MORPHOLOGY OF THE REPRODUCTIVE SYSTEM OF *STRUMETA TRYONI* (FROGGATT) (DIPTERA: TRYPETIDAE) WITH A METHOD OF DISTINGUISHING SEXUALLY MATURE ADULT MALES

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Abstract

Morphology of adult female and male reproductive systems of *Strumeta tryoni* (Froggatt) is described and figured.

Growth changes in adult male reproductive endoskeleton are described under differing conditions of temperature and light intensity. For the environmental conditions studied, physiological age was correlated with size of the male reproductive endoskeleton and a method for distinguishing teneral from sexually mature adults is given.

INTRODUCTION

There is increasing interest in the use of insect genitalia for descriptive purposes in systematics because of the unreliability of many external characters such as colour. Correct use of genitalia characters in taxonomy requires a sound knowledge of the morphology and function of the various parts of the reproductive system.

In ecological studies, it is important to be able to distinguish between teneral and sexually mature adults. Munro (1947) recognized two different ejaculatory apodeme shapes for *Isoconia rubida* Munro, but did not give any reason for such variation. Preliminary examination of the male reproductive endoskeleton of *Strumeta tryoni* (Froggatt) showed irregularities in shape and size. Studies were carried out to correlate these variations with geographic distribution, colour form and age.

METHODS AND MATERIALS

Cultures

Material of *S. tryoni* was obtained from the University of New South Wales, Sydney in February 1968. Adults were maintained in 8 in. × 8 in. × 12 in. cages after the method of Osborn and Shipp (1965). Eggs were collected in hollowed out apple skin and cultured at 25°C after the method of May (1961). All flies used in experiments were reared from one colony of adults.

Morphological studies

Reproductive systems were dissected under water. Genital ducts, ovipositors, male endoskeletons, and male 9th abdominal segments were boiled in 10 per cent. potassium hydroxide before being stained in mercurochrome and mounted in canada balsam for microscopical examination. Ejaculatory apodemes were mounted separately on flat microscope slides, and genital rings and fultellas mounted in cavity slides as *in situ*.

Geographical variation

Ten males of unknown physiological age were examined from each of six areas of Queensland, the Atherton Tableland, Ayr, Byfield, Brisbane, Toowoomba and Chinchilla.

Colour forms

Six stages of melanin formation were recognized by May (1963). Ten males of unknown physiological age of each colour stage were examined.

Development of male reproductive endoskeleton

Reproductive endoskeletal structures were dissected out, boiled in potassium

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hydroxide, stained in chlorazol black E and mounted in canada balsam as described above. The ejaculatory apodeme of each specimen was drawn using a prism camera lucida, and the length (mm) and area (sq. mm) calculated. A fixed arm planimeter was used for area measurements.

Development at 25°C and natural light intensity.—Five hundred flies which emerged between 9 a.m. and 12 noon on June 7, 1968, were held in a single cage at 25°C and exposed to natural light. The relative humidity decreased during the course of the experiment from 75 per cent. to 55 per cent. Five male flies were sampled at emergence and subsequently each evening after dusk for 24 days.

Number of pairs in copulation was recorded daily.

Development at 25°C and 120 lumens per square foot light intensity.—Five hundred flies which emerged from the same batch of pupae at the same time as the flies in the above study were held in a single cage in the light cabinet (Fig. 29) at 25°C and exposed to 120 lumens per square foot light intensity between 9 a.m. and 4.30 p.m. At 4.30 p.m. each day, the cabinet was opened to expose the flies to dusk lighting, and after the onset of dark, the cabinet was resealed. Relative humidity in the light cabinet fell to 55 per cent. each day after opening, slowly returning to 80 per cent. by 4.30 p.m. the following day. Five male flies were sampled at emergence and subsequently each evening after dusk for 24 days.

Number of pairs in copulation was recorded daily.

Development under natural conditions of temperature and light intensity.—Five hundred flies which emerged from the laboratory culture at 25°C between 9 a.m. and 12 noon on July 27, 1968, were held in a single cage in a sheltered, shady location and exposed to natural conditions. Temperatures and relative humidities were recorded by a thermohygrograph kept in the breeding cage and times of sunrise and sunset obtained for the period of sampling. Relative humidity remained between 90 per cent. and 100 per cent. for duration of the study. Five males were sampled at emergence and subsequently after dusk every third day for the first 30 days, and every second day for the next 10 days.

Number of pairs in copulation was recorded daily.

Development of musculature on the ejaculatory apodeme

A small batch of flies which emerged between 9 a.m. and 12 noon on the same day was maintained in the laboratory at 25°C, 70 per cent. relative humidity and exposed to natural light. Five male flies were sampled at emergence and subsequently each evening after dusk for 10 days. Ejaculatory apodemes and musculatures were dissected out, stained in mercurochrome and mounted in canada balsam, without boiling in potassium hydroxide.

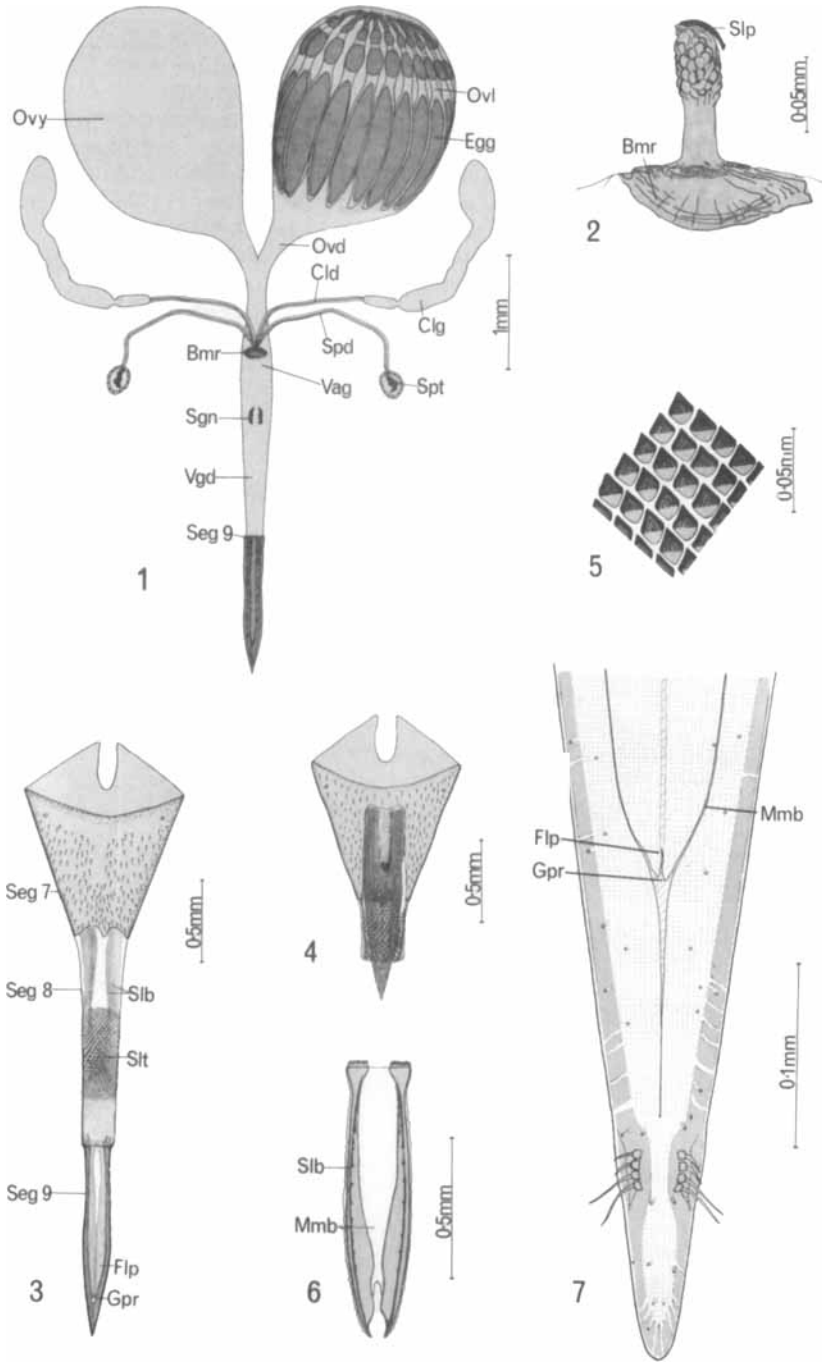
RESULTS

Female reproductive system (Figs. 1-7)

Ovaries.—The ovaries of a teneral female are small because of the immature ovarioles. At sexual maturity, an egg is formed in the basal section of each convoluted ovariole thus expanding the ovaries to fill almost the entire body cavity. The ovaries contain panoistic ovarioles bound together by a transparent membrane. Distal ends of the ovarioles meet in a point at the tip of the ovary.

Genital ducts.—Two short lateral oviducts end in a common oviduct which widens into the vagina. The vaginal duct runs into the modified 9th abdominal segment, the apical segment of the ovipositor. Two sclerotized pieces occur in the dorsal membranous vaginal duct wall. Because of their similarity to signum bursae of Lepidoptera, these sclerotized pieces have been designated as signa. A morula gland is situated on the ventral wall of the vagina. This gland is a stalked body of berry-like spherules with an apical sclerotized process.

Spermathecae.—Two spermathecae are present, functioning for reception and storage of sperm. Each consists of a small, black convoluted tube of glandular tissue surrounded by a layer of fat body. The spermathecal duct is surrounded by

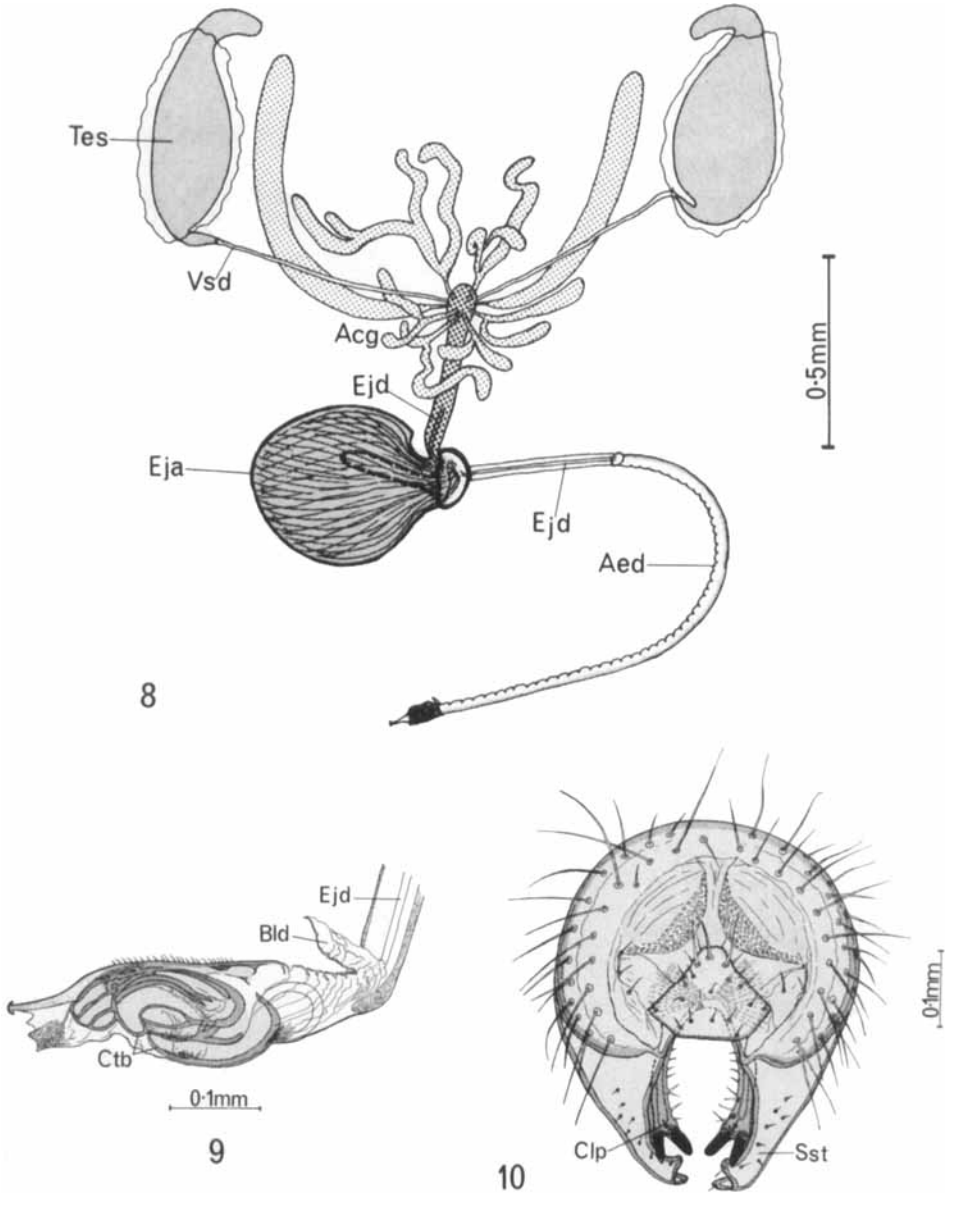


FIGS. 1-7.—*Strumeta tryoni* (Froggatt): (1) female reproductive system—Bmr., base of morula gland; Cld., duct of collaterial gland; Clg., collaterial gland; Egg, egg; Ovd., lateral oviduct; Ovl., ovariole; Ovy., ovary; Seg. 9, abdominal segment 9; Sgn., signum; Spd., duct of spermatheca; Spt., spermatheca; Vag., vagina; Vgd., vaginal duct: (2) lateral view of morula gland—Bmr., base of morula gland; Slp., sclerotized process: (3) extended ovipositor—Flp., ventral flap; Gpr., gonopore; Seg. 7, abdominal segment 7; Seg. 8, abdominal segment 8; Seg. 9, abdominal segment 9; Slb., sclerotized bands; Slt., sclerotized teeth: (4) retracted ovipositor: (5) sclerotized teeth of abdominal segment 8: (6) ventral flap of abdominal segment 9—Mmb., membrane; Slb., sclerotized band: (7) extreme tip of abdominal segment 9—Flp., ventral flap; Gpr., gonopore; Mmb., membrane.

glandular tissue and enters the vagina on the dorsal surface opposite the morula gland base.

Collaterial glands.—Two elongate, slightly convoluted collaterial glands are present. The collaterial gland duct is surrounded by glandular tissue and enters the vagina dorsally immediately posterior to the spermathecal duct.

Ovipositor.—Abdominal segments 7, 8 and 9 are modified to form the ovipositor. Segment 7, the oviscape, is setose, conical in shape, dorso-ventrally compressed and with the tergum and sternum fused laterally. The sternum is produced anteriorly to form a triangular area, the cuticle of which is very thin anteriorly, forming an oval membrane. Segment 8 is long and tubular, strengthened anteriorly by two wide, lateral sclerotized bands which thin out and disappear posteriorly.



FIGS. 8-10.—*Strumeta tryoni* (Froggatt): (8) male reproductive system—Acg., accessory glands; Aed., aedeagus; Eja., ejaculatory apodeme; Ejd., ejaculatory duct; Tes., testis; Vsd., vas deferens: (9) head of aedeagus—Bld., bladder; Ctb., sclerotized bands; Ejd., ejaculatory duct: (10) posterior view of abdominal segment 9—Clp., clasper; Sst., surstylus.

Triangular, sclerotized teeth are present on the posterior 2/3 of the segment surface. The teeth show a gradation in size, being small at the anterior and posterior extremities and well developed in the central region. Each tooth is formed by a raised anterior part of a quadrate plate, the posterior region being embedded in the segment surface. Segment 9, the aculeus, is long, dorso-ventrally compressed and pointed posteriorly, and is thus adapted for piercing the skin of fruit. The dorsal part of this segment, the tergum, is heavily sclerotized, elliptical in cross section and pointed posteriorly to form the body of the piercing organ. The sternum is represented as a ventral flap, consisting of two sclerotized bands connected by membrane along the mid line, and to the body of the piercing organ along its lateral margins. The gonopore is situated ventrally at the posterior end of the flap. On the sub-apical lateral margins of the aculeus there are four small bristles, two long and two short. The sclerotized area of the aculeus is traversed by small, irregularly placed canals which open on the surface. At rest, segment 9 is retracted inside segment 7, with segment 8 folding such that its posterior part lies anteriorly inside segment 7. During oviposition the ovipositor is extended to form a tube with the sclerotized teeth of segment 8 pointing anteriorly. During copulation, the ovipositor is partly extended and the aedeagus enters the ventral gonopore, traverses the length of the aculeus and comes to lie in the vaginal duct. Membrane along the lateral margins of the flap allows the flap to be raised during copulation and oviposition, so allowing passage of objects of greater diameter than the lumen of the aculeus.

Male reproductive system (Figs. 8-11, 25)

Testes.—The testes are yellow, pyriform bodies with their anterior ends bent over, and surrounded by a layer of fat body. The vasa deferentia lead from the posterior ends of the testes into a common duct, the ejaculatory duct, which is surrounded by glandular tissue and runs into the erecting and pumping organ. The ejaculatory duct between the sac and aedeagus is surrounded by a less developed layer of glandular tissue.

Accessory glands.—Four pairs of accessory glands enter subapically the anterior end of the ejaculatory duct. The largest pair are elongated and slightly curved, the remainder shorter and variously branched.

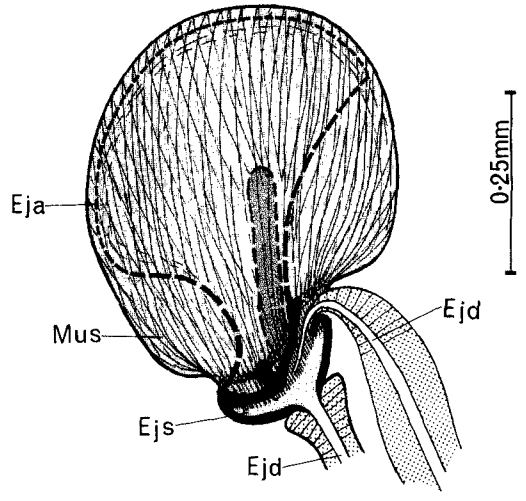


FIG. 11.—*Strumeta tryoni* (Froggatt); erecting and pumping organ—Eja., ejaculatory apodeme; Ejd., ejaculatory duct; Ejs., ejaculatory sac; Mus., muscle.

Erecting and pumping organ.—This organ is composed of a sclerotized disc, the ejaculatory apodeme, and a dome shaped ejaculatory sac with slightly sclerotized walls attached to the base of the apodeme. At the junction of the apodeme and sac, the apodeme expands into a sclerotized basal plate. The ejaculatory duct enters the sac through an aperture at the junction of the sac and apodeme basal plate and continues from the centre of the sac dome through the aedeagus. Muscles, connected to the side wall of the sac by fine fibrillae, run to the extremity of the apodeme where they are firmly attached. Contraction of these muscles draws the wall of the sac to the basal plate closing the inlet of the ejaculatory duct and reducing the sac volume. The resulting pressure on the contained seminal fluid forces it down the ejaculatory duct to the tip of the aedeagus.

Aedeagus.—The aedeagus is a long, curved, slender tube attached proximally to the internal sclerotized endoskeleton and with its distal end leaving the abdomen at the venter of segment 9 between the male claspers. At rest it is coiled and in this position the inner margin is wrinkled while the outer margin has a fine sclerotized band. The head of the aedeagus is a complicated structure with sclerotized bands and muscle attachments, probably acting as a valve serving to hold the fluid in the aedeagus, permitting pressure increase while the erecting and pumping organ is operating, thus erecting the aedeagus. A membranous bladder lies at the base of the aedeagus head.

Abdominal segments.—Abdominal segments 1-5 are well developed, segments 6-8 are absent while segment 9 is reduced and forms the genital segment. The dorsum is produced latero-ventrally to form the surstyli which are immovable non-musculated clasper-like organs. Segment 10 is membranous, invaginated inside segment 9, and has two movable, musculated claspers suspended ventrally. Each clasper possesses two small, black, terminal tooth-like structures. Surstyli and claspers, which bear sensory setae, function to hold the aedeagus during copulation.

Endoskeleton.—The endoskeleton of the reproductive system is suspended internally from the anterior wall of segment 9, and thus is an ectodermal cuticular invagination of this segment. The *genital ring* consists of a sternal apodeme anteriorly and two outwardly curved lateral arms which are connected to a bar on the anterior wall of segment 9. The *fultella* lies above the genital ring and consists of a median posterior bar which expands anteriorly into a laterally compressed phallic apodeme. At the junction of the posterior bar and phallic apodeme, two lateral arms, the vanes, are produced ventrally, and fit inside the genital ring. The vanes are slightly curved, diverge ventrally and at their ventral extremity are connected to sclerotized arms which in turn are connected to the posterior ends of the corresponding genital ring arms. The median posterior bar of the fultella has a posterior bifurcation to which is attached the basal end of the aedeagus. The endoskeleton supports a complex musculature which functions to control the aedeagus during copulation.

Geographical variation

Mean length of the ejaculatory apodeme for each area was: Atherton Tableland, 0·554 mm; Ayr, 0·546 mm; Byfield, 0·544 mm; Brisbane, 0·427 mm; Toowoomba, 0·478 mm; Chinchilla, 0·579 mm.

TABLE I

GEOGRAPHICAL VARIATION OF LENGTH OF EJACULATORY APODEME OF *Strumeta tryoni*—ANALYSIS OF VARIANCE.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F
Between areas	0·079	5	0·0158	8·77
Residual	0·04	22	0·0018	
Total	0·119	27		

The mean length of the ejaculatory apodemes of specimens from Brisbane and

Toowoomba was significantly different (5 per cent. level) from the mean length of apodemes of specimens from all other areas. The structure of the ejaculatory apodemes of specimens from Brisbane and Toowoomba, however, resembled those of immature specimens. There was no significant difference between measurements of specimens from Brisbane and Toowoomba, and between specimens from Atherton Tableland, Ayr, Byfield and Chinchilla.

Colour forms

The mean length of the ejaculatory apodeme for each colour form was: A, 0·441 mm; B, 0·421 mm; C, 0·362 mm; D, 0·343 mm; E, 0·388 mm; F, 0·330 mm.

TABLE 2

VARIATION IN LENGTH OF EJACULATORY APODEME OF COLOUR FORMS OF *Strumeta tryoni*-ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F
Between colour forms	0·051	5	0·0102	2·37
Residual	0·113	26	0·0043	
Total	0·164	31		

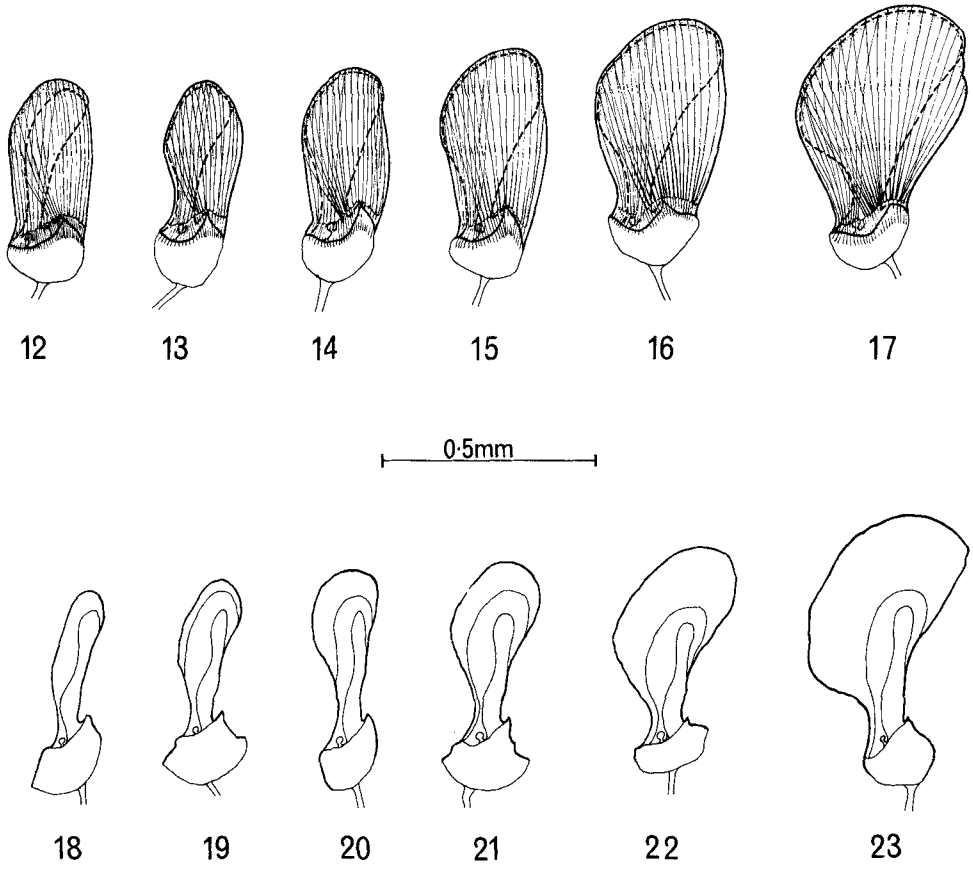
As F was not significant, it was assumed that colour form was not correlated with the mean length of the ejaculatory apodeme.

Development of male reproductive endoskeleton

Development at 25°C and natural light intensity.—Endoskeletal structures increased in size from emergence for the first 5 days, after which they remained relatively unchanged for a further 19 days. Daily growth changes of length and area of ejaculatory apodemes are shown in Figure 26. The relative daily sizes of the ejaculatory apodeme from emergence to 5 days are illustrated in Figures 18-23. At emergence, mean ejaculatory apodeme length was 0·336 mm and mean area 0·024 sq. mm, while from 5 days to 24 days mean length ranged from 0·511 mm to 0·554 mm and mean area 0·107 sq. mm to 0·131 sq. mm. These figures show an increase in length up to 1·5 times and an increase in area of up to 5·5 times that at emergence. The relative humidity range over the 5 day period was 70-75 per cent. Figure 24 shows the genital ring and fultella at emergence, and Figure 25 shows the corresponding structures 5 days later. Changes observed in these structures are an increase in thickness of the lateral arms of the genital ring, a large increase in area of the sternal apodeme, an increase in thickness of the median posterior bar of the fultella, a large increase in area of the phallic apodeme, and a widening of the vanes. Flies began copulating 5 days from emergence and copulation was observed daily at dusk, for a further 5 days. As the ratio of males to females was being constantly changed by daily sampling of males, the daily counts of numbers of flies in copulation will not be considered.

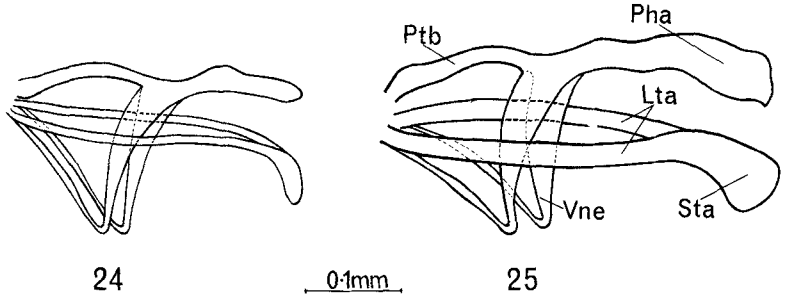
Development at 25°C and 120 lumens per square foot light intensity.—Endoskeletal structures were found to increase in size after emergence. Daily growth changes of length and area of ejaculatory apodemes are shown in Figure 27 at a light intensity of 120 lumens per sq. ft. at the top of the cage shown in Figure 29. At emergence the mean length of ejaculatory apodeme was 0·336 mm and mean area 0·024 sq. mm, while from 5 to 24 days after emergence the mean length ranged from 0·507 mm to 0·563 mm and mean area 0·101 sq. mm to 0·127 sq. mm. These figures show similar increases in size to those of the previous experiment, and flies similarly began copulating 5 days from emergence and copulations were observed daily at dusk, until the 11th day.

Development under natural conditions of temperature and light intensity.—Endoskeletal structures increased in size after emergence; growth changes in length and area of ejaculatory apodemes are shown in Figure 28. Mean length and area



FIGS. 12-23.—*Strumeta tryoni* (Froggatt), ejaculatory apodemes at various times after emergence: (12-17) with musculatures; (18-23) without musculatures: (12, 18) at emergence; (13, 19) 1 day; (14, 20) 2 day; (15, 21) 3 day; (16, 22) 4 day; (17, 23) 5 day.

reached the 5 day value of the previous experiments at 24 days with measurements of 0·534 mm and 0·108 sq. mm, respectively. Copulations were first observed at 24 days after emergence and continued each day, at dusk, until the 39th day.



FIGS. 24, 25.—*Strumeta tryoni* (Froggatt), genital ring and fultella with musculatures removed: (24) at emergence, (25) 5 days after emergence. Lta., lateral arms of genital ring; Pha., phallic apodeme; Ptb., median posterior bar; Sta., sternal apodeme; Vne., vane.

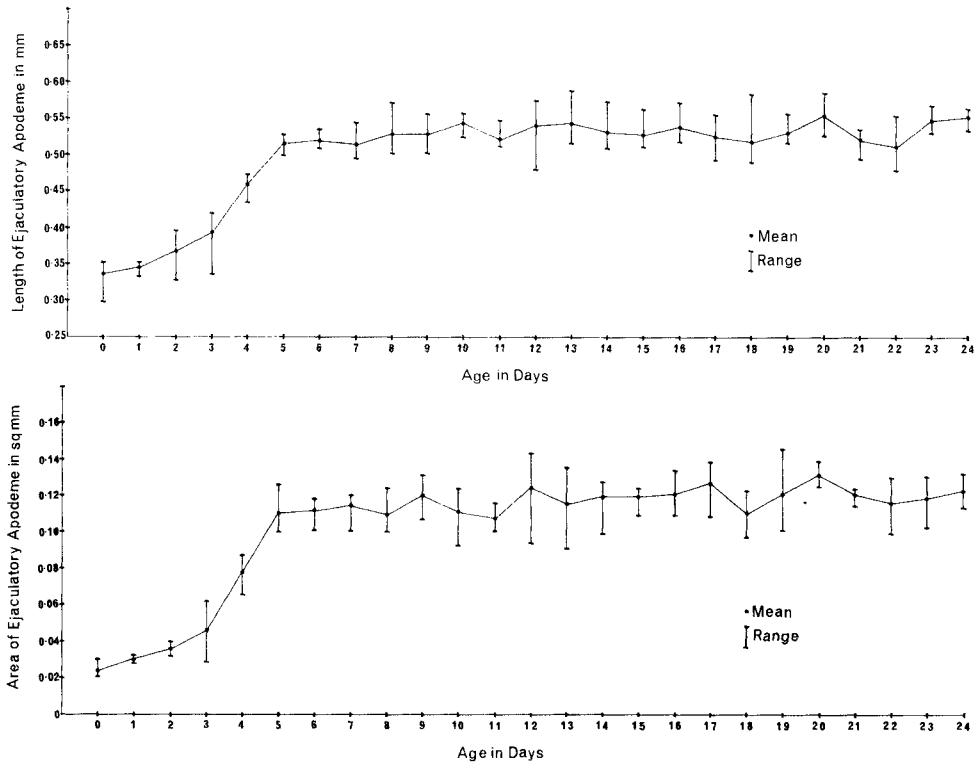


FIG. 26.—Change in length and area of ejaculatory apodeme of *Strumeta tryoni* (Froggatt) with age at 25°C and natural light intensity.

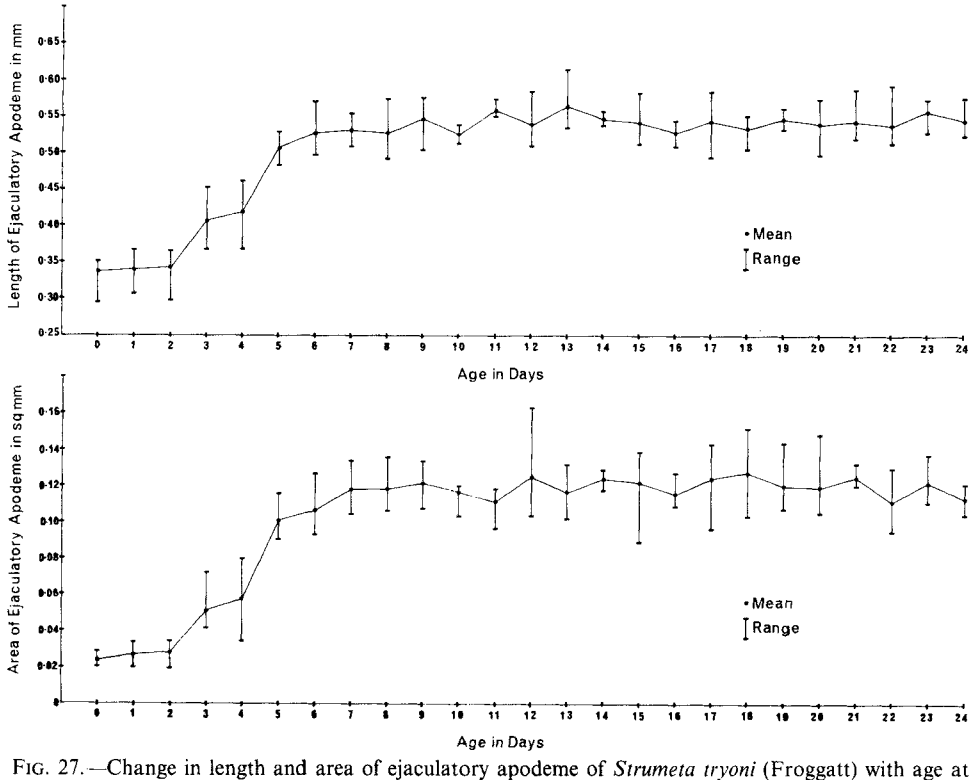


FIG. 27.—Change in length and area of ejaculatory apodeme of *Strumeta tryoni* (Froggatt) with age at 25°C and 120 lumens per square foot light intensity.

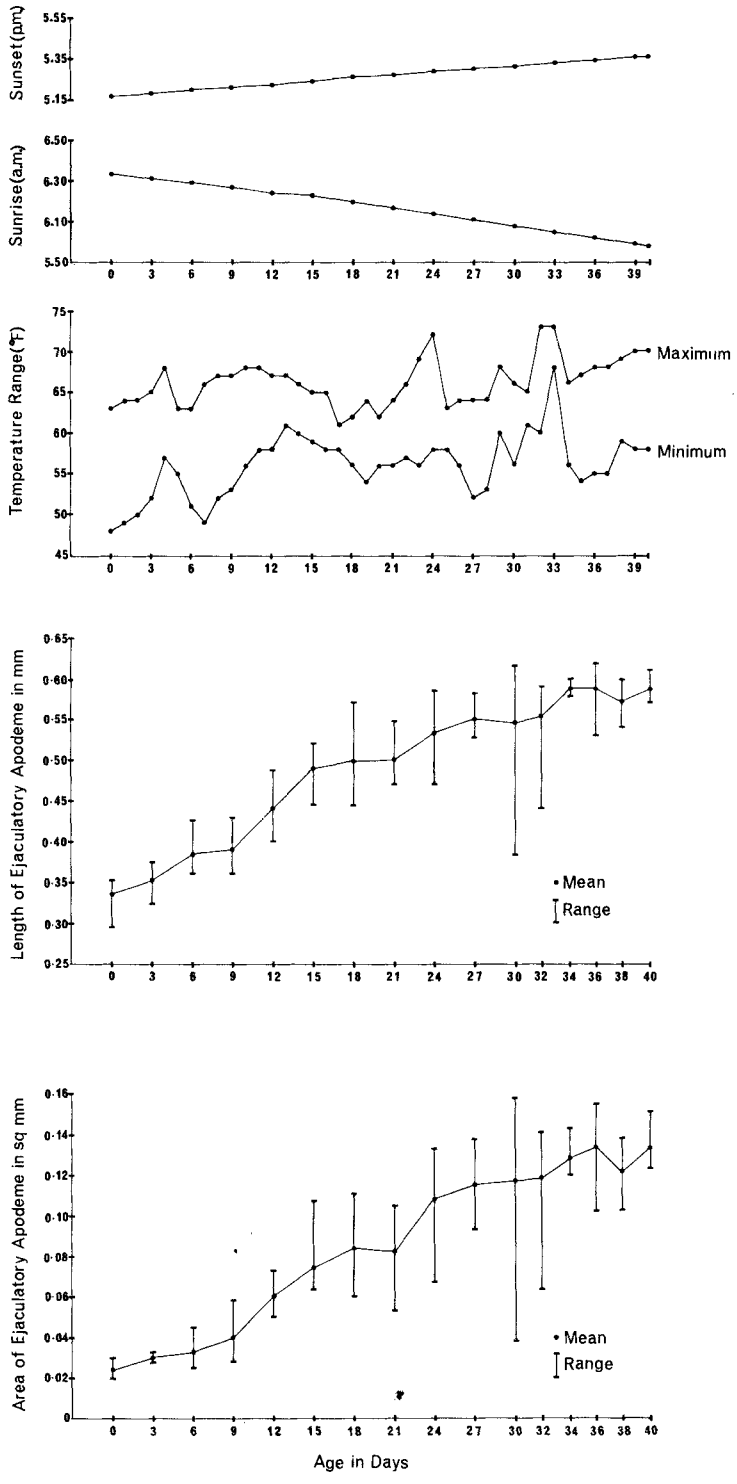


FIG. 28.—Changes in length and area of ejaculatory apodeme of *Strumeta tryoni* (Froggatt) with age, under natural conditions of temperature and light intensity. Maximum, minimum temperatures and sunrise, sunset times are figured.

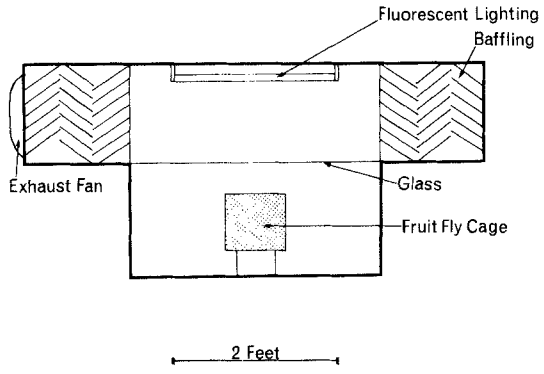


FIG. 29.—Front elevation of light cabinet. All walls light proofed; light proof baffling in upper section allowed air flow from exhaust fan to disperse heat from fluorescent tube; light ballasts located away from cabinet to reduce heat load.

Development of musculature on the ejaculatory apodeme

The relative daily sizes of the ejaculatory apodeme and its musculature from emergence to 5 days, of adults held at 25°C and natural light intensity, are illustrated in Figures 12-17. At emergence the apodeme had a small musculature which increased in size with the apodeme until 5 days old. At each stage, the musculature covered the apodeme.

DISCUSSION

At 25°C, 70-75 per cent. relative humidity and natural light conditions, flies were sexually mature and began copulating 5 days after emergence. Sexual maturity was therefore correlated with size of the ejaculatory apodeme, and both length and area of the apodeme gave an accurate estimation of this parameter. Because of the greater increase in area than length, from emergence to sexual maturity, area was found to provide a better index than length, though in practice length was easier to measure and would be sufficient. Because the endoskeletal structures function as supports for muscles which control the mechanics of copulation, the flies were not able to copulate until the structures had reached a certain size and so supported well developed musculatures.

The flies maintained at 25°C, 55-80 per cent. relative humidity and 120 lumens per square foot light intensity developed a mature ejaculatory apodeme 5 days after emergence and also began copulating at this age. Thus the change in light regime to which the flies were subjected did not affect either the development of endoskeletal structures or the age at which the flies began to copulate.

These results differ from those of Barton Browne (1957) who stated that the age at which males became active was influenced by illuminance and daily period, and that at 25°C, 75 per cent. relative humidity and 120 lumens per square foot for $7\frac{1}{2}$ hours per day, 50 per cent. of males were first sexually active after 31·6 days.

Under the variable conditions of external temperature and light intensity, the flies were not sexually mature until 24 days old and there was a greater daily range in apodeme measurements than under the stable 25°C temperature.

Comparison of these four sets of results does not implicate temperature, relative humidity or light as the critical factor determining the different rates of development to sexual maturity. Undetermined differences in intensity and duration of dusk lighting may have been a factor possibly interrelated with temperature and high relative humidity.

Significant differences between means of samples from different geographical areas (Table 1) were probably due to differences in physiological age, and not geographical variations.

The growth changes of the endoskeletal structures caused corresponding changes in shape. Great care must therefore be taken in attempting to use characters of these structures for descriptive purposes in systematics.

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