



**THE MALE REPRODUCTIVE SYSTEM IN *PAPILIO POLYTES POLYTES* LINNAEUS,
1758 (LEPIDOPTERA: PAPILIONIDAE)**

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ABSTRACT

Observations on the male reproductive system in *Papilio polytes polytes* L. were carried out in the 5th larval instar and 3-4 day old male adult. They were dissected and were fixed in Bouin's fluid. The tissues were routinely processed and sections were taken at 5-7 μ and stained doubly in Haematoxyline and Eosin. The paired testes are the only male reproductive organs in fifth instar larvae which are reddish, elongated and oval. The reproductive system of adult male consists of reddish, fused single testis, vasa-differentia, seminal vesicle, ejaculatory duct, aedeagus and paired accessory glands. There are four testicular follicles in each larval testis and eight testicular follicles in adult testis. Each testicular follicle in both testes shows the entire process of spermatogenesis, dividing follicle into four zones viz., germarium, the zone of growth, zone of maturation and zone of transformation. The spermatozoa are of eupyrene and apyrene types and grouped together in the form of numerous bundles. The testes and vasa-differentia are mesodermal showings no intima whereas remaining reproductive organs are ectodermal as they are lined by intima. The male genital aperture is located on the 9th abdominal segment.

KEYWORDS: Testes, Follicles, Vas deference, Ejaculatory duct, Aedeagus, *Papilio polytes polytes*.

INTRODUCTION

The taxonomists, morphologists, and physiologists have much interest in the anatomy of external and internal reproductive organs in animals. The taxonomists and morphologists described and have used the structural features for animal classification. With the help of anatomical details, physiologists, on the other hand, have elucidated the function of the system.^[1] Morphological studies substantiated by histological observations of the genital organs of the members of Lepidoptera present a wide scope of the investigation.^[2] The literature available on the reproductive system of Lepidoptera is widely spread out. Earlier contributions^[3-14] on the reproductive organs of moths and butterflies are of great significance. The information available indicates that most of the work is pertaining to anatomy and histology of reproductive system of moths and the very scanty information is available on the butterflies. Therefore, to fill this gap in existing knowledge, it has been decided to work on details of the male reproductive system in *Papilio polytes polytes* Linnaeus, (Papilionidae) which is one of the important pests of curry leaf, *Citrus* spp. and other Rutaceae. The present study will add basic knowledge in the reproductive system of Lepidoptera and will also help in the formulation of pest control strategies against the species under study.

MATERIAL AND METHODS

Field collected larvae of *P. polytes polytes* were reared up to the adult stage in rearing cages on leaves of *Murraya koenigii* under laboratory conditions. The fifth instar larvae and 3-4 day old males were dissected in chilled insect ringer solution^[15] for male reproductive anatomical studies. The reproductive organs were fixed in Bouin's fixative for 24 h. They were washed in water and dehydrated gradually using ethyl alcohol. After dehydration, tissues were cleared in xylene, infiltrated and embedded in paraffin wax at 54^oC. Tissues were sectioned at 5-7 μ . Sections were double stained with Haematoxyline-Eosin.^[16] Observations were made and microphotography was done.

RESULTS

Anatomical observations

In the fifth instar larvae of *P. polytes polytes*, the male reproductive system is represented by a pair of testis only (Fig.1A). The testes are reddish, elongated oval shaped measuring about 2mm long and 1mm in diameter. They are situated dorsolaterally in the 5th abdominal segment on either side of the dorsal vessel. They are surrounded by fat bodies and richly supplied with the trachea.

In adults, the male reproductive system consists of the unpaired testis, vasa differentia, ejaculatory duct, aedeagus and paired accessory glands (Fig.2A). The

testis is roundish, unpaired, measuring about 2mm in diameter and situated dorsally in the body. The unpaired testes in the adult are formed by the fusion of paired, red-colored testes of the larvae (Fig. 2A, a). It is situated below the terga of the fifth and sixth abdominal segments in the midline and retains its red color in the adult. The vasa deferentia are a pair of long, narrow tubes which leave the testis near their posterior end just under the coils of intestine. A small efferent duct, the vas efferens communicates with the vas deferens of its anterior end which leaves from the base of each testicular follicle. Proximally, each vas deferens enlarged into two successive bulbous regions, the seminal vesicles. Vas deferens measures about 7-9 mm in length. Beyond this, the vas deferens narrows and enters the paired ejaculatory ducts (ductus ejaculatorius duplex) which later on combine to form median ejaculatory duct (Fig.2Ac). The median ejaculatory duct (ductus ejaculatorius complex) is very much coiled measures about 4.5 – 5 cm long and terminates at the base of the intromittent organ, the aedeagus. Proximally, a median ejaculatory duct is slightly colorless, then its middle part is whitish spongy and distally it is again colorless (Fig. 2A d,e). The aedeagus is a brownish sclerotized tube. It broadens anteriorly and posteriorly is provided with musculature at the base (Fig. 2A g). It opens in the 9th abdominal segment ventrally. Accessory glands are a pair of elongated coiled tubes. Each gland opens at the anterior end of the forked arm of the ejaculatory duct and their junction is marked by a constriction. The accessory glands are 4.8 – 5 cm long, proximally milky white and distally colorless (Fig.2A h).

Histological observations

Each larval testis is externally covered by the peritoneal sheath containing small rounded nuclei at equal distance. The external coat of testis is compact and fibrous spongy and show a true nature of the connective tissue differentiated into outer tunica externa and inner tunica interna. This testicular tunica interna sends 3 transverse septa which divide the testis into 4 chambers, the testicular follicles. The successive stages of development of the germ cells are distinguishable in each testicular follicle (Fig.1B). In the follicles, spermatogenesis is well distinguished showing both types of spermatozoa, eupyrene, and apyrene. The apical portion of each follicle shows germarium (Apical cell) followed by a zone of growth having spermatogonia in the form cyst and zone of maturation containing spermatocytes. The sperms of each cyst remain grouped together in a number of bundles in the zone of the transformation of the testis. Overall the testis show number of sperm bundles (Fig.1CD).

In adults, the entire testis is covered by the peritoneal sheath and a capsular coat. As in case of the larval testis, the capsular coat of adult testis is differentiated into outer theca externa and inner theca interna. This coat is made up of closely packed cuboidal cells with a very spongy and granular cytoplasm. The plasma membranes are not

distinct. The testicular coat not only surrounds the testis but its theca interna enters in the substance of testis by means of six septa dividing the testis internally into eight chambers, testicular follicles and externally correspond to the transverse marking on it. The median longitudinal septum indicates the line of fusion of the two larval testes (Fig.2B). Each testicular follicle shows successive stages of development of the germ cells. They are arranged so systematically that represent entire process of spermatogenesis from apical to distal end of the follicle. On the basis of which testicular follicle is divided into four zones *viz.* germarium, a zone of growth, a zone of maturation and zone of transformation. The apical cell is not clearly distinct in the apical region of the follicle. The zone of growth shows the number of spermatogonia encysting into a cellular envelope, the sperm cyst and undergoes multiplication. Zone of maturation of follicle shows the spermatocytes in abundant number. The zone of transformation shows spermatozoa without sperm cyst. The sperms are of eupyrene and apyrene types and grouped together in the form of numerous bundles. The head and tail regions of sperms are distinct (Fig.2CD).

The anterior portion of the vas deferens shows slightly folded epithelium which is made up of tall columnar epithelial cells showing granular cytoplasm around the central nucleus. The inner surface of the epithelium is lined by a prominent brush border and is limited by a basement membrane (Fig.3A). The distal region of vas deferens show cuboidal epithelial cells and these cells have no brush borders. Cellular organization of the seminal vesicle is similar to those of the anterior region of the vas deferens except the absence of a brush border of the epithelium. Apically the epithelium of the seminal vesicle shows a large number of sperms and serves as their storehouse. The vas deferens, as well as seminal vesicle, is externally surrounded by a single layer of circular muscle fibers.

The ductus ejaculatorius duplex is made up of cuboidal cells surrounded by a single layer of circular muscle. The lumen shows the granular secretions and sperm bundles (Fig.3B). The ductus ejaculatorius simplex or median ejaculatory duct is distinguished into a non-muscular glandular and a muscular chitinous region. The nonmuscular region is further distinguished into three successive parts based on the nature of secretions. The first glandular region of ejaculatory duct shows cuboidal epithelial cells based on the well-developed basement membrane. It is the largest region of the ejaculatory duct. It shows viscous secretion in the lumen and appears as a mass of vacuolated material arranged in concentric circles at the periphery and is distributed radially in the lumen (Fig.3C). The second and third glandular regions of the ejaculatory duct have the same tissue layers as the first glandular part. The secretion which is seen in the lumen of second glandular part shows granular and vacuolated appearance (Fig.3D) and third glandular part of ejaculatory duct shows crystalline secretion (Fig.3E).

The muscular chitinous region of the ejaculatory duct show non-secretory folded epithelium which is lined with thick intima. In this region, the muscularis is very well developed and shows an inner and outer longitudinal layer of muscle fibers with the circular layer in between (Fig.3F).

The aedeagus is sclerotized tube invaginated inside the phallosome distally and it is attached to it by an aedeagal apodome which arose from the base of aedeagus. The

endophallus is membranous, highly folded reversible tube within the aedeagus, which is in continuation of the ejaculatory duct (Fig.3G).

The accessory glands show columnar epithelium surrounding the wide lumen. The cytoplasm is granular and forms a dense layer at the base of the cells. Free ends of cells are vacuolated. The basement membrane is well developed (Fig.3H).

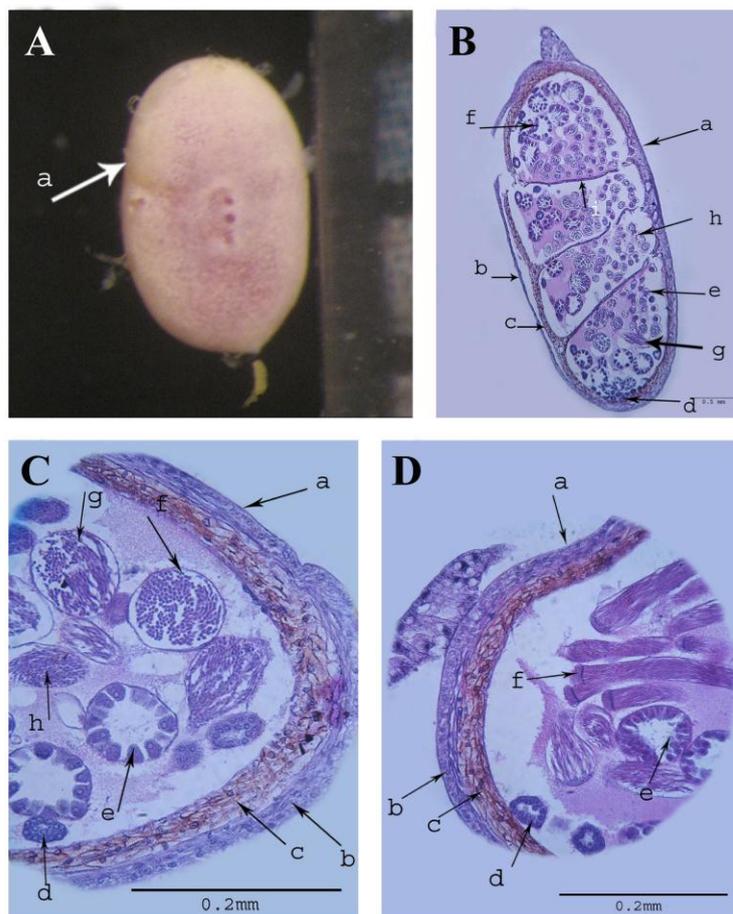


Fig. 1: A Larval testis in *Papilio polytes polytes* after dissection (a). B L.S. of larval testis. Note the peritoneum (a), tunica externa (b), tunica interna (c), spermatogonia (d), spermatocytes (e), sperm cyst (f), sperm bundles (g) and testicular follicle (f). C L.S. of larval testis (magnified) showing the peritoneum (a), tunica externa (b), tunica interna (c), spermatogonia (d), sperm cyst (e), spermatids (f,g) and spermatozoa (h). D Cross section of larval testis. Note the peritoneum (a), tunica externa (b), tunica interna (c), sperm cyst (d,e) and sperm bundles (f).

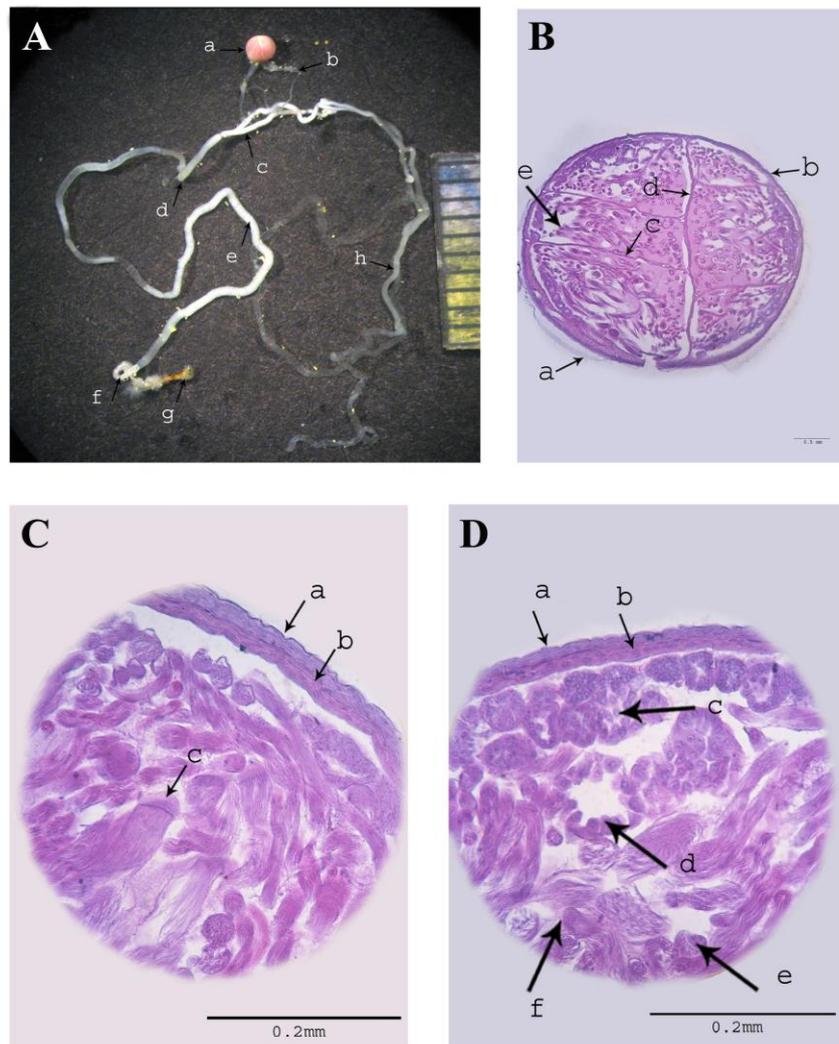


Fig. 2: A General organization of the male reproductive system in *Papilio polytes polytes* adult after dissection. *Note* the testis (*a*), vas deferens (*b*), duplex ejaculatory duct (*c*), glandular ejaculatory duct (*d,e*), muscular ejaculatory duct (*f*), aedeagus (*g*) and accessory glands (*h*). B T. S. of adult testis depicts peritoneum (*a*), capsular coat (*b*), horizontal septum (*c*), longitudinal septum marks fusion of larval testes (*d*) and testicular follicles (*e*). C T. S. of the adult testis (magnified). *Note* the tunica externa (*a*), tunica interna (*b*), and a bundle of spermatozoa (*c*). D T. S. of the adult testis (magnified). *Note* the tunica externa (*a*), tunica interna (*b*), spermatogonia (*c*), sperm cyst (*d*), spermatids (*e*) and sperm bundles (*f*).

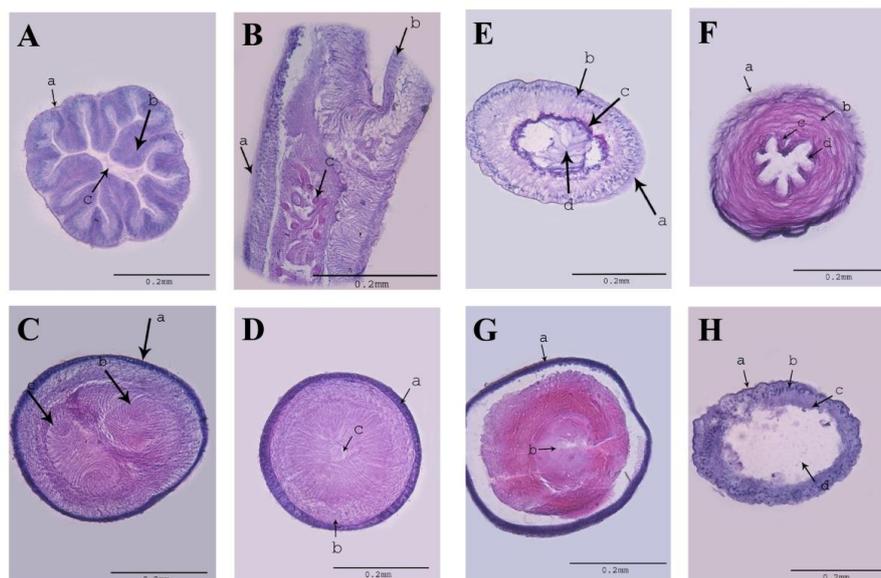


Fig. 3. A T. S. of vas deferens. Note the muscle layer (a), folded epithelium containing columnar epithelium (b) and a lumen with secretion (c). B L.S. of a duplex ejaculatory duct showing circular muscles (a), epithelium (b) and a fluid-filled lumen containing sperm bundles (c). C T.S. of the glandular ejaculatory duct (proximal region). Note the cuboidal epithelium (a) and luminal secretion indicating a flow of secretion from the duplex ejaculatory duct (b, c). D T.S. of the glandular ejaculatory duct (middle region). Note the epithelium (a), luminal secretion (b) and lumen (c). E T.S. of the glandular ejaculatory duct (distal region). Note the circular muscles (a), epithelium (b), intima (c) and fluid coating on spermatozoa in the lumen (d). F T.S. of a muscular ejaculatory duct. Note the thick coat of circular muscles (a,b), folded epithelium (c) and intimal lining (d). G T.S. of aedeagus showing highly sclerotized chitinous coat (a) and aedeagus cavity containing secretion (b). H T.S. of an accessory gland. Note the circular muscles (a), columnar epithelium (b) thin intima (c) and secretion in a lumen (d).

DISCUSSION

The male reproductive organs in the *P. polytes polytes* are basically similar in their pattern with other moths and butterflies studied by Williams^[17], Callahan and Chaplin^[4], Tedders and Calcote^[7], Davis^[8], Ruckes^[18] and Goyle.^[11] In general, the male reproductive system in the species under study is more or less similar to that of *Plathypena scarba*^[9], *Papilio demoleus demoleus*^[11], *Leucoptera coffeella*^[12] and *Dioryctria rubella*.^[14] The results of the present study are more or less similar as described for Lepidoptera i.e. testis separate in larva and fused in the adult, the pair of vasa deferentia, two seminal vesicles, one ejaculatory duct and paired accessory glands.^[5]

In Lepidoptera, the two testes are bound together in a single capsule.^[19,20] The larval testes of *P. polytes polytes* grow gradually in size throughout the larval instar and then it is followed by a period of rapid growth during the pre-pupa and pupa which consider with the maturation of a large number of cysts. This is in agreement with the sequence of testicular growth and differentiation reported for another Lepidoptera *Heliothis virescens*.^[21] The septum formation and subsequent division of testis follicle into several lobes have been described by Krause^[22] in the wood-eating beetle, *Passalus cornutus*. In the adult, testis consists of eight transverse spermatid tubes enclosed under a common scrotum is a specialized feature of the Lepidoptera and has been reported in *Pieris*^[18], *Plathypena scarba*^[9], *Leucoptera coffeella*^[12]

and *Dioryctria rubella*.^[14] However, in *P. scarba* testicular follicles are not distinguishable^[9] and in *L. coffeella* testis is made up of a large number of follicles.^[12] It is interesting to note that, in *Spodoptera litura*^[23], the spermatogenesis is completed in the larval stage itself. Similarly, in the larval testis of species under study, the entire process of spermatogenesis is nearly completed. According to the Wigglesworth^[20] the mature sperm break through their cyst wall to enter genital duct and they are often still held together in bundles (spermatodesms) of various forms, their heads being inserted into a cap or rod of gelatinous material. Similar types of sperm bundles are observed in both, larval and adult testis of *P. polytes polytes*. The moths and butterflies are unusual in that they normally produce two types of spermatozoa, nucleated eupyrene and anucleated apyrene.^[24] The larval and adult testis of *P. polytes polytes* also showing apyrene and eupyrene spermatozoa. Generally, besides germ cells, the testicular follicles contain a large distinct cell or nucleated mass of cytoplasm at the apex called the Versonian cells or apical cells. However, in the present study, such apical cells are not observed clearly. According to Friele^[25], an apical cell is a conspicuous structure in *Psychoda altenata* during the larval stages, but it degenerates and disappears in the pupa and is not to be found in the adult testis.

In many insects, the vas deferens is a narrow tube, enlarged posteriorly to form seminal vesicle and non-chitinous.^[19,20,26] In *P. polytes polytes* also, the vas

deferens shows a similar pattern and it is not lined with chitinous intima internally and the paired vas deferens enters the respective paired median ducts, the duplex ejaculatory ducts in *P. polytes polytes*. The lumen of seminal vesicle and the vas deferens is filled with secretion where as in lumen of vas deferens and seminal vesicles; the sperms are found to be intermixed with the fluid. Similarly, in *P. scarba*^[9] and *D. rubella*^[14] the ductus ejaculatorius duplex which unites posteriorly into a single duct, the ductus ejaculatorius simplex and giving rise to the accessory glands at their anterior terminal. The observations on ductus ejaculatorius simplex in *P. polytes polytes* are in accordance with observations of Wang *et al.*^[14] who reported 2 morphological areas, the primary segment and cuticular segment of ductus ejaculatorius simplex in *D. rubella*. On the basis of morphological as well as histological observations, the proximal section of the median ejaculatory duct in species under study is glandular showing secretion in the lumen and differentiated into three areas. However, in *P. scarba*^[9] proximal section of ductus ejaculatorius simplex represents the first secretory and second secretory area. The aedeagus in present species is similar to that described by Snodgrass.^[26] The walls of the ejaculatory duct are three-layered; outer circular muscles, middle of longitudinal muscle and inner thick epithelium. The thickness of epithelium suggests the glandular function. The ejaculatory duct enters the genital capsule where it meanders through the various layers of muscles associated with the base of the aedeagus before terminating at its tip. Accessory glands are uniform and paired in *P. polytes polytes* and open at the anterior end of the duplex ejaculatory duct separately. Similarly, in *P. scarba*^[9] and *D. rubella*^[14], each arm of ductus ejaculatorius duplex is continuous with the long thin accessory gland. In other Lepidoptera, the function of accessory glands has been hypothesized to be the production of seminal fluid.^[4,27]

From the foregoing discussion, the results obtained on the male reproductive system of the species under study show close similarity with other Lepidoptera in morphological, anatomical and histological structures. The presence of separate testis in larva and enclosed in the scrotum in a fused form in the adult is also a common characteristic of Lepidoptera. The completion of spermatogenesis in larval testis is an interesting character which is not a common character for all Lepidoptera.

CONCLUSION

The present study will help to establish and confirm the basic knowledge in the lepidopteran insects and to know the physiology and development of lepidopteran insects. The study will also help to formulate control strategies against the species under study and related species.

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