{X}$, mm.), intercept (a) and slope (b), correlation coefficient (r) and its significance level, of collected female autogenous and anautogenous tabanids.

N	Species	$\overline{X} \pm S.D.$	$\overline{Y} \pm S.D.$	a	b	r	Significance Level
1/ Autogenous species:							
11	*C. frigidus*	7.07±0.410	0.16±0.09	- 0.91	0.15	.17	P > 0.05
6	*C. mitis*	9.46±0.351	0.55±0.68	- 14.06	1.54	.43	P > 0.05
2	*C. nubiapex*	7.82	0.30				
12	*H. frontalis*	12.16±0.569	1.15±0.76	- 4.52	0.47	.38	P > 0.05
2/ Anautogenous species:							
10	*C. furcatus*	8.60±0.632	0.34±0.08	- 0.08	0.05	.09	P > 0.05
12	*H. illota*	11.15±0.444	1.08±0.49	- 2.50	0.32	.35	P > 0.05
6	*H. typhus*	10.26±0.677	0.77±0.22	- 2.07	0.28	.92	P = 0.01

Table XXVI. Proportions of water, lipid and soluble protein in reared male (RM), reared female (RF) and collected female (CF) tabanids; autogenous species.

Sex	Species	% Water Mean ± S.D.	% Lipid Mean ± S.D.	% Protein Mean ± S.D.
RF	*T. reinwardtii*	69.4	19.2	
RF	*H. americana*		16.5	
CF	" "	73.9	3.9	
RM	*C. frigidus*	74.8±1.63	11.70±2.95	22.90±5.42
RF	" "	72.74±1.67	16.86±2.78	27.80±1.35
CF	" "	73.07±3.05	4.33±2.36	
RM	*C. mitis*	72.30	10.20	
RF	" "	70.45	20.50	
CF	" "	72.10	2.75	
RF	*C. nubiapex*	70.00	18.10	
CF	" "	73.27	5.35	
RM	*H. frontalis*	70.86±2.55	15.41±3.07	20.14±2.57
RF	" "	70.20±1.51	16.74±3.45	19.16±2.89
CF	" "	73.23±2.48	5.33±2.61	
RF	*H. itasca*	68.60	15.45	

Water as % wet weight, Lipid as % dry weight, Soluble protein as % dry weight.

Table XXVII. Proportions of water, lipid and soluble protein in reared male (RM), reared female (RF) and collected female (CF) tabanids; anautogenous species.

Sex	Species	% Water Mean ± S.D.	% Lipid Mean ± S.D.	% Protein Mean ± S.D.
RM	*C. excitans*	74.79±0.87	6.84±1.51	25.64±1.19
RF	" "	74.53±0.89	6.53±2.53	29.71±3.03
RF	*C. furcatus*	77.0	5.00	
CF	" "	71.31	5.08	
RM	*H. epistates*	68.60	19.10	
RF	" "	75.68±6.22	4.73	28.80
CF	" "	70.40		
RM	*H. illota*	73.69±1.30	11.98±3.64	24.35±2.29
RF	" "	72.84±1.25	9.04±4.59	25.41±4.15
CF	" "	70.86±1.35	5.65±1.70	
RF	*H. lurida*	73.5	8.65	
RF	*H. typhus*	74.4	4.30	
CF	" "	71.63	4.69	

Water as % wet weight, Lipid as % dry weight, Soluble protein as % dry weight.

Table XXVIII. Probabilities of the observed differences between mean wing lengths, mean wet weights, mean dry weights, mean lipid weights, and mean soluble protein weights of reared male (RM), reared female (RF) and collected female (CF) tabanids occurring by chance; intraspecific comparisons.

Species	Sexes	Probabilities				
		Wing	Wet	Dry	Lipid	Protein
Autogenous species:						
Atylotus "A"	RM vs. RF	>0.05	>0.9			
C. frigidus	RM vs. RF	<0.05*	<0.05*	<0.05*		<0.01*
	RM vs. RF	>0.4			>0.1	
	RF vs. CF	<0.05*	>0.05	>0.05	<0.001*	
H. frontalis	RM vs. RF	>0.5	<0.05*	<0.001*	<0.05*	<0.001*
	RF vs. CF	<0.02*	<0.05*	<0.001*	<0.05*	
Anautogenous species:						
C. excitans	RM vs. RF	<0.01*	<0.001*	<0.001*	>0.4	<0.001*
H. illota	RM vs. RF	<0.02*	<0.05*	<0.05*	>0.5	>0.05
	RF vs. CF	<0.05*	<0.001*	>0.05		
	RF vs. CF	>0.1			<0.05*	

* Probabilities less than 5%, difference considered significant.

Table XXIX. Probabilities of the observed differences between the mean proportions of water, lipid and soluble protein of reared male (RM), reared female (RF) and collected female (CF) tabanids occurring by chance; intraspecific comparisons.

Species	Sexes	Probabilities		
		Water	Lipid	Protein
Autogenous species:				
C. frigidus	RM vs. RF	<0.02*	<0.05*	<0.05*
	RF vs. CF	>0.05	<0.001*	
H. frontalis	RM vs. RF	>0.05	>0.2	>0.4
	RF vs. CF	<0.05*	<0.001*	
Anautogenous species:				
C. excitans	RM vs. RF	>0.2	>0.5	<0.05*
H. illota	RM vs. RF	<0.05*	>0.1	>0.4
	RF vs. CF	<0.001*	<0.05*	

* Probabilities less than 5%, differences considered significant.

Water as % wet weight

Lipid as % dry weight

Soluble protein as % dry weight.

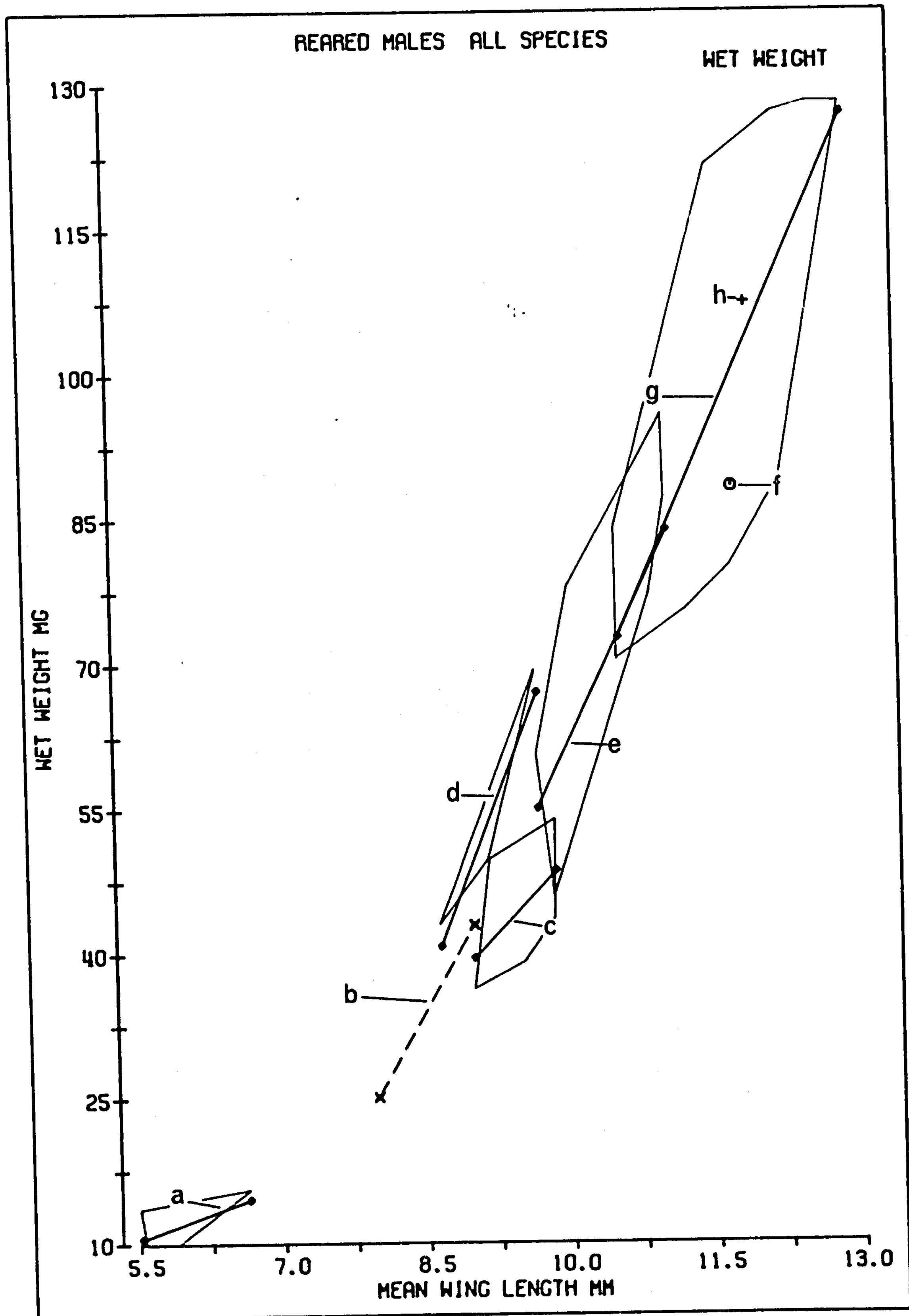
Fig. 3 Wet weight vs. wing length of reared male tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops mitis*	(Autogenous)
c	*Chrysops excitans*	(Anautogenous)
d	*Atylotus* "*A*"	(Autogenous)
e	*Hybomitra illota*	(Anautogenous)
f	*Hybomitra epistates*	(Anautogenous)
g	*Hybomitra frontalis*	(Autogenous)
h	*Tabanus reinwardtii*	(Autogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.



REARED MALES ALL SPECIES
WET WEIGHT
WET WEIGHT MG
MEAN WING LENGTH MM
130
115
100
85
70
55
40
25
10
5.5
7.0
8.5
10.0
11.5
13.0
a
b
c
d
e
f
g
h

Fig. 4 Dry weight vs. wing length of reared male tabanids.

a *Chrysops frigidus* (Autogenous)

b *Chrysops mitis* (Autogenous)

c *Chrysops excitans* (Anautogenous)

d *Hybomitra illota* (Anautogenous)

e *Hybomitra epistates* (Anautogenous)

f *Hybomitra frontalis* (Autogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.

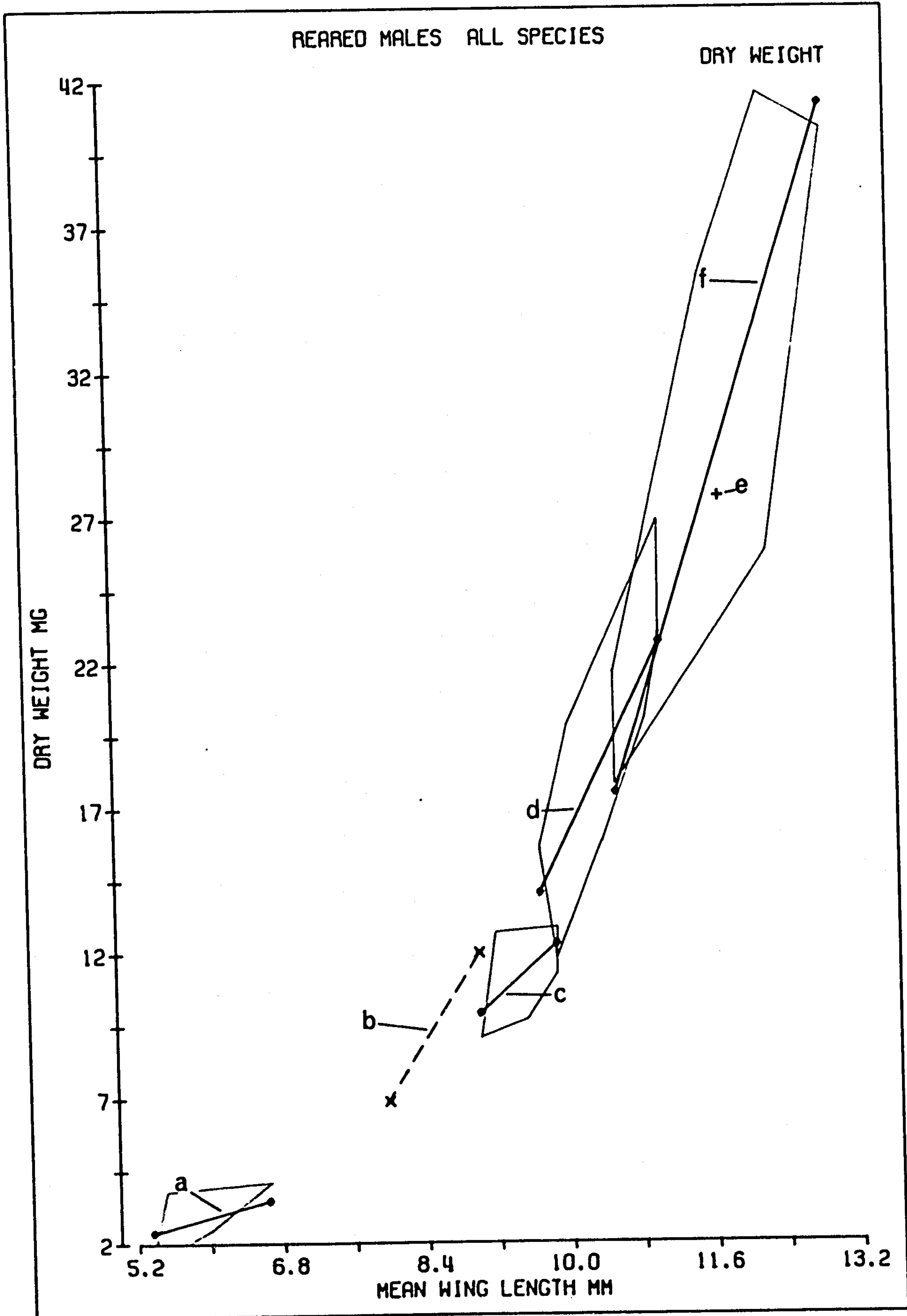

REARED MALES ALL SPECIES
DRY WEIGHT
42
37
32
27
22
17
12
7
2
DRY WEIGHT MG
5.2
6.8
8.4
10.0
11.6
13.2
MEAN WING LENGTH MM
a
b
c
d
e
f

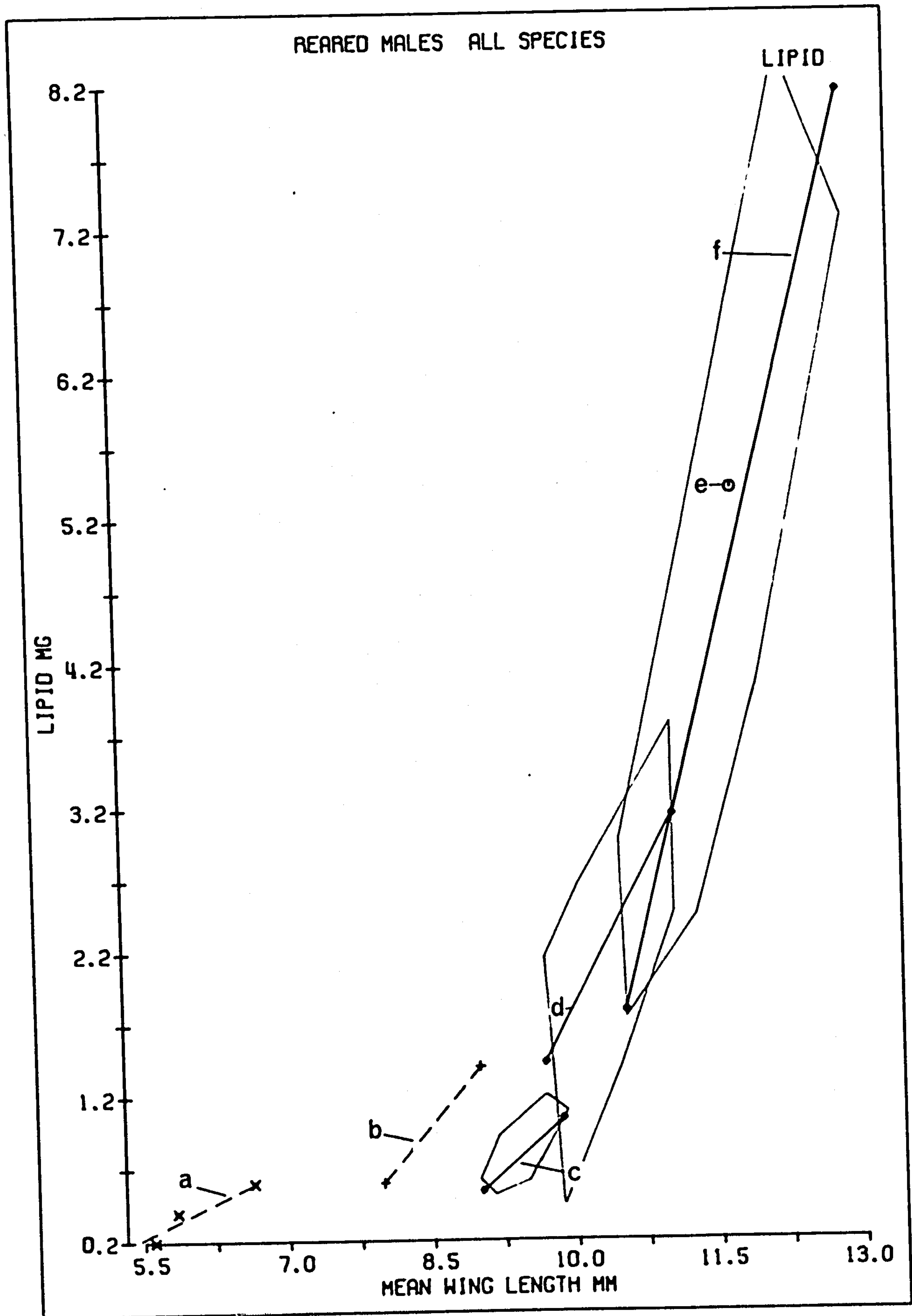
Fig. 5 Lipid weight vs. wing length of reared male tabanids.

a	Chrysops frigidus	(Autogenous)
b	Chrysops mitis	(Autogenous)
c	Chrysops excitans	(Anautogenous)
d	Hybomitra illota	(Anautogenous)
e	Hybomitra epistates	(Anautogenous)
f	Hybomitra frontalis	(Autogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.



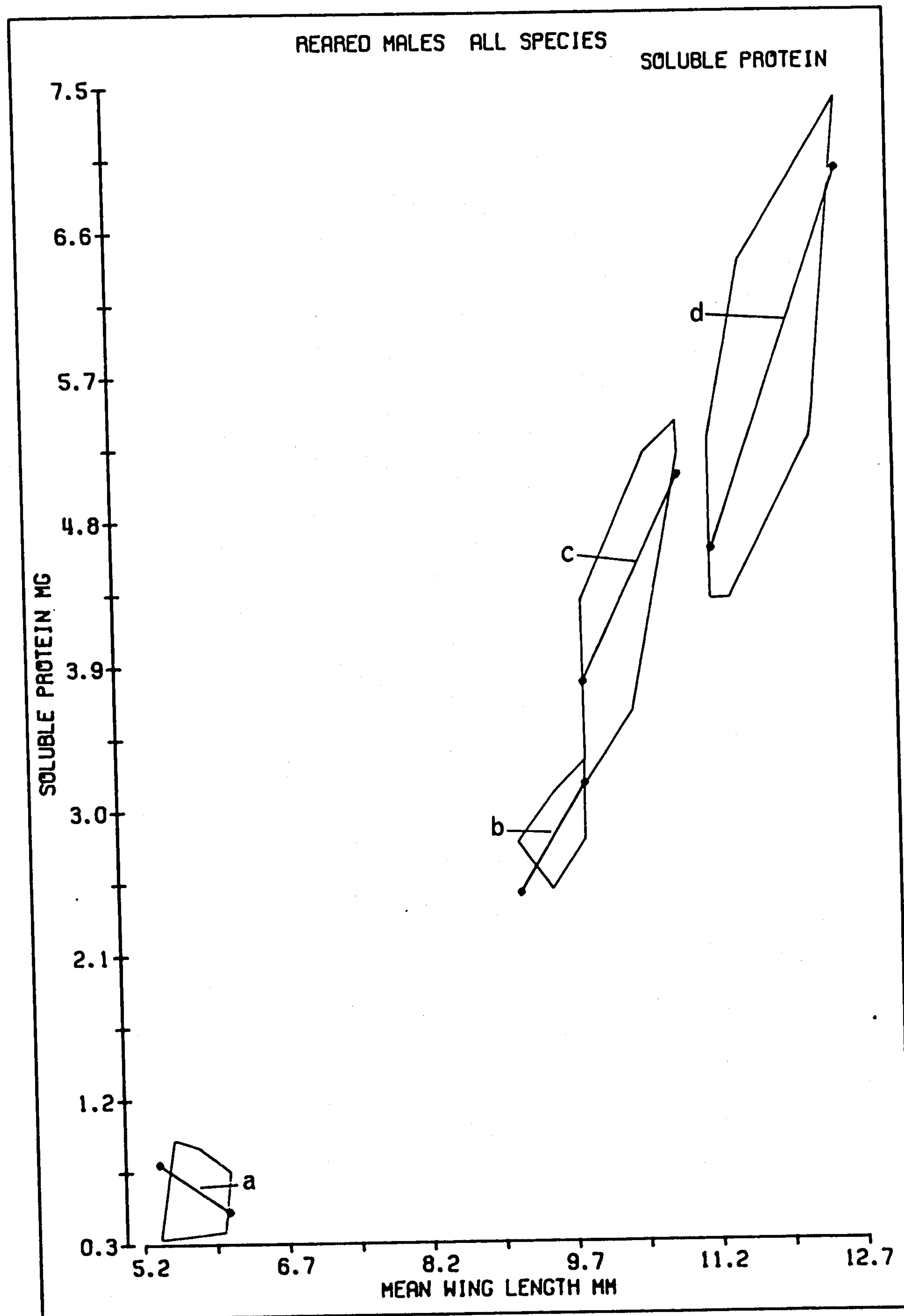
REARED MALES ALL SPECIES
LIPID
LIPID MG
8.2
7.2
6.2
5.2
4.2
3.2
2.2
1.2
0.2
5.5
7.0
8.5
10.0
11.5
13.0
MEAN WING LENGTH MM
a
b
c
d
e
f

Fig. 6 Soluble protein weight vs. wing length of reared male tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops excitans*	(Anautogenous)
c	*Hybomitra illota*	(Anautogenous)
c	*Hybomitra frontalis*	(Autogenous)

continuous line = fitted regression line.

Polygons indicate range of individuals' weights.



REARED MALES ALL SPECIES
SOLUBLE PROTEIN
7.5
6.6
5.7
4.8
3.9
3.0
2.1
1.2
0.3
SOLUBLE PROTEIN MG
5.2
6.7
8.2
9.7
11.2
12.7
MEAN WING LENGTH MM
a
b
c
d

Fig. 7 Wet weight vs. wing length of reared female tabanids.

a *Chrysops frigidus* (Autogenous)

b *Chrysops nubiapex* (Autogenous)

c *Atylotus "A"* (Autogenous)

d *Chrysops mitis* (Autogenous)

e *Hybomitra itasca* (Autogenous)

f *Hybomitra frontalis* (Autogenous)

g *Tabanus reinwardtii* (Autogenous)

h *Chrysops furcatus* (Anautogenous)

i *Hybomitra typhus* (Anautogenous)

j *Chrysops excitans* (Anautogenous)

k *Hybomitra illota* (Anautogenous)

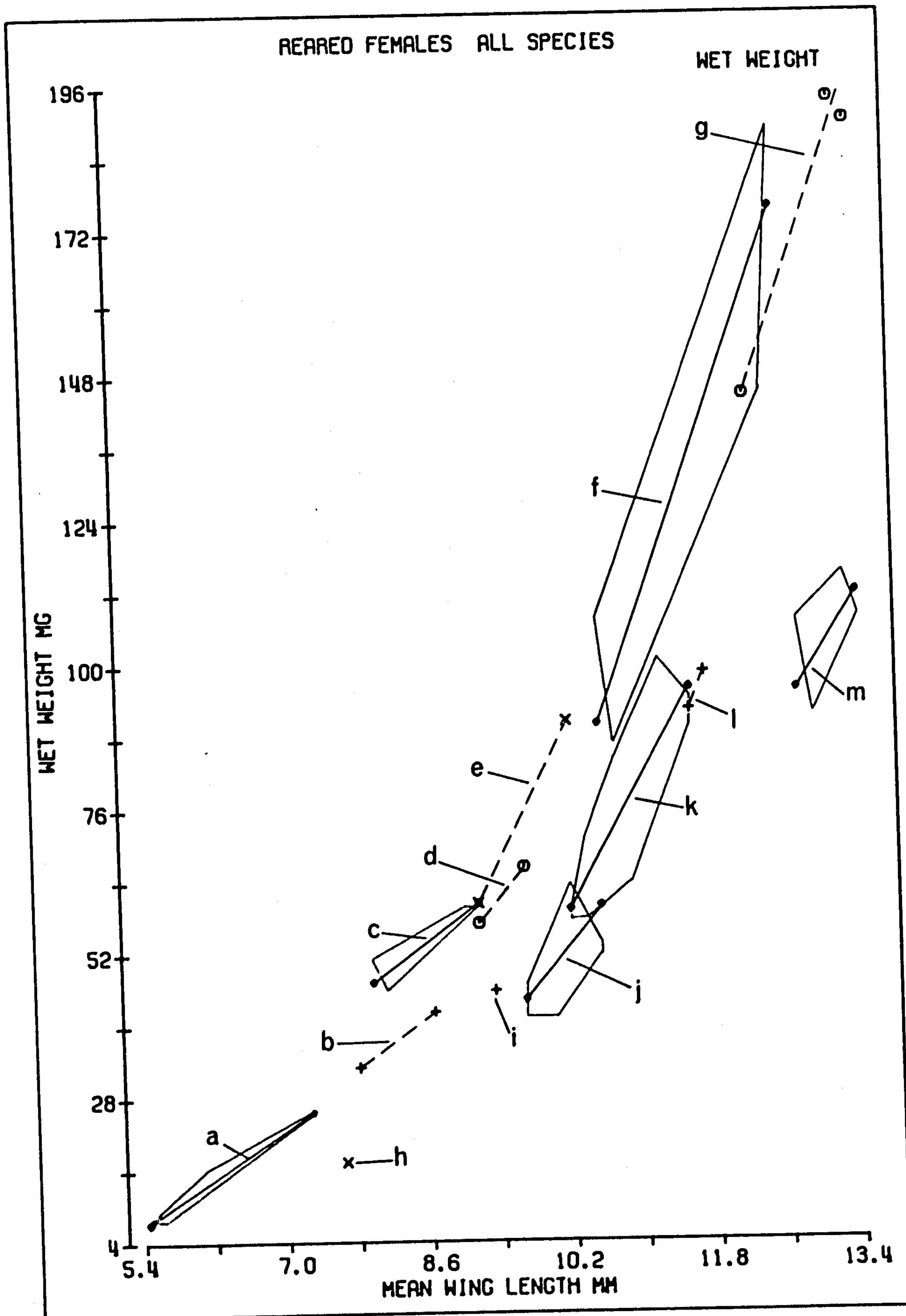
l *Hybomitra lurida* (Anautogenous)

m *Hybomitra epistates* (Anautogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.



REARED FEMALES ALL SPECIES
WET WEIGHT
WET WEIGHT MG
196
172
148
124
100
76
52
28
4
5.4
7.0
8.6
10.2
11.8
13.4
MEAN WING LENGTH MM
a
b
c
d
e
f
g
h
i
j
k
l
m

Fig. 8 Dry weight vs. wing length of reared female tabanids.

a *Chrysops frigidus* (Autogenous)

b *Chrysops nubiapex* (Autogenous)

c *Haematopota americana* (Autogenous)

d *Chrysops mitis* (Autogenous)

e *Hybomitra itasca* (Autogenous)

f *Hybomitra frontalis* (Autogenous)

g *Tabanus reinwardtii* (Autogenous)

h *Chrysops furcatus* (Anautogenous)

i *Hybomitra typhus* (Anautogenous)

j *Chrysops excitans* (Anautogenous)

k *Hybomitra illota* (Anautogenous)

l *Hybomitra lurida* (Anautogenous)

m *Hybomitra epistates* (Anautogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.

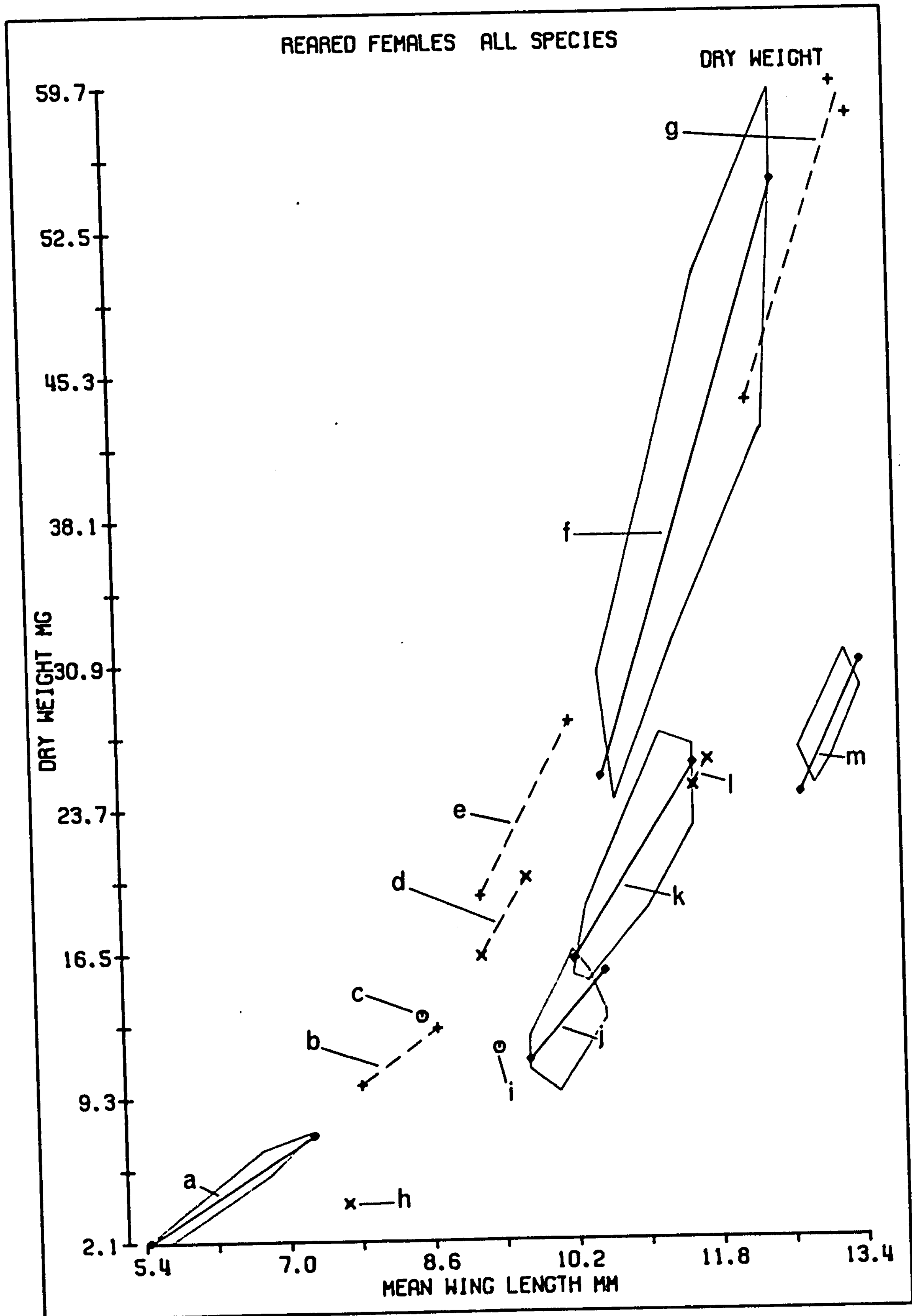

REARED FEMALES ALL SPECIES
DRY WEIGHT
DRY WEIGHT MG
59.7
52.5
45.3
38.1
30.9
23.7
16.5
9.3
2.1
5.4
7.0
8.6
10.2
11.8
13.4
MEAN WING LENGTH MM
a
b
c
d
e
f
g
h
i
j
k
l
m

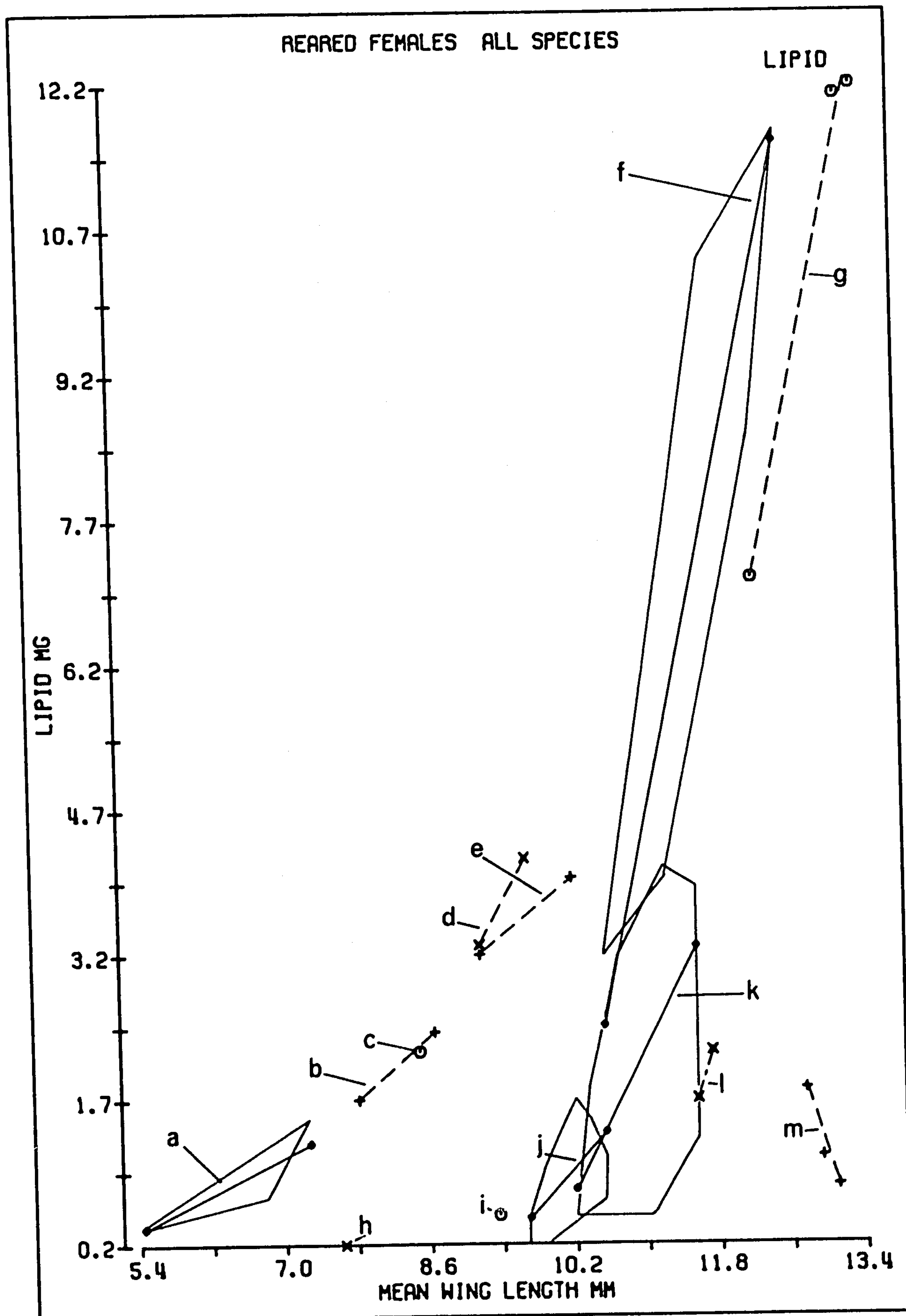
Fig. 9 Lipid weight vs. wing length of reared female tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops nubiapex*	(Autogenous)
c	*Haematopota americana*	(Autogenous)
d	*Chrysops mitis*	(Autogenous)
e	*Hybomitra itasca*	(Autogenous)
f	*Hybomitra frontalis*	(Autogenous)
g	*Tabanus reinwardtii*	(Autogenous)
h	*Chrysops furcatus*	(Anautogenous)
i	*Hybomitra typhus*	(Anautogenous)
j	*Chrysops excitans*	(Anautogenous)
k	*Hybomitra illota*	(Anautogenous)
l	*Hybomitra lurida*	(Anautogenous)
m	*Hybomitra epistates*	(Anautogenous)

continuous line = fitted regression line

dashed line used when too few individuals reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.



REARED FEMALES ALL SPECIES
LIPID
LIPID MG
MEAN WING LENGTH MM
12.2
10.7
9.2
7.7
6.2
4.7
3.2
1.7
0.2
5.4
7.0
8.6
10.2
11.8
13.4
a
b
c
d
e
f
g
h
i
j
k
l
m

Fig. 10 Soluble protein weight vs. wing length of reared female tabanids.

a *Chrysops frigidus* (Autogenous)

b *Chrysops excitans* (Anautogenous)

c *Hybomitra illota* (Anautogenous)

d *Hybomitra frontalis* (Autogenous)

e *Hybomitra epistates* (Anautogenous)

continuous line = fitted regression line

dashed line used when too few individuals were reared for the fitting of a regression line.

Polygons indicate range of individuals' weights.

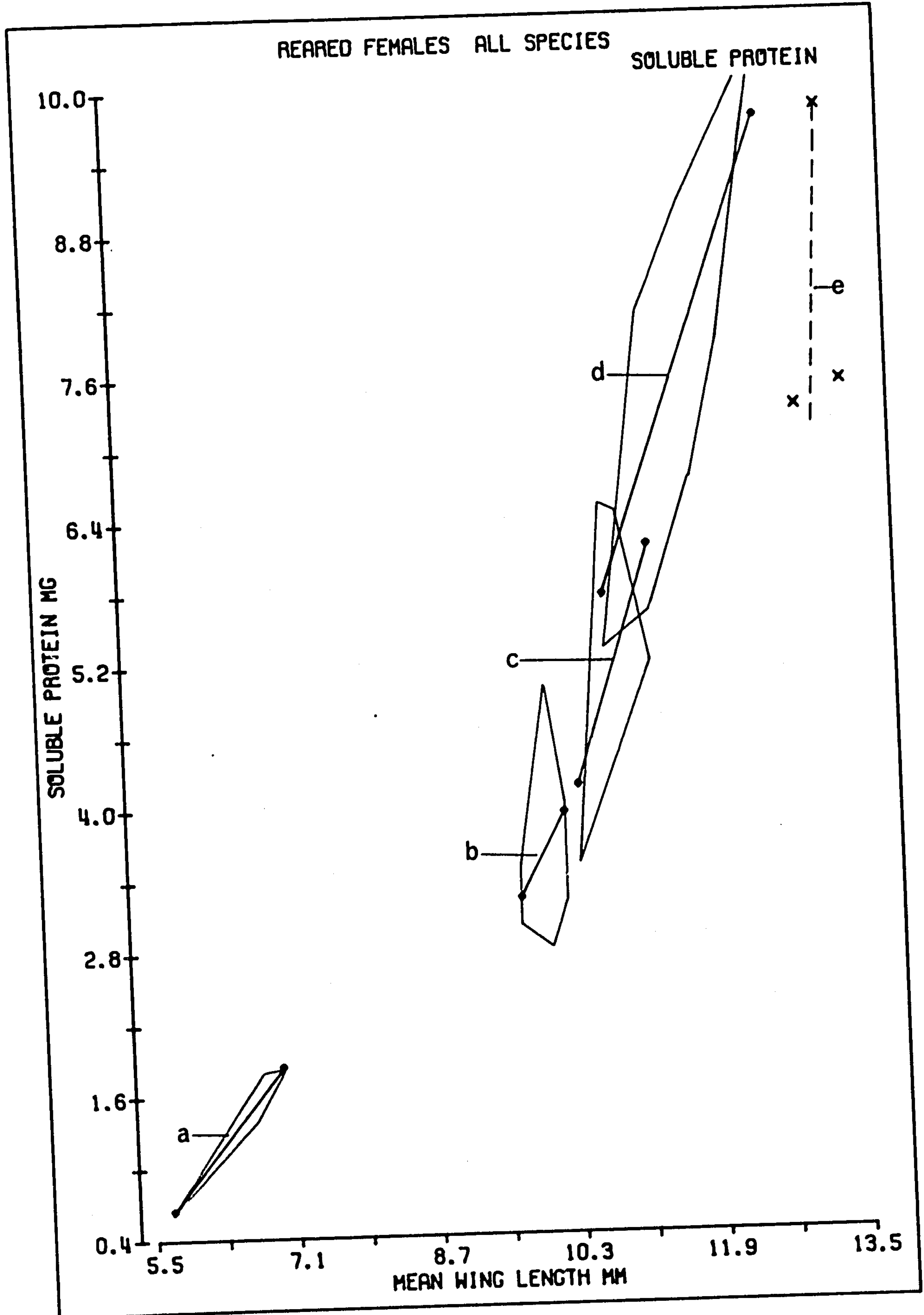

REARED FEMALES ALL SPECIES
SOLUBLE PROTEIN
SOLUBLE PROTEIN MG
10.0
8.8
7.6
6.4
5.2
4.0
2.8
1.6
0.4
5.5
7.1
8.7
10.3
11.9
13.5
MEAN WING LENGTH MM
a
b
c
d
e

Fig. 11 Wet weight vs. wing length of collected female tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops nubiapex*	(Autogenous)
c	*Chrysops furcatus*	(Anautogenous)
d	*Haematopota americana*	(Autogenous)
e	*Chrysops mitis*	(Autogenous)
f	*Hybomitra typhus*	(Anautogenous)
g	*Chrysops excitans*	(Anautogenous)
h	*Hybomitra frontalis*	(Autogenous)
i	*Hybomitra epistates*	(Anautogenous)
j	*Hybomitra lurida*	(Anautogenous)
k	*Hybomitra illota*	(Anautogenous)
l	*Hybomitra lasiophthalma*	(Anautogenous)
m	*Hybomitra tetrica hirtula*	(Anautogenous)
n	*Hybomitra rupestris*	(Autogenous)
o	*Hybomitra affinis*	(Anautogenous)
p	*Hybomitra nuda*	(Anautogenous)

continuous line = fitted regression line

dashed line used when too few individuals collected for the fitting of the regression line.

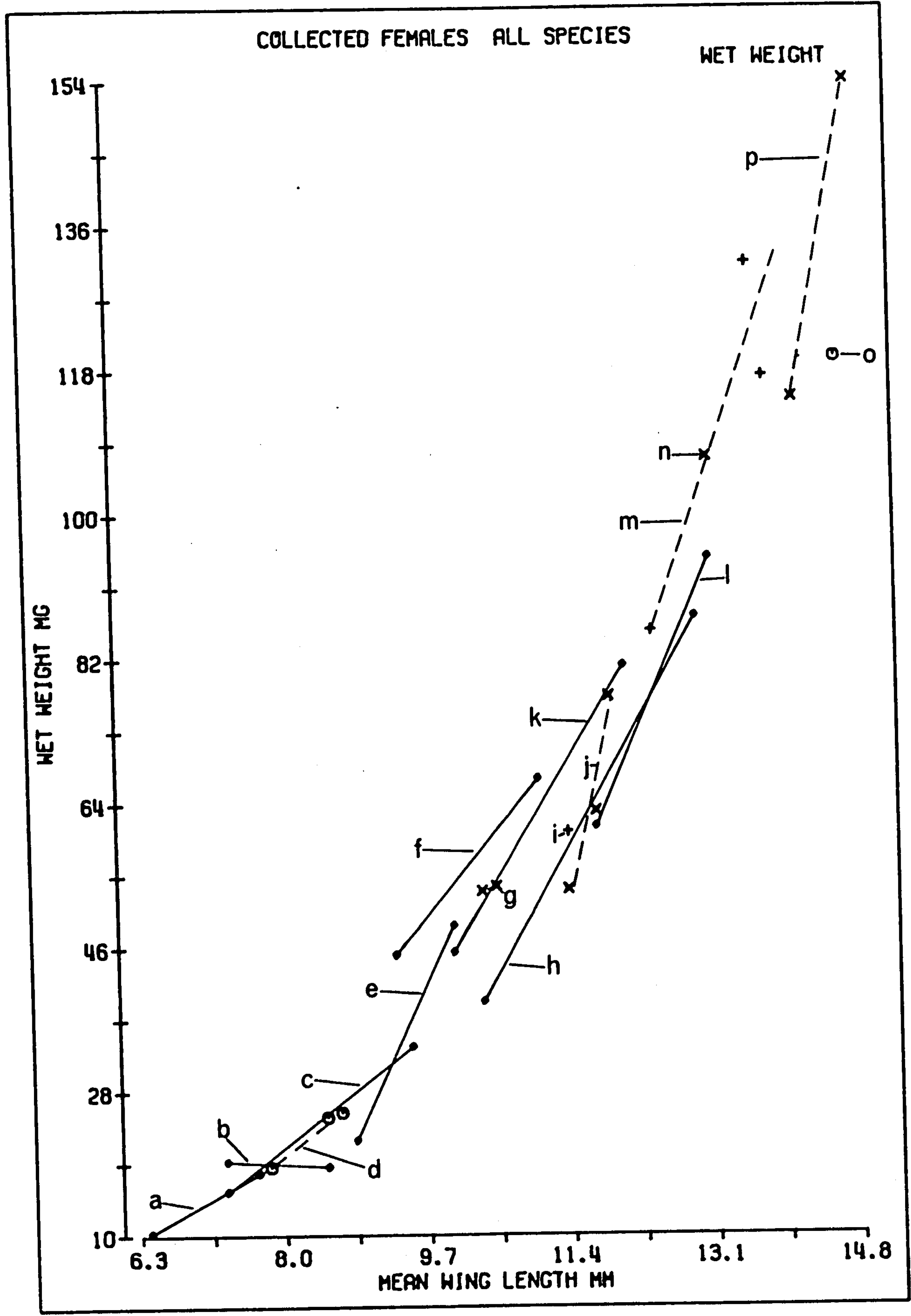

COLLECTED FEMALES ALL SPECIES
WET WEIGHT
WET WEIGHT MG
MEAN WING LENGTH MM
154
136
118
100
82
64
46
28
10
6.3
8.0
9.7
11.4
13.1
14.8
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p

Fig. 12 Dry weight vs. wing length of collected female tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops nubiapex*	(Autogenous)
c	*Haematopota americana*	(Autogenous)
d	*Chrysops furcatus*	(Anautogenous)
e	*Chrysops mitis*	(Autogenous)
f	*Hybomitra typhus*	(Anautogenous)
g	*Chrysops excitans*	(Anautogenous)
h	*Hybomitra illota*	(Anautogenous)
i	*Hybomitra epistates*	(Anautogenous)
j	*Hybomitra frontalis*	(Autogenous)

continuous line = fitted regression line

dashed line used when too few individuals collected for the fitting of a regression line.

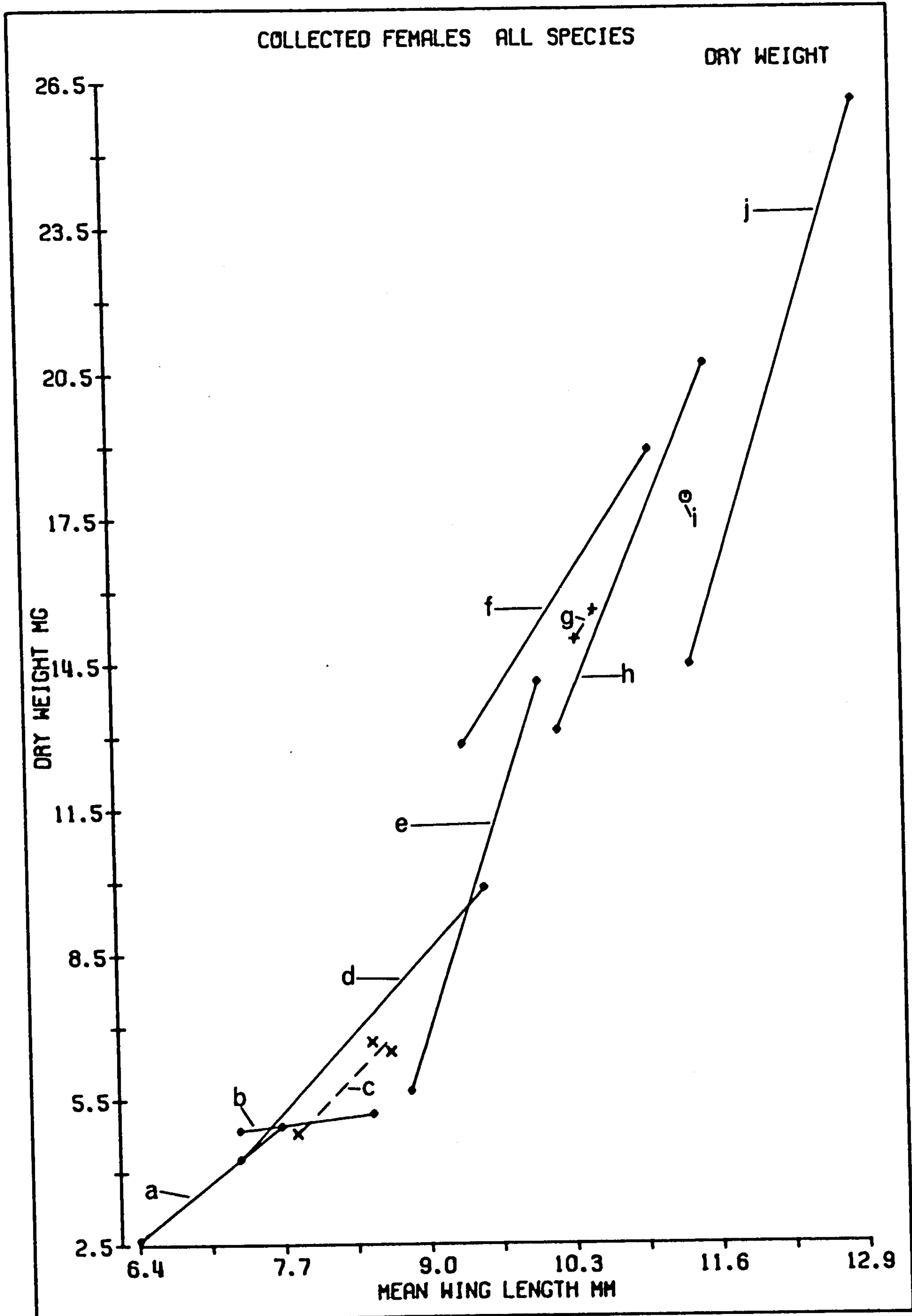
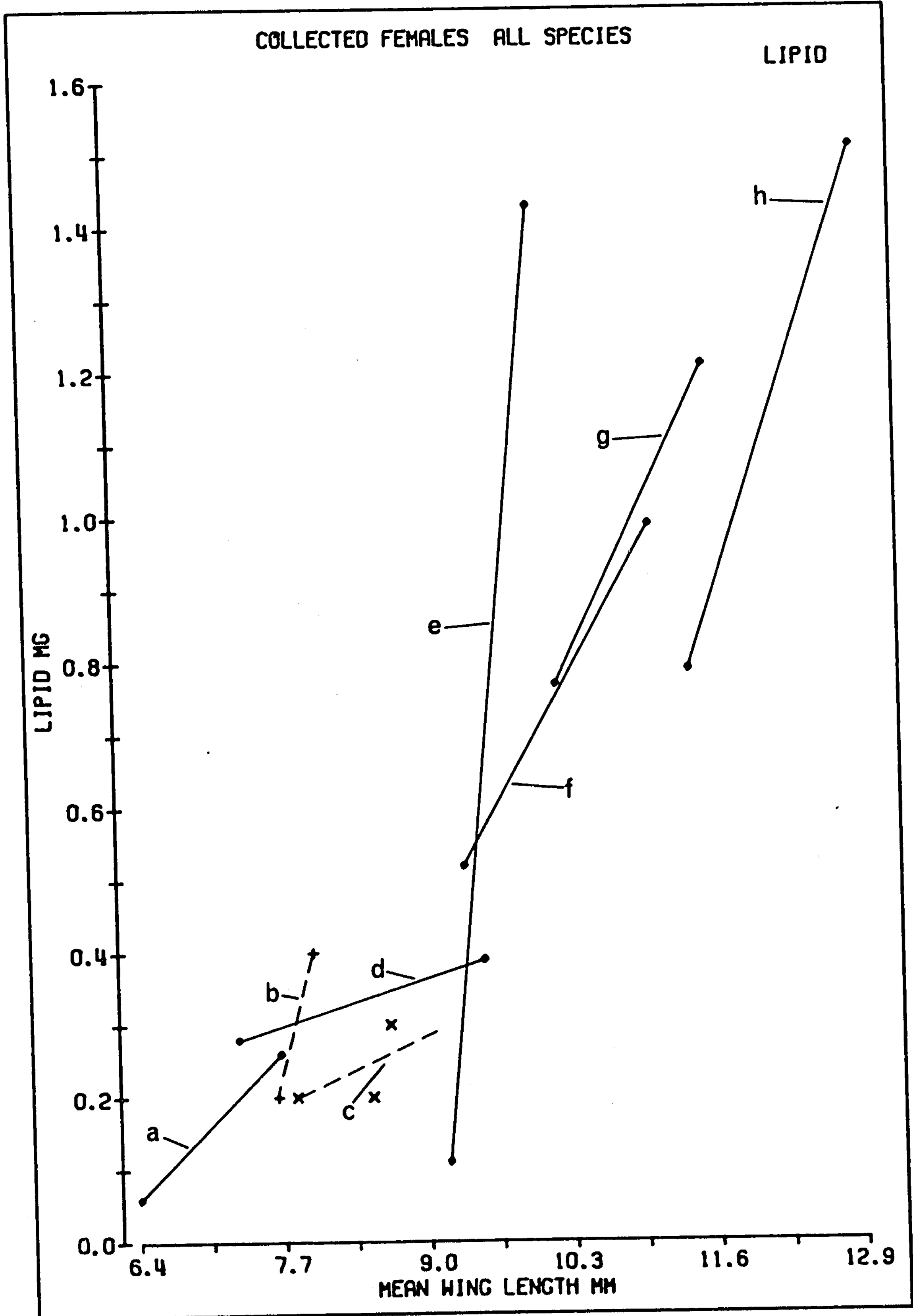

COLLECTED FEMALES ALL SPECIES
DRY WEIGHT
DRY WEIGHT MG
MEAN WING LENGTH MM
26.5
23.5
20.5
17.5
14.5
11.5
8.5
5.5
2.5
6.4
7.7
9.0
10.3
11.6
12.9
a
b
c
d
e
f
g
h
i
j

Fig. 13 Lipid weight vs. wing length of collected female tabanids.

a	*Chrysops frigidus*	(Autogenous)
b	*Chrysops nubiapex*	(Autogenous)
c	*Haematopota americana*	(Autogenous)
d	*Chrysops furcatus*	(Anautogenous)
e	*Chrysops mitis*	(Autogenous)
f	*Hybomitra typhus*	(Anautogenous)
g	*Hybomitra illota*	(Anautogenous)
h	*Hybomitra frontalis*	(Autogenous)

continuous line = fitted regression line

dashed line used when too few individuals collected for the fitting of a regression line.


COLLECTED FEMALES ALL SPECIES
LIPID
LIPID MG
MEAN WING LENGTH MM
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
6.4
7.7
9.0
10.3
11.6
12.9
a
b
c
d
e
f
g
h

c. Discussion

One objective of this part of the study was to compare food reserves within a species but the major objective was to compare reserves between autogenous and anautogenous species. Since autogenous species produce eggs without a previous blood meal the emphasis is on why the anautogenous species require a blood meal. The simplest hypothesis is to suggest that they lack enough food reserves; i.e. they are obligatory anautogenous. Another possibility is that they have sufficient food reserves but lack the neuro-hormonal stimulus for their mobilization. The first hypothesis was chosen as the working hypothesis.

The relationship between lipid content and sex showed no constancy in the two groups of reared flies. In the autogenous species there was no significant difference in the amount of lipid between male and female _Chrysops frigidus_ although the females had proportionally more lipid than the males; whereas in _Hybomitra frontalis_ females had significantly more lipid than males but there was no difference in the proportion of lipid between the two sexes. In the anautogenous species there was no significant difference in the amount or proportion of lipid between male and female _Chrysops excitans_ or between male and female _Hybomitra illota_. In other species, sample sizes were so small that no tests of significance were carried out. However, in the autogenous _Chrysops mitis_ two males averaged 1.00 mg of lipid (10.2% dry weight) while two females averaged 3.75 mg (20.5%); whereas in the anautogenous _Hybomitra epistates_ one male had 5.4 mg of lipid (19.1%) and three females averaged 1.33 mg (4.7%).

Rozeboom and Twohy (1958) demonstrated that no difference existed between adult males and females, respectively, of anautogenous *Culex pipiens* and autogenous *Culex molestus* in the amount of food reserves. Lang (1963) confirmed this in *Culex molestus*, but found that rearing temperature differentially affected the lipid content of male and female *Culex pipiens*. Normally there was no difference, but at 25 C males contained almost twice as much lipid as females. In Lepidoptera, adult males contain more lipid than adult females (Dutkowski and Ziajka, 1970).

Except in *Hybomitra epistates*, male tabanids contained more water than females. The difference was significant in *Chrysops frigidus* and *Hybomitra illota*. This agrees with the general principle that the amount of water present is inversely related to the amount of lipid (Christophers, 1960; p. 416).

In general, the relationship between lipid content and wing length was faithfully reflected in the relationships between wet weight and wing length, and dry weight and wing length. Thus if one sex had a heavier wet weight for a given wing length then it also has a heavier dry weight and a heavier lipid weight.

The relationship between soluble protein and sex showed no constancy in the two groups. Reared females of *C. frigidus* and *H. frontalis* had significantly more soluble protein than their respective males. On a proportional basis, only *C. frigidus* females had more protein than males. *Chrysops excitans* females had significantly more and proportionately more soluble protein than males; no significant differences could be detected in *H. illota*.

In the interspecific comparisons between reared males there

was no distinct separation within the wet, dry, lipid or soluble protein weights of the autogenous and anautogenous species for a given wing length. A possible exception being the lipid content of Chrysops excitans (anautogenous), its line of best fit falling below the curve formed by the other five species. Whereas the proportion of lipid in the other species ranged from 12% to 19%, that of C. excitans was only 6.8%.

These results can only be compared with those of Rozeboom and Twohy (1958) and Lang (1963) on the Culex pipiens complex since no other workers have compared food reserves between male autogenous and anautogenous insects. They found that the autogenous Culex molestus had a significantly heavier dry weight and lipid weight than the anautogenous Culex pipiens. Culex molestus males also had a significantly higher percentage of lipids. However, C. pipiens males had a greater percentage of nitrogen than C. molestus.

In the interspecific comparisons between reared females there were two distinct groups of flies. For any given wing length the autogenous species had greater wet, dry and lipid weights than the anautogenous species. Because of the nature of the regression (model II), these results can be interpreted the other way, i.e. for any given lipid weight autogenous species had shorter wings than anautogenous species. However, a look at the proportions of lipid in the two groups lends support to the former statement. In the autogenous females, lipid content ranged from 16.5% to 20.5% of the dry weight whereas in the anautogenous species it ranged from 4.3% to 9%. Thus, the separation of the two groups seen when lipid weight is plotted against wing length is not due to the anautogenous flies having disproportionately

longer wings than the autogenous species but represents a true difference in the amount of lipids. Differences in wet and dry weights reflect this difference in lipid weight between the two groups. In the soluble protein content of reared females, the separation of Hybomitra epistates from the other four species does appear to be caused by the specimens having disproportionately longer wings as the proportion of soluble protein (28.8%) was greater than that of all of the other species except Chrysops excitans (29.7%).

Once again these results can only be compared with Culex pipiens and Culex molestus (Twohy and Rozeboom, 1957; Lang, 1963) with which they are in agreement.

The water:lipid relationship of the reared females held true. In six autogenous species water content ranged from 68.6% to 72.7%. In six anautogenous species it ranged from 73.5% to 77.0%. Perhaps by a comparison of wet weights and dry weights plotted against wing length, or by just simply comparing water content (as a percentage of wet weight) one could predict which species of tabanids are autogenous.

The question of the mechanism involved in autogenous egg development was raised by Rozeboom and Twohy (1958). From their data they concluded that the accumulation of food reserves was a characteristic common to both males and females and was thus independent of the mechanism involved in autogenous egg development. Lang (1963) took this a stage further. Although he agreed that the larvae of the autogenous species were able to accumulate more lipid reserves than those of the anautogenous species he showed that the females of the anautogenous species were unable to maintain their lipid content during metamorphosis at 25 C. He suggested that the ability to conserve lipid reserves

during metamorphosis may be a biochemical characteristic of autogeny. Conversely, the inability to maintain lipid would characterize anautogeny. Any attempt to make a general statement about tabanids is probably unwise. Nevertheless, an attempt will be made. Since reared males of both autogenous and anautogenous species had the same lipid content (Fig. 5), and males of the anautogenous species had more lipid than their females (Table XXVII) it is tempting to speculate that anautogeny in tabanids is made possible by the females' ability to use lipid during metamorphosis. Unfortunately, no data on lipid content of larvae is available.

In the comparison of collected females some species were collected as adults but were not reared. The wet weights of these individuals have been plotted along with the wet weights of the other species (Fig. 11). This figure includes 16 species and indicates how well the principle, that heavier flies have longer wings, holds true; thus enforcing the contention that wing length is a satisfactory measure of size. No distinct separation of species into groups is obvious although the relative position of the species in the two groups being compared (autogenous and anautogenous) is the reverse of that seen in the figure comparing wet weights of reared females (Fig. 7). Thus for any given wing length the autogenous species are less heavy than the anautogenous species. This trend is less clear in the dry weight and lipid weight comparisons. Collected autogenous flies are parous while anautogenous flies are most often nulliparous. Thus even after laying one batch of eggs autogenous females weigh only slightly less than nulliparous anautogenous flies.

A comparison of the lipid content of reared and collected

autogenous flies gives an indication of how much lipid is used during a gonotrophic cycle. Data are available for five species. Before oviposition, lipid content ranged from 16.7% to 20.5% of the dry weight, whereas after oviposition it ranged from 3.9% to 5.4% (Table XXVI). Recently emerged anautogenous flies had between 4.3% and 9% of lipid. Thus it would appear that they lack sufficient lipid to develop and deposit a batch of eggs. A similar conclusion was reached for *Culex pipiens* (Twohy and Rozeboom, 1957).

The emphasis throughout this section has been on the difference in the amount of lipid present. Yet the need for a blood meal in hematophagous insects is explained as the need to obtain protein for egg development (Clements, 1963) and yolk in oocytes is chiefly protein yolk (Chapman, 1969; p. 291). Presumably lipid is converted to protein before deposition as yolk. Evidence is available which supports the reverse conversion. Eldridge (1968) showed that blood fed *Culex pipiens* either stored the meal as lipid or deposited it as protein in the oocytes. Tsetse flies also store their blood meals as fat (Bursell, 1961).

VII. GENERAL DISCUSSION AND CONCLUSIONS

The main objective of this study was to determine the incidence of autogeny in tabanids. The limitation of time made it necessary to restrict the study to a few localities in Alberta. Because of the paucity of information on autogeny in tabanids an extensive study using as many species as could be collected was attempted rather than an intensive study of one or two species. From what evidence there is of autogeny in mosquitoes (see Spielman, 1971) and biting midges (Linley, 1966) it would be unwise to extend the results of this study to other populations of the same species, even in Alberta.

Evidence has been presented which suggests that 15 species of tabanids do not need a blood meal to develop their first batch of eggs, i.e. they are autogenous, and 16 species do require a blood meal, i.e. they are anautogenous. These characters are associated with the amount of lipid present in recently emerged flies. Females of autogenous species had more lipid than females of anautogenous species.

Since Downes' (1958) paper, many authors (e.g. Oldroyd, 1964; Spielman, 1971) have accepted his conclusion that Diptera evolved as blood feeding insects. Autogeny in the blood feeding families is looked upon as a secondary adaptation and even as being abnormal (Downes, Downe and Davies, 1960). Human nature being what it is, the tendency has been to accept the normal without question and attempt to explain the abnormal. Thus autogeny has been explained as a method of coping with an unfavourable adult environment (Spielman, 1971). Unfavourableness is usually associated with the difficulty of obtaining a blood meal either because of the weather, e.g. windswept environments, or the

fluctuations in host populations (Downes et al.,1962; Corbet, 1964c; Downes, 1965). Such an explanation represents false reasoning since it does not account for the presence of autogenous species in favourable habitats (see Spielman, 1971). Also, the difficulty of obtaining a blood meal can only explain the absence of anautogenous species. Unless there is some competitive exclusion between the larvae, with the anautogenous larvae being successful, the presence of autogenous species in unfavourable environments is in no way related to this unfavourableness. It is not a cause and effect relationship. Thus the high proportion of autogenous species in such environments is due solely to the low proportion of anautogenous species. I believe that not enough emphasis has been placed on discovering why blood feeding exists in the Diptera. Perhaps autogeny is the "normal" condition and anautogeny the "abnormal".

The need of a blood meal, or other protein source, by an adult is generally looked upon as being a method of supplementing an inadequate larval diet (Davies et al., 1965 ; Oldroyd, 1964); although Snodgrass (1961) thought of the larva as a means of supplementing the food of the adult. Oldroyd (1964) emphasizes that the larval dipteran is the principal feeding stage and the function of the adult is to lay eggs as quickly as possible. If this is true, why would such a potentially hazardous behaviour as obtaining blood from an often unwilling host have evolved? If one accepts Downes' (1958) hypothesis that the original Diptera were biting flies, then blood feeding in extant species can be looked upon as the perpetuation of this ancient habit. If, however, the view of Mackerras (1954) is adopted, i.e. tabanids evolved as flower feeding insects which later developed the

blood feeding habit, blood feeding becomes secondary and its development requires explaining. A case can be made for the evolution of blood feeding in mosquitoes and blackflies as a means of supplementing an inadequate larval diet. The larvae of these species are indiscriminate particulate feeders, ingesting particles of suitable size (Pucat, 1965; Chance, 1970). Much of this material may be useless as food. If the larval stage was subject to mortality, through predation or drying of the habitat, a reduction in the length of the larval life will lessen the chance of such mortality, and thus prove beneficial to the species. However, this would probably mean that the adults would have to supplement the amount of food stored by the larvae. In the Culex pipiens complex of mosquitoes, the anautogenous form has a shorter larval period than the autogenous form (Twohy and Rozeboom, 1957). Thus the advantage of non-blood-feeding adults has to be weighed against the disadvantages of a longer larval life. Tabaninae larvae are predaceous, feeding upon the body contents of other animals. Presumably this food has a high nutrient value. Coupled with this, the larvae have few limiting environmental factors (Fairchild, 1969), even to the extent of being able to survive dry conditions (Lamborn, 1930; Schomberg, 1952). Why, then, should such flies need a blood meal to develop eggs?

There are two opposite views on the evolution of tabanids, each by an eminent worker. Oldroyd (1964) considers those tabanids which have given up blood feeding (he follows Downes' (1958) view that blood feeding is primitive) as having made little evolutionary advance and declining in numbers, whereas those groups which still feed on blood are evolving into "a multitude of species in a great burst of evolution" (p. 99). Direction of evolution has followed adult preferences

whereas in the Nematocera and the Cyclorrhapha the larval feeding habits have been the dominating factors. The other view (Fairchild, 1969) is far less dogmatic. He cautiously states that most tabanids appear to need a blood meal to develop eggs but recognizes the possibility that autogeny may occur in these. He further states that dependence on vertebrates has probably not been an absolute limiting factor to the distribution of tabanids.

This study has shown that anautogenous species have a greater probability of multiple gonotrophic cycles than autogenous species. Presumably, the ability to develop and lay eggs without a blood meal leaves the insect in such a weak condition that it is then unable to obtain a blood meal. The blood meal obtained by anautogenous species allows for egg development, oviposition and storage of some yolk in the next batch of oocytes. It may also supply some energy, as a blood meal does in mosquitoes (Clements, 1955), and thus the necessity of finding a sugar source may be eliminated. Linley's (1968) observations on the sandfly *Leptoconops bequaerti* are pertinent. He observed that parous autogenous females spent over 10 min. on the skin, probed frequently but were consistently unsuccessful in taking blood. He accredited this to be an effect of starvation following production of the first batch of eggs. Anautogenous females were successful in obtaining blood.

In conclusion, it is suggested that tabanids evolved as flower feeding flies. Some developed the ability to utilize lipid during metamorphosis. These flies then lacked sufficient food reserves to develop eggs. Blood feeding occurred which permitted increased adult longevity and the possibility of multiple gonotrophic cycles.

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