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GENESIS OF THE REPRODUCTIVE SYSTEM OF MOSQUITOES IV: THERMAL MODIFICATION OF *AEDES STIMULANS*[1]

William R. Horsfall, Maria C. Ronquillo[2]
and William J. Patterson[3]

Department of Entomology, University of Illinois,

Urbana, Illinois 61801 U.S.A.

ABSTRACT

Growth of male imaginal parts from primordia present at the end of embryogeny of certain mosquitoes is inhibited when abnormally high temperature prevails during subsequent ontogeny. This report deals with stasis in morphogenesis of these parts and explains reasons for their replacement by female parts in *Aedes stimulans* (Walker) (Diptera: Culicidae). The basic facts are that male imaginal discs fail to express definitive form while the female ones are uninhibited. Not only is development of solely male imaginal discs suppressed but expression of maleness of dimorphic parts (antennae, palpi for example) is thwarted. Embryos are either primordially female (unisexual) or are provided with imaginal discs for both male and female parts (bisexual). Bisexual embryos of *Aedes stimulans* as well as those of several other aedine mosquitoes give rise to males when reared at temperatures characteristic of melt water in vernal pools. They produce definitive females when ontogeny proceeds at abnormally high temperature. Inhibition of male organization is caused by heat and is a result of dysfunction within the cells involved.

INTRODUCTION

For several years much of the effort of this laboratory has been devoted to study of the effect of thermal conditions on anomalous morphology of mosquitoes. While most attention has been given to *Aedes stimulans* (Walker), effects on some 15 other species have been observed. Early work dealt with gross morphological changes as seen in the adult when caused by different temperatures and different sequential exposures acting during postembryogeny (Horsfall and Anderson '61, '63; Anderson and Horsfall '63, '65; Horsfall, Anderson and Brust '64, and Brust and Horsfall '65). Histological

1. This research was supported by Public Health Service research grant CC—00335, from the Center for Disease Control, Atlanta, Georgia.

2. Present address: Department of Biology, University of New Mexico, Albuquerque. N. Mexico.

3. Present address: 3rd Army Medical Laboratory, Atlanta, Georgia.

changes occurring during normal development of female and male reproductive systems were detailed by Ronquillo and Horsfall ('69) and Horsfall and Ronquillo ('70). The third report on abnormal histogenesis of male reproductive appendages was that of Voorhees and Horsfall ('71). Effects of thermal stress on embryonic development was discussed in Anderson and Horsfall ('65a) and Cupp and Horsfall ('70), ('70a). Results presented in this paper show degenerative and hypertrophic histological changes that occur in the component from which the male adult is normally produced when constant high temperature (28.4°C) persists throughout larval and pupal life. The discussion is based on these as well as prior results recounted in antecedent papers. The appended bibliography includes only references to papers originating from this laboratory; other relevant papers have been cited in papers noted above.

METHODS

Rearing techniques were essentially those described by Patterson ('68). Incubators of local design permitted regulation of temperature to ±0.05°C. Monitoring devices assured maintenance of this temperature. Larvae were fed live yeast daily, and fouling of rearing media was avoided by moving larvae to newly prepared media frequently. Histological preparations were made in the manner described by Ronquillo ('68).

Larvae were placed at 28.4° within 12 hours after hatching at room temperature of 25°. Times of ecdysis were known within 1 hour, and larvae of the same age at the beginning of each instar were reared together. Larvae for histological preparation were killed at intervals of 5 hours beginning with instar 2 and ending at termination of instars 2, 3, 4 and during the pupal stage.

RESULTS

Histological preparations were made of the primordia and rudiments of the reproductive system of that half of the population equipped at the end of embryogeny to produce males. Embryos bearing primordia for parts of the male system also bear them for those of the female system. They are thereby primordially bisexual. At intervals of 5 hours beginning with instar 2 larvae were killed and fixed, sectioned, stained and examined for significant histological changes of the parts in abdominal segments 5–9. The major changes are depicted and recounted herein.

The gonadal masses of bisexual embryos show one influence of abnormal temperature early in larval instar 2. The cellular state of the testis (Te, fig. 1) at this time is unchanged, but the ovary is more distinct than it is in a larva reared at 21° (Horsfall and Ronquillo, '70, fig. 12). Generative cells of the testes fail to develop even to the point of stratification at 28.4° which they do early in instar 3 at 21° (idem, fig. 13). Instead the germinal cells are abnormal (figs. 1 to 4) and fail to increase in number. The sheath of gonadal fatbody (Fb), a male feature, does become thicker (figs. 1 to 5) as it does normally but fails to lengthen just as the testis it covers fails to lengthen. In time it becomes loosened and even detached (figs. 5 and 6) instead of becoming a jacket in the usual manner. At no time does it encase or even encroach onto the ovarian portion. With advancing age, each ovary (O) and its associated lateral

oviduct (LOa) assume rudimentary states by the time the larval tissue has attained instar 4 (figs. 2 to 4).

While continued exposure to 28.4° results in complete statis of the germ-cell section of the testis (fig. 3 to 4), the anterior lateral oviduct continues to elongate and in time encloses the testicular rudiment. The fatbody persists into the adult stage as a loosely attached mass in the region of the testicular rudiment.

The ovarian rudiment becomes progressively differentiated into normal constituent parts during instar 4 (fig. 4). It begins to expand in diameter as more ovarioles are differentiated during pupal life (fig. 5 and 6). In *Aedes stimulans* the maximum number of ovarioles to be expected is about 20 in each ovary whereas an ovary in a female derived from a unisexual embryo may have 60 or more ovarioles as a tubular mass (Ronquillo and Horsfall '68). A bulbous configuration characterizes the shape of the definite ovary of bisexual origin (fig. 6). When all other features indicating bisexual origin of an adult have been obliterated by thermal treatment this one is diagnostic.

Efferent tracts and accessories

Primordia for the canals efferent from the gonads of both sexes and those for accessory appendages are present in segments 8 to 9 (figs. 7 to 16) of the bisexual component. Male imaginal discs in segment 9 (figs. 7 and 14) include 2 podal buds (9Bu) from which the normal gonapophyses are derived and 2 ampullae (9I) which at 21° or lower give rise to the lower tracts (seminal vesicles and vasa deferentia) and accessory glands. Extending from the ampullae in segment 9 to the testes in segment 6 during larval life are the posterior filaments (PF). These filaments provide the means for uniting vasa deferentia to the vasa efferentia (Horsfall and Ronquillo '70) when they contract during pupal life. The median genital plate of segment 9 (9GP of fig. 14 from which the ejaculatory canal is derived at low temperature) forms a bridge in the midventral region between the podal buds. The 2 podal buds of segment 8 (8Bu of fig. 8) under certain circumstances produce gonapophyses (Voorhees and Horsfall '71).

Caudal parts of the female efferent system are derived from the median genital plates in each of segments 8 and 9 (figs. 9 to 15). The common oviduct (CO, fig. 10) begins as an anterior invagination of the median genital plate of segment 8 (8GP of fig. 10). Spermathecae arise from this plate by invagination of a part behind that producing the oviduct. The vaginal area is created from the caudal part of 8GP when the abdominal segments telescope in pupal life (figs. 12 and 13). At the same time 9GP is drawn forward to the post-vaginal area to form the atrium with its sac-like bursa in the manner of the normal ♀ system (see also Ronquillo and Horsfall '68).

Gonapophyses

The two gonapophyses are derived from the podal buds (9Bu) of segment 9 at 21° (see also Horsfall and Ronquillo '70). At 28.4° they start to differentiate and develop as far as formation of the transverse sulcus (9Bu of fig. 8) in instar 3. No further development occurs (figs. 9 to 13). In the adult they are represented by 2 bilobed masses laterocaudad of the genital atrium (fig. 12). In other species they may be entirely eliminated by high temperature. Under some thermal conditions supernumerary gonapophyses may be derived from podal buds in segment 8 as well (see also Voorhees and Horsfall '71).

Cerci

Cerci (strictly female parts) are derived during stress of the bisexual component from primordia that appear laterally in segment 10 during larval instar 3 of the bisexual component. Development is progressive at 28.4°, and cercal rudiments are prominent during larval instar 4 (fig. 16) as they are in the unisexual component. In the adult they are as fully formed in females derived from bisexual embryos as they are from unisexual ones when rearing occurs at temperatures over 27°. Cerci show progressively more definitive form as rearing temperatures are increased above 24° as was shown in earlier papers.

Dimorphic imaginal parts

Adults derived from embryos of whatever sexual state have all other external parts in common, and they are dimorphic when development occurs under normal conditions. All of the dimorphic parts become feminized in *Aedes stimulans* reared at 28.4° (see Anderson and Horsfall, '63). Body form, oral stylets and other cephalic appendages and claws, are indistinguishable from those of the normal female with only one exception. The maxillary palpus of stressed forms is always atypical of the unisexual female condition (see Anderson and Horsfall, '63). It is slightly longer than that of a normal female palpus even when reared at the highest temperature permitting survival. Because of this difference it serves as an external recognition feature of a thermally induced female. Histological changes in these parts have not been included herein.

DISCUSSION

A dual population of normal males and females appears when *Aedes stimulans* grows to maturity in its usual haunts of vernal woodland pools in eastern North America where the water temperature is between 4 and 20° nearly all of the time. A population may mature at temperatures as high as 28+°, but the species cannot be perpetuated because, in ranges above constant 23°, the definitive male system has deficiencies. The female system develops normally at all temperatures. While developmental states described herein are those in *Aedes stimulans*, other species of *Aedes* exhibit similar responses to abnormal thermal environment imposed on larval and pupal stages.

Dimorphism of mosquitoes is an adult feature and is associated with sexual functions. It exists because some parts are unique to each sex and because of differences in appearance of homologous parts. The definitive female has ovaries, oviducts, spermathecae, bursa copulatrix, accessory gland and cerci that have no homologous parts in the male. The definitive male has testes, vasa efferentia, vasa deferentia, seminal vesicles, accessory glands, ejaculatory canal, gonapophyses and paraprocts that are uniquely male. Extensively dimorphic homologous parts are antennae, palpi, oral stylets and claws. Other imaginal features are less conspicuously dimorphic.

The dimorphic state of imaginal mosquitoes has been shown by others to be determined by the presence or absence of genetic determiners for maleness imparted to the zygotes (see literature review in Ronquillo and Horsfall, '68 and Horsfall and

Ronquillo '70). The female component is endowed with only female determiners so that only the female form may be derived therefrom. Zygotes which are capable of producing males bear determiners for male features paired with ones for uniquely female features and are thereby heterozygous for sexual features. Embryos derived from heterozygous zygotes are provided with primordia for both male and female parts and are bisexual. Embryos derived from homozygous zygotes have female primordia only and are unisexual as well as homozygous for features denoting sex.

Development of the dimorphic parts to the primordial state during embryogeny is independent of thermal influence although infrequent and erratic elimination of some parts of both sexes may be induced (Anderson and Horsfall '65a. Cupp and Horsfall, '70 and '70a). Expression of features of maleness beyond the primordial state is progressively minimized at temperatures in excess of 23° (Horsfall and Anderson '63). It is virtually prevented in *Aedes stimulans* at constant temperatures in excess of 28°. Postembryonic differentiation of female parts is not affected by temperatures that inhibit maleness.

Parts obviously affected by growth at abnormal temperature are those that are elongate as are gonads, gonapophyses, antennae, palpi et cet. They become differentiated earlier at their bases or proximal ends than at their apices or distal ends. Low temperature (23° or less) allows maleness to be expressed from base to apex in all parts (Ronquillo and Horsfall '69, Horsfall and Ronquillo '70). Temperatures in excess of 23° and below 28.4° may allow expression of maleness in the basal portions but may prohibit it apically (Horsfall and Anderson '63). The extent of terminal inhibition is a function of degree and duration of exposure. The lower the temperature the fewer the parts affected and the less pronounced the effect from base to apex.

Some parts such as the testes require favorable temperature throughout ontogeny to produce fully functional parts. Ovaries begin development slightly later but also require a long time span. The lower parts of the efferent tracts develop late in larval life and are unaffected by temperature early in larval life. The gonapophyses and cerci begin midway of larval life and mature slowly. All other parts become specialized during the pupal period. Ampullae attached to disc 9 become the seminal vesicles, vasa deferentia and accessory glands in the male at normal temperatures as the attached posterior strand contracts toward the testes. At 28.4° ampullae fail to develop beyond a ball of cells, and late in ontogeny they become detached from disc 9 and are drawn by the contracting filaments to the junction of the lateral oviducts where they appear in the adult as lateral nodules. Development of any of these parts may be totally or partially inhibited by timing of thermal stress.

In the primordial state, testes are larger than ovaries in the bisexual embryo, and they have such structural features as walls and distinct generative sections. Each primordial ovary appears as only a patch of similar cells with a tubular filament devoid of nuclei extending anteriorly (see part I for nature of filament in the unisexual form). When development of the testicular primordium is held in abeyance, the ovarian primordium begins to elongate, and soon it differentiates into an anterior generative portion and a posterior lateral oviduct. Development of ovarian tissue in unisexual embryos and in bisexual ones is concurrent at 28.4°. The extent of development of the ovary at the time of emergence of the adult differs in the two. An ovary derived from the unisexual embryo is elongate, cylindrical, tapered anteriorly and may have 60+ ovarioles. One derived from a bisexual embryo has 16 to 20 ovarioles and is bulbous

(fig. 6). The reason for this difference lies in the fact that the anterior portion of the ovary of the former is derived from cells in the anterior filament, while no such cells occur in the embryonic ovary of the latter (see Ronquillo and Horsfall '69 and Horsfall and Ronquillo '70).

All elements of the lower reproductive tract and accessory parts (bursa, spermathecae, atrium et. cet.) or parts of the lower male tract are differentiated late in larval life or early in the pupal stage. At constant 28.4° only the uniquely female ones become definitive. However, both male and female tracts and accessories may be present by varying degree and timing of the thermal stress. Extent of growth of any male primordium is related to temperature in effect at the time a part is differentiating.

Differentiation of testicular primordia is not required to stimulate maleness in the lower tract or development of gonapophyses. These parts will assume the male state when thermal treatment is relieved during the interval of their development even though abnormal temperature persisted long enough to prevent testicular growth.

The occurrence of supernumerary male appendages on segment 8 (see also Voorhees and Horsfall, '71) indicates also that elements of maleness may develop in the absence of testicular growth. Gonapophyses on segment 8 are derived from the bilateral or podal buds as they are in segment 9. The buds become evident during larval instar 3 whether from bisexual or unisexual embryos. They atrophy in both during instar 4 at normal rearing temperatures. They may differentiate into appendages in the former provided abnormal temperatures prevail throughout early development and normal temperature prevails during subsequent development during larval instar 4 and pupal stage. The median genital plate of segment 8 under these conditions may invaginate and even become partially differentiated into caudal parts of the median oviduct and spermathecae. Regardless of whether this happens, the podal buds of segment 8 become rudimentary gonapophyses. Subsequent exposure to temperature below 23° causes them to respond to the inherent male influence as do the serially homologous buds in segment 9, and they develop into gonapophyses, also. Buds of segment 9 become fully developed appendages while those of segment 8 are aberrant but clearly recognizable (Voorhees and Horsfall '71) possibly because those of segment 9 are more advanced earlier in larval life.

Direction of expression of dimorphic imaginal parts from primordia in the bisexual embryo toward male or female form proceeds late in ontogeny according to the temperature prevailing at the time they are actively growing. The male form of appendages may be expressed in an individual with female gonads and vice versa. Male appendages appear in the one case and female ones in the other because the male genetic code is dominant at low temperature in differentiating cells, and the female one dominates development at high temperature. The overall effect of continuous high temperature (28.4°) is that the female derived from a bisexual embryo resembles one derived from unisexual embryo externally except for those parts where definition began very early in ontogeny as is the case with the palpi and gonapophyses of segment 9.

Development in the primordially bisexual component of *Aedes stimulans* is a response of each part to thermal conditions and not to growth of other parts. Testes may become differentiated to the level of sperm formation in conjunction with or in the absence of external evidence of maleness by the time and degree of thermal stress.

They may become mature in part in association with ovarian development. Ovaries, too, may be produced regardless of the form of external features or whether some testicular development has occurred. Ovotestes form at temperatures intermediate between 23 and 28°.

The influence of high temperature on course of development beyond the embryo of the bisexual component of *Aedes stimulans* seems to be one of creating dysfunction in genetic decoding of male signals. Very high temperature (28.4°) prohibits male signals from being active in any part. Presumably any cell under thermal stress prior to and during differentiation will fail to respond to its intrinsic male signals. All parts that become differentiated toward maleness from base to apex (as do gonads and appendages) require high temperature early to create dysfunction in decoding in basal cells. The same temperature applied later when apical portions are differentiating will cause anomalous development in them only thereby resulting in full expression of maleness basally and in partial or no expression of maleness apically. Temperatures between 28.4° and 23° produce a gradient in response in degree of maleness so that apical parts of antennae, palpi, and dististyle of gonapophyses are demasculinized. In the absence of intrinsic male signals a cell reacts to the female ones, and the female form is expressed.

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ABBREVIATIONS

BC, bursa copulatrix (derived from median genital plate of segment 9).

8Bu, podal or bilateral bud segment 8 (primordium of secondary gonapophysis).

9Bu, podal or bilateral bud segment 9 (primordium of primary gonapophysis).

Ce, cercus (also rudiment).

CO, common oviduct (also rudiment).

F, (see fig. 5) – ovariole.

Fb, fatbody (gonadal).

8G, terminal ganglion of ventral nervous system (7 and 8G, united 7 and 8 ganglia).

8GP, median genital plate segment 8.

9GP, median genital plate segment 9.

9I, ampulla (primordium of vas deferens, seminal vesicle and accessory gland).

IG, caudal end of digestive tract.

LOa, anterior lateral oviduct (also rudiment).

LG, larval gut.

LT, larval trachea.

O, ovary.

PF, posterior filament (Y-shaped strand between gonadal mass and segments 7 and 9).

9Sc, sclerous plate on segment 9 of larval cuticule.

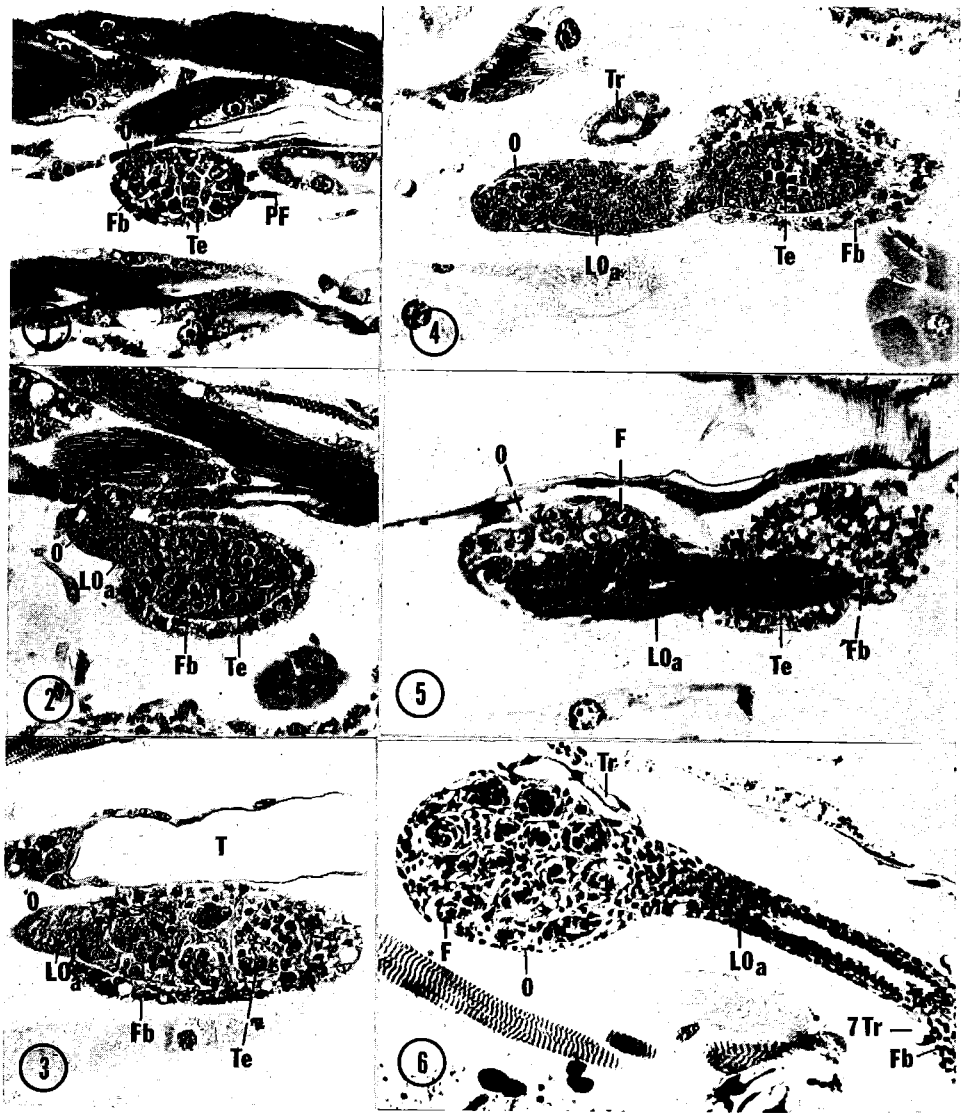
Sp_1, spermatheca.

SpD, spermathecal ducts.

T, dorsal trachea.

Te, testis (also primordium and rudiment).

Tr, trachea (7 Tr, tracheal branch in segment 7).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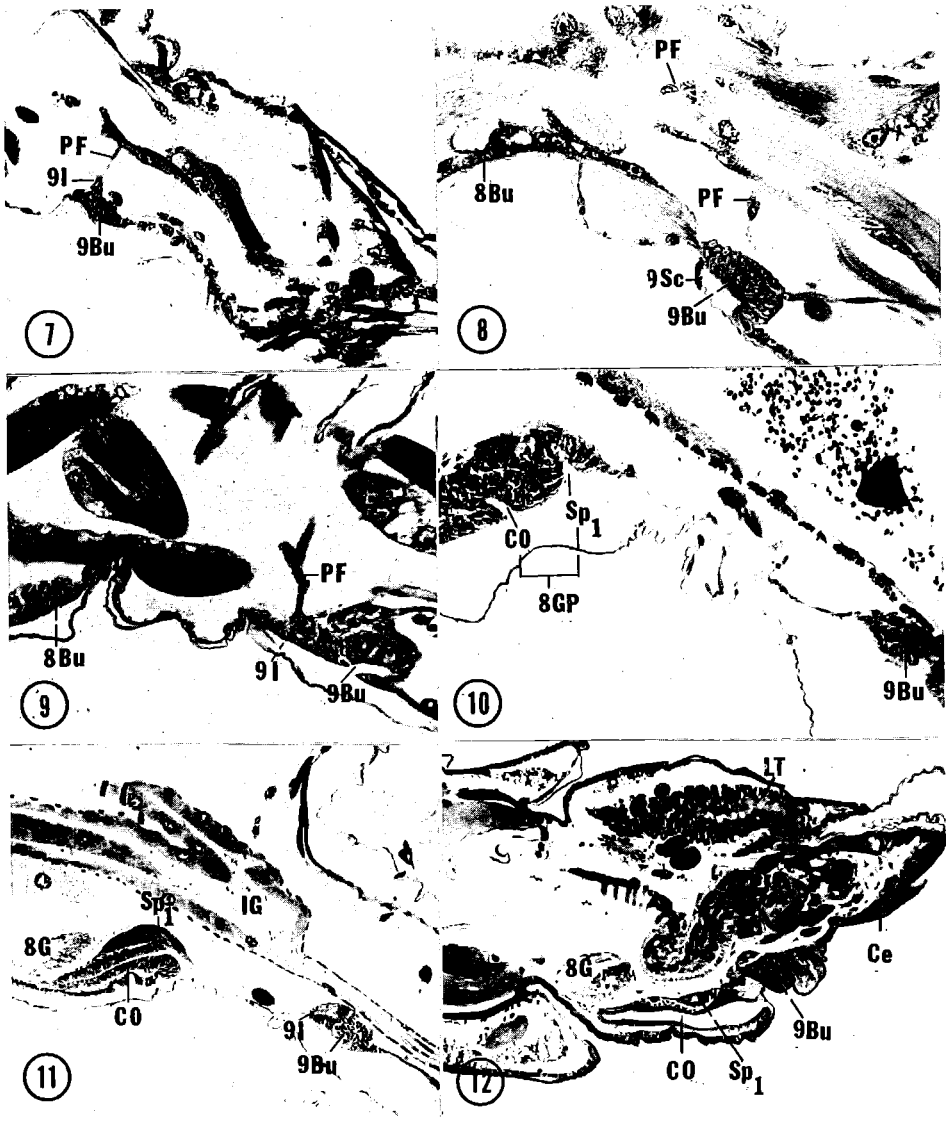
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Explanations of Figures 1–12

1 to 6. Gonadal states attained during larval and pupal development of the primordially bisexual component at 28.4°C; caudal end to right; longitudinal section.

1. Gonadal complex with ovarian cap (O), testis (Te), fatbody (Fb) and part of the posterior filament (PF) during early hours of instar 2; X–120.

2. Same with rudimentary ovary and anterior lateral oviduct (LOa); undeveloped testis (Te) with sheath of fatbody 5 hours into instar 3; X–150.

3. Anomalous state of testis in instar 4 at 15 hours; X–150.

4. Ovary with rudimentary ovariole, lengthened anterior lateral oviduct; anomalous testis with thickened fatbody in instar 4 at 55 hours; X–180.

5. Ovary with rudimentary ovarioles (F) and anterior lateral oviduct with cavitation; testicular area indicated by cup of fatbody in pupa at zero hours; X–200.

6. Ovary with numerous ovarioles; lateral oviduct with lumen; testis not recognizable; fatbody as mass in pupa at 55 hours; X–210.

7 to 13: Sequential states of the lower tract and gonapophyses in larval and pupal segments 8 and 9 of the primordially bisexual component at 28.4°C; caudal end to right; longitudinal section.

7. Primordial gonapophysis (9Bu), ampulla (9I) and caudal end of posterior filament (PF) in segment 9 of larval instar 2 at 15 hours; X–85.

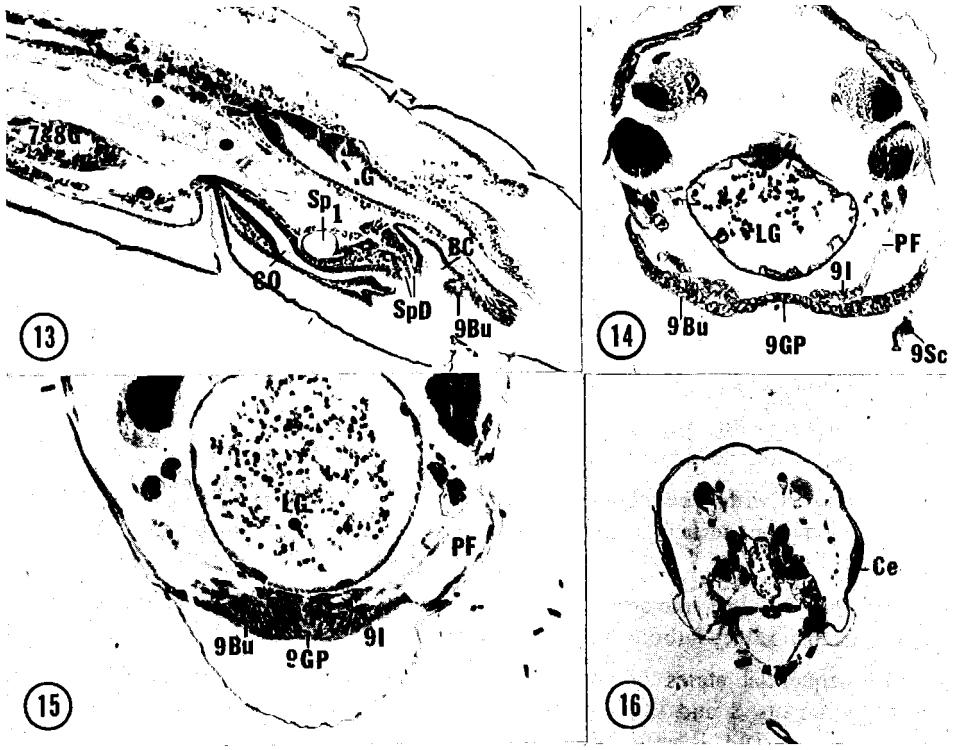
8. Rudimentary gonapophysis (9Bu) with transverse ventral sulcus; parts of posterior filament (PF); primordial podal bud (8Bu); instar 3 at 10 hours; X–150.

9. Gonapophysis (9Bu) with sulcus, attached ampulla (9I) and attached posterior filament; gonapophysis (8Bu) as thickened disc; instar 4 at 5 hours; X–250.

10. Gonapophysis (9Bu) static; genital plate (8GP) with invaginations for common oviduct (CO) and spermatheca (Sp_1) in instar 4 at 35 hours; X–260.

11. Gonapophysis (9Bu) static; common oviduct and spermathecal development extensive in segment 8 of instar 4 at 60 hours; X–135.

12. Gonapophysis (9Bu) static; female tract complete across segment 8 in pupa at 5 hours and before telescoping of caudal segments; X–110.



13. Fully developed female parts in adult within pupal cuticula at 30 hours; X–110.

14 to 16. Development of parts in larval segments 9 and 10 during instars 3 and 4; cross section.

14. Imaginal disc of segment 9; gonapophysis rudiment (9Bu) with ampulla (9I) and attached posterior filament (PF); median genital plate (9GP) as single layer of cells in instar 3 at 25 hours; X–115.

15. Same disc in larval instar 4 at 35 hours; X–250.

16. Cerci in larval segment 10 of instar 4 at 35 hours; X–85.