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Testicles Fusion of *Diatraea saccharalis* F. (Lepidoptera; Crambidae) During Post-embryonic Development

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ABSTRACT

The sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae), is an insect of great economic importance. The larvae are fed on sugarcane, causing damages to sugar and alcohol industry. In order to increase the knowledge concerning the internal anatomy, testicles of *D. saccharalis* were subjected whole mount method and processed by using scanning electron microscopy to describe its anatomy during post-embryonic development. This organ suffers fusion during pupae stage (chrysalis), differing from some Lepidoptera species with respect to localization, size, form and coloration. Four testicular follicles are present in larval stage; nevertheless, in adult stage this number is variable. Despite the observed differences, general anatomical patterns are very similar to those described in other Lepidoptera, and the divergent characteristics may be important for phylogenetics and taxonomics studies.

Key words: Sugarcane borer, anatomy, larvae, pupae, adult, morphology.

INTRODUCTION

The sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae), is an insect of great economic importance, since its larvae are fed on grass culms such rice, maize and especially sugarcane causing servere damages to sugar and alcohol industry (Gallo 2002; Nava 2009). The insect's life cycle lasts about 58 to 70 days (Nakano *et al.*, 1981). After copulation the female lays its eggs on the leaves of sugarcane. The larvae feed on leaves. Then enter and start feeding on the culms. In feeding, the larvae will form longitudinal or transverse galleries, which can cause the drop in new plants or decreasing the concentration of sucrose in older plants (Marucci 2006). After about 40 days feeding, the larvae make a cocoon with leaf pieces of galleries formed and become pupae. This stage lasts about 10 days and the moths emerge, with reproductive function (Polanczyk *et al.,* 2004; Maranhão 1978; Lara 1992, Nakano *et al.,* 1981; Moreira and Marucci 2008).

Information regarding internal morphology of such insect during its post-embryonic development is important to enhance control mechanisms and comparative studies

among species (Quicke *et al.,* 1992). Generally, in larval stage, the testicles are paired structures located close to central vessel at the fifth abdominal segment (Chapman, 1998; Eaton, 1988; Snodgrass, 1993). In some species they remain separated during adult stage (King and Akai, 1982), but in others they are merged as in *Alabama argillacea* (Lepidoptera: Noctuidae) (Medeiros, 1986) *Grapholita molesta* (Lepidoptera: Tortricidae) (Morais *et al.,* 2009) *Ephestia kuhniella* (Nowock, 1973) *Heliothis virescens* (Lepidoptera: Noctuidae) (Meola and Loeb, 1995), *Methona themisto* (Lepidoptera: Ithomiidae) (Corsato-Alvarenga *et al.,* 1987) *Manduca sexta* (Lepidoptera: Shingidae) (Reinecke *et al.,* 1983) and *Calpodes ethlius* (Lepidoptera: Hesperiidae) (Lai-Fook,1982), originating a sole structure (Phillips, 1970; King and Akai, 1982). This fusion occurs at the final larval stage or in the beginning of pupal stage. This work aimed at observing testes fusion in *D. saccharalis*, as well as the anatomical changes along its post-embryonic development. These data will serve future to consider mechanisms of biological control.

MATERIALS AND METHODS

Larvae, prepupae, pupae and adults of *D. saccharalis* were supplied by the Laboratory of Cellular Biology and Genetics, Department of State University of Maringá, where were kept at breeding room at 25±1ºC, 70±10% RH, 12h photoperiod and fed on artificial diet (Hensley and Hammond, 1968). Male insects were anesthetized with ether vapour, transferred to Petri dishes and dissected through the ventral region in saline solution for insects (Martinez, 2002) using Zeiss Esteroscopic Microscope. After removing fat body, digestive tract and releasing trachea, we identified the testicles, stained them in the insect body using Light-Green and Toluidine Blue and submitted them to preparation in whoule mount on glass slide (Chen, 1984; Martinez, 2002). Afterwards, morphometrical analyses were carried out and documentations were done using Sony Cyber-Shot digital camera.

For scanning electron microscopy were used 80 individuals, 20 testicles were removed from of larvae, 20 of prepupae, 20 of pupae and 20 of adults were prepared according to Rigoni *et al.,* (2004). The samples were dehydrated in ethanol crescent solutions (70-100%) and submitted to critical point dryer Baltec CPD/030. After metallization with a 10 nm layer of gold, they were examined and photographed in Shimadzu SS-550 scanning electron microscope at State University of Maringá, Parana State, Brazil.

RESULTS

Morphometrical and anatomical analysis in total assemblage and scanning electron microscopy showed that during post-embryonic development. The testes of *D. saccharalis*, merged and undergo changes related to the shape, size and number of testicular follicles (Table 1, Figs. 1 and 2).

In the larval stage were observed two testes located in the dorsal region, at the fifth abdominal segment, close to the median line and arranged one on each side

of the insect body. They have reniform shape with length of 0.2 mm in larvae of the second stage and 1.85 mm in larvae of the fifth stage, and a width of 0.2 mm in the second stage larvae and 0.9 mm in the fifth larval stage. They have white colour and are surrounded by trachea from the fifth abdominal spiracle (Table 1, Figs. 1A and 2A). The organ has whitish appearance and oxygen is carried by branches of the tracheal system, from the fifth abdominal segment.

Anatomic Parameters of testicles		Developmental stages			
		Larvae - 2nd to 5th instars	Prepupa	Young Pupa	Elderly/AdultPupa
Amount of testicles		2	2 (close)	1 (under fusion)	1
Size (mm)	Length	0.5 - 1.85	1.85	2.0	1.75/1.5
	Width	0.2 - 0.9	11.5	2.0	1.75/1.5
	Thickness	-	-	1.5	1.5/1.5
Shape		Reniform	Oval	Spherical	
Colour		White			
Number of follicles		4		8	10-14

Table 1. Testicles of *D. saccharalis* along the development post-embryonic.

In prepupae, the testes are very close to each other, present oval shape, with length of 1.85 mm and width of 1.15 mm. They have white colour and are significantly large in relation to those in larvae (Table 1, Figs. 1B and 2B). The fusion was found in young pupae (Table 1, Figs. 1C and 2C).

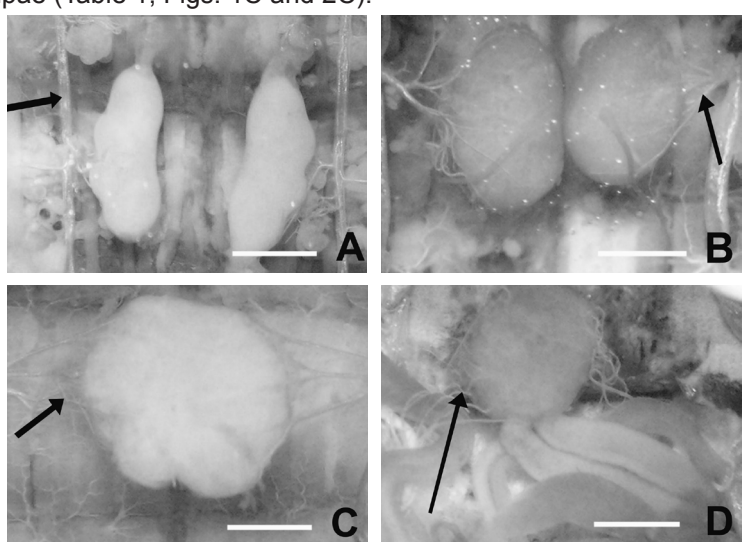

Fig. 1. Testicles of *D. saccharalis* at different post-embryonic stages Observed in stereoscopic microscope. Whoule mount technique, light-green staining. (A) 3rd instar, (B) evidencing prepupa Approximation of testicles; (C) Young pupa, where the testicles are under advanced fusion; (D) Adult, evidencing the sole testicle after fusion. T = testicle, Tr = tracheae, Tq = tracheoles, vs = seminal vesicle, vd = vas deferens, ga = accessory glands. Scale bar: A = 500µm, B-D = 1000µm.

For older pupae and adult insects, we observed only one white, spherical testis at the same location, with length of 2.0 mm, and width of 2.0 mm, and thickness of 1.5 mm (Table 1, Figs. 1D and 2D). At this stage, the torsion of the testicle occurred.

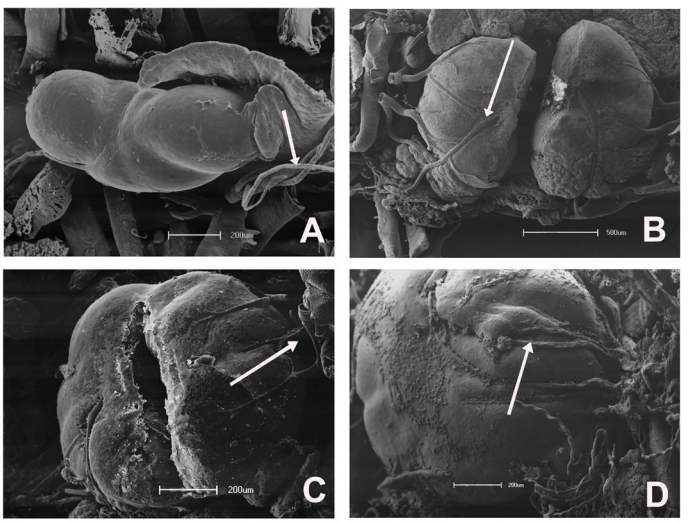

Fig. 2. Testicles of *D. saccharalis* under SEM (A) 5th instar; (B) prepupa; (C) with Pupa testicle under fusion (D) Adult. T = testicle, G = Fat body, Tq = tracheoles. Scale bar: A, B and C = 200µm, D = 500µm.

Macroscopically, we can see other organs that comprise the male reproductive system (*vasa deferentia*, seminal vesicles, accessory glands and ejaculatory duct) (Fig. 1D).

Testicular follicles varied in number according to developmental stage: four in prepupae, eight during the fusion in young pupae, and ten or fourteen pupae and adults older (Table 1).

DISCUSSION

Anatomical aspects of *D. saccharalis* testes are, in general, similar to those mentioned in other Lepidoptera. The presence of two reniform testes in larvae and one spherical testis in old pupae and adult of *D. saccharalis* was also described for *A. argillacea* (Medeiros, 1986), *C. ethlius* (Lai-Fook, 1982), *M. sexta* (Reinecke *et al.,* 1983), *Leucoptera coffeella* (Lepidoptera: Lynotiidae) (Alves *et al.,* 2006) and *G. mellonella* (Polanska *et al.,* 2005). *Ephestia kuhniella* has two testes united by a common tissue before the merger (Musgrave, 1937). However, the white colour for the testes observed in all developmental stages of *D. saccharalis* do not occur in other Lepidoptera species. In larvae of *M. sexta* the testes are transparent and in pupae and adults they present a cream colour (Reinecke *et al.,* 1983). According to Lai-Fook (1982) the testes of *C. ethlius*, vary from transparent to opaque in larvae and from yellow to red in pupae and adults. Adults of *E. hegesia* (Mancini and Dolder, 2004) and *M. themisto* (Corsato-Alvarenga *et al.,* 1987) present red testes. Caspari

and Blomstrand (1958) observed a yellow pigment in the lining epithelium of testes of *Ephestia* larvae, which disappears along the insect development - a characteristic not observed for *D. saccharalis*. In *A. argillacea* the testis has color green (Medeiros 1986). In *G. molesta* color testes varies from yellow to violet (Morais *et al.,* 2009).

In respect the testes position in the insect body, it may vary to different species of Lepidoptera. In larvae of *Bombyx mori* (Lepidoptera: Bombycidae) (Omura, 1936), *M. sexta* (Reinecke *et al.,* 1983), *A. argillacea* (Medeiros, 1986), *G. molesta* (Morais *et al.,* 2009); *M. themisto* (Corsato-Alvarenga *et al.,* 1987) and *H. virescens* (Meola and Loeb, 1995), the testes are found at the fifth abdominal segment as observed in *D. saccharalis*. However, in *C. ethlius* (Lai-Fook 1982), they are found at the sixth abdominal segment.

The testes are aerated by branches of the tracheal system from the sixth (Reinecke *et al.,* 1983) or fifth abdominal spiracle (Lai-Fook, 1982; Meola and Loeb, 1995), as in *D. saccharalis*.

Testes size may vary in Lepidoptera and according to Omura (1936) such variation is related to the species and its developmental stage. In the last instar testis size of *D. saccharalis* is 1.85mm long and 0.9 mm wide, greater than described for the last instar stage of *M. sexta* (1.6 mm long and 0.8 mm wide). However, in pupae of *M. sexta* the testis is greater (3.8 mm long and 3.5 mm wide) (Reinecke *et al.,* 1983) than that found for *D. saccharalis* (1.75 mm long and 1.5 mm wide). In adults of *A. argillacea* the testes presents 2 mm of diameter (Medeiros, 1986).

Testes fusion occurs in many Lepidoptera and it was described for several species as *E. kuhniella* (Musgrave, 1937), *L. coffella* (Alves *et al.,* 2006), *H. virescens* (Meola and Loeb, 1995), *E. hegesia* (Mancini and Dolder, 2004)*, M. sexta* (Reinecke *et al.,* 1983)*, C. ethlius* (Lai-Fook, 1982), *G. molesta* (Morais *et al.,* 2009) and *G. mellonella* (Polanska *et al.,* 2005). Nevertheless, in others as *B. mori* such fusion does not take place (Omura, 1936). The approximation and union of testes are described in fifth instars larvae of *M. sexta* ready for the commencement of pupation, although the fusion has not taken place yet (Reinecke *et al.,* 1983) as also observed for prepupae of *D. saccharalis*. Testes fusion in *M. sexta* (Reinecke *et al.,* 1983) occurs in the prepupae stage, approximately one or two hours before the pupa stage, similarly observed for *D. saccharalis.* However, in *C. ethlius* and *G. molesta* the fusion occurs in the fifth instar stage, twelve hours before pupation (Lai-Fook, 1982). In *G. molest* the fusion also occurs in fifth instars larvae (Morais 2009). Torsion of the testis seen in *D. saccharalis* was also described in *M. sexta* (Reinecke *et al.,* 1983) and *Ephestia kuhniella* (Nowock, 1973).

According to Nation (2002) every testis is comprised a number of tubes and testicular follicles in which spermatozoids are maturated. The number of follicles, according to Uvarov (1966) and Nation (2002), vary widely between species and presents taxonomic importance. In larvae of *B. mori* (Omura, 1936), *C. ethlius* (Lai-Fook, 1982), *M. sexta* (Reinecke *et al.,* 1983), *G. mellonella* (Polanska *et al.,* 2005) and *D. saccharalis* we observed four follicles. However, the amount of testicular

follicles in adults, reported for *C. ethlius* (Lai-Fook, 1982), *G. molesta* (Morais *et al.,* 2009) and *E. kuhniella* (Musgrave, 1937) is eight, while for other Lepidoptera as *L. coffeella* (Alves *et al.,* 2006), and *E. hegesia* (Mancini and Dolder, 2004) it is variable. In *D. saccharalis* varies from ten to fourteen in old pupae and adults. Every testicular follicle is bound to a vas deferens through an efferent vessel (Maranhão, 1978; Vanetti, 1983; Gillot, 2005).

The results support the existence of differences as regards location, size, shape and colour of *D. saccharalis* testes when compared to other Lepidoptera. However, despite such differences, the general anatomical and morphological patter is very similar for the entire Order. Thus, works related to testicular ultrastructure and spermatogenesis must be encouraged to broaden the knowledge regarding the male reproductive system along post-embryonic development of *D. saccharalis*.

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