Article

Histoanatomy of the male reproductive system of the adult *Deudorix isocrates* (Fab.) (Lepidoptera: Lycaenidae)

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Abstract

The pomegranate butterfly, *Deudorix isocrates* (Fab.) is the serious pest of pomegranate causes considerable economic loss. The present light microscopic studies on the male reproductive system of the adult *D. isocrates* (Fab.) describe the anatomical and histological details which have not been reported earlier. These aspects of the pest studies have greater significance in pest management because reproductive organs are related with the development and life cycle of the pest. Anatomically the male reproductive system of *D. isocrates* consists of fused testes, a pair of thin walled vasa deferentia in continuation of proximal end of fused testes, a pair of seminal vesicles as a simple dilation of vasa deferentia, distal paired portion of ejaculatory duct which is referred as ductus ejaculatorius duplex, proximal single tubular ductus ejaculatorius simplex and the paired accessory glands. The genital tracts are complexly coiled. Histologically the fused testis is composed with spirally coiled longitudinal seminiferous follicles exhibit spermatogenesis in the numerous cysts. Longitudinally folded tall columnar epithelium of vas difference, cuboidal epithelium of seminal vesicle and low columnar epithelium of ejaculatory duct in *D. isocrates* are evidential in other lepidopterist species. The wall of male accessory gland is composed of columnar epithelium and muscle coat. These observations are concurred with some reports of the pest species of moths. In the accessory glands the nuclei of the columnar epithelial cells are round and located basally. The apical cytoplasm of columnar cells is vacuolated suggests secretory nature of accessary gland.

Keywords *Deudorix isocrates*; pest, anatomy; histology; reproductive system.

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1 Introduction

Deudorix isocrates (Fab.) Lepidoptera, Lycaenidae is a severe economic pest on pomegranate and guava (Kumar et al., 2017; Devi et al., 2021). It is the most injurious to the pomegranate causes 10 to 38% yield loss in Maharashtra (Shevale,1997).There is considerable research work has been carried out on its bio-ecology and

management but there is no any report available on its histoanatomical studies of reproductive organ system. The reproductive organs are related with the development of the pest and its life cycle due to which their studies have great importance in the pest management. The studies have been concentrated on the anatomical and histological features of reproductive organs to understand reproductive biology of the lepidopteran pests (Marti and Carpenter, 2007; Pezenty et al., 2021 Richmond and Tomescu, 1987). Number of times such studies have been undertaken to evaluate the effect of different biological and chemical formulations (Hatem et al., 2007; Ahemad et al., 2015, Cruz et al., 2015; Ibrahim, 2022; Malberg et al., 2022; Scudeler et al., 2022) and physical factors (Sallam et al.1996; Hamza et al., 2022) on reproductive organs to find control measures against the lepidopteron pests. The morphological study on reproductive organ is also important to predict control period of the insect pests (Zhang et al., 2017). Similarly the male reproductive system may provide significant evidence for the taxonomic and phylogenetic analyses of insects (Hua et al., 2020). Our study offers the first report on the detailed anatomical and histological description of the male reproductive system of *D. isocrates* adult. The present study provides the fundamental anatomical and histological characteristics of the male reproductive organs of *D. isocrates* which is useful as a basis of further research in the reproductive biology, physiology, systematics and management of this pest.

2 Materials and Methods

The specimens were obtained by laboratory stock reared on artificial diet. For anatomical study, the fresh adults were sacrificed by putting them in hot water. The sacrificed specimens were opened along with the mid dorsal line in insect saline under stereoscopic dissecting binocular microscope. The morphometric observations were made using ocular micrometer. These observations were presented as average with SE (Standard Error).

The live male specimens from laboratory reared stock were also used to make histological preparations. The wings and legs were cut and the scales from the abdomen were removed by camel hair brush. The specimens were dissected alive in a 0.7% saline. After dissecting out the complete reproductive system, each reproductive organ was separated and immediately transferred to different vials containing fixatives like aqueous Bouin's, Carnoy's, formal saline and chilled Acetone. Out of these fixatives, Bouin's was the best and hence it was used for the further histological studies. The organs were kept in Bouin's for 12 hrs., then washed under running tap water for 5 to 6 hrs., dehydrated in ascending grades of alcohol, cleared in Benzene and infiltrated with paraffin wax (M. P. 52^{o}C – 54^{o}C) for two and half hours. The longitudinal serial sections and cross serial sections were cut at 7 µ with a rotary microtome. Sections were spread on pre-egg albumin smeared slides.

The staining of tissue sections was done with Delafield's haematoxylene and 1% alcoholic eosine for the histological studies. The stained sections were mounted in DPX after clearing in xylene. The histological observations were made using light microscope under 10X and 45X magnifications.

3 Results

Anatomically the male reproductive system of *Deudorix isocrates* consists of fused testes, a pair of vasa deferentia, a pair of seminal vesicles, ductus ejaculatorius duplex, ductus ejaculatorius simplex and the paired accessory glands (Fig. 1). The genital tracts are complexly coiled.

Testes are fused, due to which anatomically appears unpaired single testis. It is more or less round in shape and yellowish green in pigmentation. It is wider than its length. In the anterior half of 5th abdominal segment the testis is situated along the midline and dorsal to the ileum of alimentary canal. On an average the testis is 0.89 mm (SE±0.053) long and 0.99 mm (SE±0.075) wide. The testis bears testicular follicles. The testicular follicles are arranged spirally (Fig. 2). The follicles open into the vas deferens proximally. Externally, the testis

is covered by peritoneal sheath. The nuclei of peritoneal sheath are darkly stained and are spherical in shape. Each testicular follicle is packed with the various stages of spermatogenesis from the distal to proximal end. These zones are germarium, zone of growth, zone of reduction divisions and zone of transformation. The meiotic figures are visible in zone of reduction (Fig. 3). The zone of transformation reveals the spermatids and sperm bundles. The spermatids are small round cells with round nucleus, which is subjected for changes in shape during further steps of spermeiogenesis.

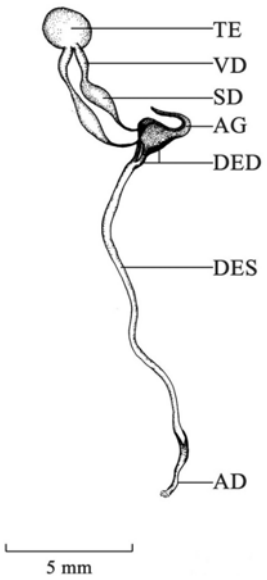

Fig. 1 Male reproductive system of *D. isocrates* adult; AD: Aedeagus, **AG:** Male Accessory gland, **DED:** Ejaculatorius duplex, **DES:** Ejaculatorius simplex, **SD:** Seminal Vesicle, **TE:** Testis, VD: vas deferens.

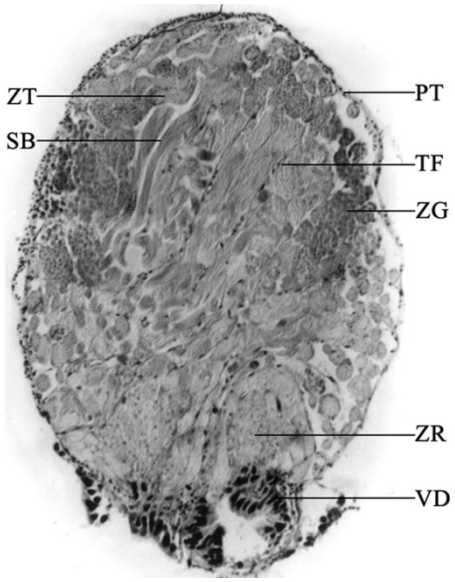

Fig. 2 L.S. of testis, *D. isocrates* adult x143. PT: Peritoneal sheath, **SB:** Sperm bundle, **TF:** Testicular follicle, **VD:** Vas deferens, **ZG:** Zone of growth, **ZR:** Zone of reduction, **ZT:** Zone of transformation.

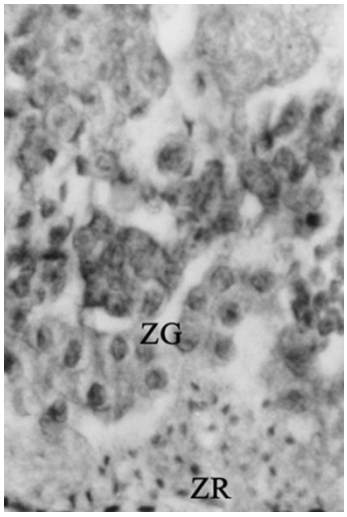

Fig. 3 Magnified portion of testis, *D. isocrates* adult x125. ZG: Zone of growth, **ZR:** Zone of reduction.

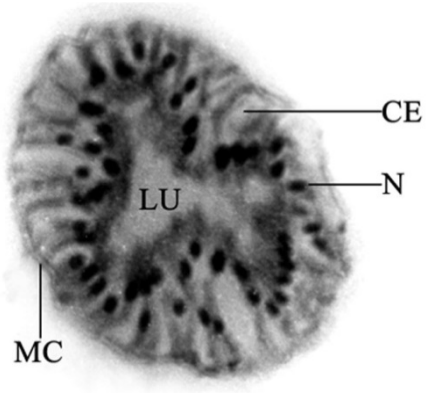

Fig. 4 T.S. vas deferens, *D. isocrates* adult x313. CE: Columnar epithelium, **MC:** Muscle coat, **N:** Nucleus.

Vasa deferentia and seminal vesicles are the paired organs (Fig. 1). A pair of vasadeferentia arises from ventral side of the testis and run posterior wards up to 6th abdominal segment. In 6th abdominal segment, portions of vasa deferentia are distended and forms the pear shaped sacs. These sacs are of seminal vesicles. Posterior to seminal vesicles the vasadeferentia are further extended to open into the ductus ejaculatorius duplex. The portion of vasa deferens anterior to the seminal vesicle is thicker; on an average 0.27 mm (SE±0.019) than that posterior to the seminal vesicle. It is about 0.10 mm (SE±0.006) in diameter. Including the seminal vesicle the average length of vas deferens is 6.22 mm (SE±0.39). The seminal vesicle is 2.38 mm (SE±0.125) long and 0.43 mm (SE±0.029) wide, at the middle. Lengthwise, the vas deferens takes smooth 'U' shaped turn due to which the seminal vesicle lies crosswise in the 6th abdominal segment. After the bent, the vas deferens extends up to 4th abdominal segment and opens into the, ductus ejaculatorius duplex. Histologically, the wall of vas deferens is composed of tall columnar epithelium and outer thin muscle coat (Fig. 4). The epithelium is thrown into a number of folds. The lumen of seminal vesicle is loaded with loosely arranged sperms. The lumen of the seminal vesicle is much wider than that of the vas deferens. The epithelium of seminal vesicle is lined with small cuboidal cells. The nucleus of cells is compact and round. The epithelium of seminal vesicle not folded like that of the vas deferens. A thin muscle coat encircles the

epithelium of seminal vesicle.

Ductus ejaculatorius duplex is the anterior paired portion of ejaculatory duct (Fig. 1). It is situated extremely lateral in body cavity, on right side to the testis and it lies deep in the ventral part of 4th abdominal segment. Anteriorly, each arm of the ductus ejaculatorius duplex is present in the form of conspicuous, globular region, which is filled with thick, milky white secretion. Vasa deferentia open into ductus ejaculatorius duplex at this region. The ductus ejaculatorius duplex is posteriorly present in the form of thick tubes. These tubes extend posteriorly for short distance and opens into ductus ejaculatorius simplex at the posterior margin of 5th abdominal segment. Along with the length the ductus ejaculatorius duplex from each side are closely adnated laterally to each other. The average length of ejaculatory duplex is 0.71 mm (SE±0.033) while its average diameter is 0.52 mm (SE±0.017) at the globular portion. The wall of ductus ejaculatorius duplex is thin and translucent but region appears milky white, due to the thick secretion stored in its lumen. The ductus ejaculatorius duplex has the widest lumen filled with packed sperm bundles and the secretion (Fig. 5). The wall is composed with the low columnar epithelium. The epithelial cells bear round centrally placed nucleus.

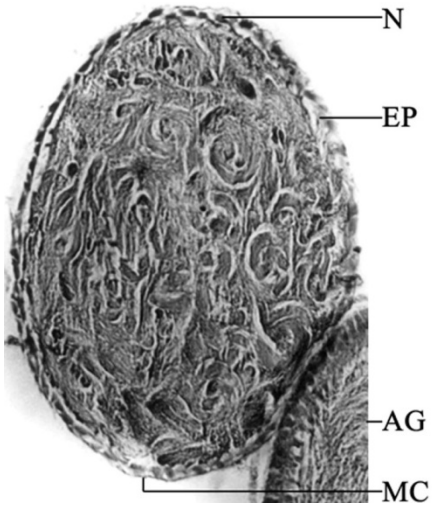

Fig. 5 T.S. of ductus ejaculatorius duplex, *D. isocrates* adult x153. EP: Epithelium, **MC:** Muscle coat, **N:** Nucleus.

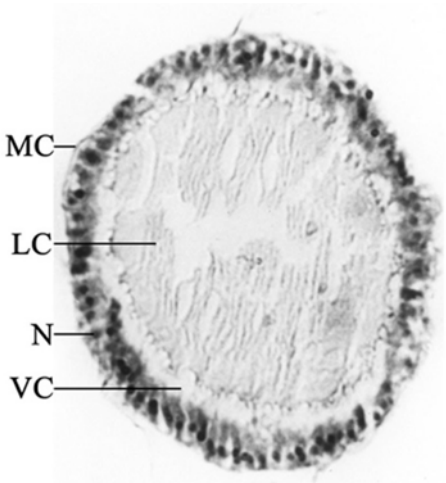

Fig. 6 T.S. of ductus ejaculatorius simplex (anterior portion), *D. isocrates* adult x269. LC: Luminal content, **MC:** Muscle coat, **N:** Nucleus, **VC:** Vacuolated cytoplasm.

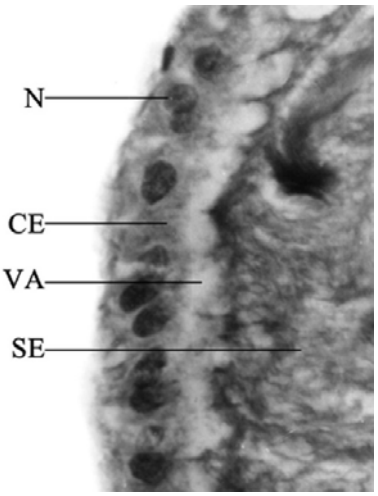

Fig. 7 T.S. of male accessory gland in part (Magnified), *D. isocrates* adult x675. CE: Columnar epithelium, **N:** Nucleus, **SE:** Secretion, **VA:** Vacuolated cytoplasm.

The two arms of ductus ejaculatorius duplex open in to a common duct at a particular point, which defines anterior end of ductus ejaculatorius simplex. At this portion the ductus ejaculatorius simplex takes 'U' shaped turn and runs slightly anterior. It passes to ventral side of the ileum of the alimentary canal and occupies left half of the 5th abdominal segment. Then it runs posteriorly up to the phalobase. Throughout the length, ductus ejaculatorius simplex is uniform in diameter. Its wall is relatively thick than rest of the reproductive organs. The translucent wall of this organ is covered by fat bodies. On an average ductus ejaculatorius simplex is 11.13 mm (SE±0.521) long and 0.28 mm (SE±0.015) in diameter. The ductus ejaculatorius simplex shows slight histological changes in its anterior and posterior regions. The intima of the anterior region is relatively very thin than that present in the posterior region. The wall of ejaculatorius simplex is composed of columnar cells with basally situated round nucleus. At the anterior region of simplex, the cytoplasm of cells is darkly stained basally while apically it is vacuolated (Fig. 6). In posterior region the apical cytoplasm is not vacuolated. Throughout the length of ejaculatory duct the epithelial lining is surrounded by muscle coat.

A pair of male accessory glands arises from anterior part of ductus ejaculatorius duplex and is situated anterior to it. They reach up to 3rd abdominal segment. Each gland is short and curved distally so that the blind distal end of gland turns towards the midline of the abdominal cavity. The accessory gland appears white due to the thick, milky white luminal content however, its wall is translucent. Proximally the gland is wider with which it opens into ductus ejaculatorius duplex. The gland gradually narrows towards distal end. Each gland from either side is adnated closely to each other. On an average each gland is 4.14 mm (SE±0.171) long. Its average diameter at proximal end is 0.33 mm (SE±0.012) while is 0.10 mm (SE±0.005) at the distal end. The lumen of accessory gland is filled with secretion. The wall of male accessory gland is composed of columnar epithelium and muscle coat. The nuclei of the columnar epithelial cells are round and located basally. The apical cytoplasm of columnar cells is vacuolated (Fig. 7).

4 Discussion

In *D.isocrates*, the male reproductive system comprises the fused testes (where spermatogenesis occurs), paired vas deferens, seminal vesicles, accessory glands, ejaculatorius duplex and unpaired ejaculatorius simplex opens into aedeagus as a generalised lepidopterist characteristic (Mari et al., 2018; Feng Bo et al.,2020) with some species specific characteristics. The paired testes are fused and composed with spirally arranged

follicles is characteristic in *D. isocrates* while follicles filled with many cysts reveal sequential stages of spermatogenesis are general histological features concurred with the report from other lepidopterist species (Alves, 2006). Longitudinally folded tall columnar epithelium of vas difference, cuboidal epithelium of seminal vesicle and low columnar epithelium of ejaculatory duct in *D. isocrates* is evidential in other species (Drecktrah, 1966). The lumen of ejaculatorius simplex is narrower than that of the duplex and full with secretion. It suggests secretory nature of epithelium of the ejaculatorius simplex. The vacuolated apical cytoplasm of the epithelium also proves the same. The similar report (Amaldoss, 1990) has been made in other species of the lepidopterist pests. In *D. isocrates* the accessory gland is thick, short and curved unlike to other lepidopterist species in which it appears long tubular (Mathur, 1966; Richmond and Tomescu, 1987). The wall of male accessory gland is composed of columnar epithelium and muscle coat. These observations are concurred with that reported in moth species (Fernandez and Landim, 2005).The nuclei of the columnar epithelial cells are round and located basally. The characteristic secretory epithelium of the accessory glands is reported in other lepidopterist species (Lai-fook, 1982). In *D. isocrates* the apical cytoplasm of columnar cells is vacuolated suggests secretory nature of accessary gland. The anatomical part of present report reveals expected details of male reproductive organs but suggests need of EM studies to report more histological details of reproductive ducts and accessory glands because some regions are not anatomically differentiated clearly.

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