Postembryonic study on the development of the female efferent genital system in the lemon-butterfly, *Papilio demoleus* L. (Lepidoptera)


Department of Zoology, Banaras Hindu University, Varanasi-221005, India

*Present address and address for reprint request: Department of Plant Protection, College of Agriculture, University of Guilan, Rasht, Iran

Abstract. Development of the female efferent genital system was studied during the larval-pupal transformation in the lemon-butterfly, *Papilio demoleus*. Two pairs of genital histoblasts (imaginal discs) — a pair each in the 8th and 9th abdominal segments of the 5th (ultimate) instar larva — are the precursors of this system. These histoblasts fuse to form a composite elongate structure, the parent body, which by late larval stage produces rudiments of the various components of the system: the oviduct, bursa copulatrix, spermatheca, vagina and accessory glands. These rudiments grow and differentiate into their definitive (adult) forms and sizes during the first 4 of the 6-7 day pupal life. While the external opening of the parent body on the 9th abdominal segment becomes the primary gonopore or the ovipore, the secondary (functional) gonopore or the ostimum bursae develops *de novo* as an invagination on the 8th abdominal sternum that meets the bursal rudiment above, possibly by mutual growth of the two. The bursal rudiment subsequently gets detached from the parent body except in the mid-posterior region where it retains its connection with the former. This connection later elongates to give rise to the adult seminal duct. By day 4 of the pupal stage, the adult efferent genital system is fully formed.

Key Words: female efferent genital system, development, *Papilio demoleus*

Introduction

Development of the efferent genital system in female Lepidoptera has been studied by several workers (Jackson, 1889; Verson and Bisson, 1895; Du Bois, 1931; Dodson, 1937; Ammann, 1954; Brunold, 1957; Srivastava and Srivastava,1959; Wittig, 1960; Joubert,1964; Leclercq-Smekens,1976; Sethi and Dhillon, 1981). However, details and timings of the development of various components of the system, particularly those of the bursa copulatrix, its detachment from the parent body and formation of two separate gonopores are largely lacking. We have attempted to throw some light on these aspects in the following paper.

Materials and Methods

Young larval stages of *P. demoleus* were collected from the field and raised in a BOD
incubator at 28 ± 1 °C, 75-80 per cent RH and 16 hr photophase. Newly moulted 5th (ultimate) instar larvae were sorted from culture jars with different ages of the instar under study reckoned from moult. The 5th instar lasts for 5 days inclusive of a one day prepupal stage followed by a 6-7 day pupal stage. Observations on the development of the efferent genital system were carried out from day 2 of the 5th instar larva to day 4 of the pupal stage; at which time all components of this system are fully formed. Insects of the desired ages were dissected in insect Ringer (Ephrussi and Beadle, 1935) which was then replaced by Bouin’s fluid for better visibility after clearing unwanted tissues from around the developing organs. For histological study, the tissues were routinely processed and cut at 7 and 5μm respectively and double stained in haematoxylin and eosin.

Fig. 1. Diagrams of the stages in the development of the EGS in Papilio demoleus. Details in the text.
RESULTS

Development during the larval stage

The development of the female efferent genital system (EGS) in *P. demoleus* begins with two pairs of rounded ectodermal invaginations, the genital histoblasts (GH = imaginal discs), a pair in each of the 8 and 9th abdominal segments close to the posterior margins of the respective sternum (Figs. 1a-c and 2). Being invaginations, each histoblast has its own lumen and external opening underlaid by the old larval cuticle (Figs. 1a-c, 3 and 4). Changes in these structures commence in day 4 larva when the intrasegmental histoblasts, which are separated in earlier instars, are drawn closer in order to fuse with each other, possibly due to shrinkage of the segments. Following fusion, the cavities and external openings of the histoblasts are reduced to one in each pair (Fig. 1b). On day 5, further fusion also occurs between the (previously) fused intersegmental histoblasts of the 8th and 9th segments, giving rise to a composite elongate structure named here as the parent body (= genital pouch of Leclercq-Smekens, 1976, Figs. 1c and 5). The parent body has a single cavity and a single external opening that constitutes the primary gonopore, the ovipore of the adult female (Fig. 1c, also Fig. 7). The parent body hereafter starts sending out rudiments of the various components of the EGS. In the middle-aged (12 hr) prepupa, rudiments of the oviduct (ODR) and bursa copulatrix (BCR) are produced from the anterior-half of the parent body while those of the vagina (VGR) and accessory glands (AGR), from the posterior-half (Fig. 1d). The rudiment of the oviduct is in the form of a tube projecting anterior to the bursal rudiment and occupying the anterior-third of the parent body, that of the bursa copulatrix, in the form of a rounded body dorsal to the former, that of the vagina, in the form of an oblong tube behind the oviduct accessory gland rudiments, in the form of two posteriorly directed and inwardly curved arms at the end of the vaginal rudiment. In the late (24 hr) prepupa, a longitudinal groove appears medially on the dorsal surface of the oviduct rudiment and a finger-like spermathecal rudiment (SPR) behind the bursal rudiment (Fig. 1). Appearance of the spermathecal rudiment completes the formation of all the precursors of the EGS.

Development during the pupal stage

In the newly emerged (0 hr) pupa, the groove on the surface of the oviduct rudiment sinks deeper to split it into two ectodermal components of the definitive lateral oviducts (LOD), as well as to demarcate the rudiment of the common oviduct (CODR) behind them (Figs. 1f and 6). The ectodermal LOD subsequently join their pre-existing mesodermal counter parts (not shown in the figures) to produce the LOD of the adult. By 12 hr pupal stage, the accessory gland rudiment produces a short duct (RDR) from its basal part (Fig. 1g). At 25 hr this structure is differentiated into three definitive parts namely, the reservoir duct (RD), reservoir (RES) and accessory glands (AG) (Fig. 1h). During the same period, an invagination occurs on the 8th
abdominal segment that grows upwards to meet the COD rudiment on its ventral aspect. The external opening of the invagination becomes the secondary (functional) gonopore, the ostium bursae (OB) and its upper passage, the ductus bursae (DB) (Figs. 1h and 7). By 36 hr pupal stage, the bursal and ductus components meet, possibly by mutual growth toward each other, at the same time retaining their connections with the COD (Fig. 8).

In the same stage, a small proximal outgrowth from the spermathecal rudiment marks out the lagena (LAG) and the utriculus (UT). The anterior end of the utriculus subsequently elongates and differentiates into a considerably long (and convoluted) spermathecal gland (SPG) of the adult during the remainder of the developmental period (Figs. 1i-l). By 48 hr pupal stage, the bursa with its ductus starts detaching itself from the COD part of the parent body while leaving the middle region of the latter still connected. This connected portion represents the future seminal duct (SD) (Fig. 1j, Fig. 8). Hereafter, all rudiments grow and differentiate only to acquire their adult forms and sizes so that by day 4 of the pupal stage, the EGS of the adult is fully developed (Fig. 1k, 1l and Fig. 10).

**DISCUSSION**

Despite development of the female EGS in Lepidoptera having been a subject of several studies, its descriptions and interpretations have differed with different workers. Jackson (1889) derived the median oviduct (or COD) component of the system in Vanessa from an unpaired median longitudinal groove, while Dodson (1937) derived the same in Zygaena in three parts: an anterior, from the fused posterior ends of the larval (mesodermal) LOD; a median, from a ventral ectodermal groove; and a posterior, from a thickened ectodermal band. All three subsequently joined to a bursal rudiment to produce the definitive COD. Srivastava and Srivastava (1959), on the other hand, derived the entire system in Leucinodes from two unpaired median invaginations present on the 8th and 9th abdominal segments which they called unterine and spermathecal rudiments respectively. However, what they called paired ectodermal longitudinal grooves on these segments, and interpreted as the remnants of gonapophyses, most probably represent the imaginal discs of other workers according to Matsuda (1976). Most other workers, however, derive the EGS from imaginal discs rather than from longitudinal grooves. The number of these discs has varied from two pairs present on the 8th and 9th abdominal segments (Verson and Bisson, 1896; Du Bois, 1931; Wittig, 1960; Leclercq-Smekens, 1976) and three pairs present in the 7-9 segments (Ammann, 1954; Brunold, 1957). Ammann (1954) in Solenobia reported that the discs of the 7th segment fuse to become an unpaired genital passage, the common oviduct (COD), while other components of the EGS namely, the bursa copulatrix and spermatheca, are produced from the 8th segment, and vagina and accessory glands by the 9th segmental discs after they formed a dorsoventrally superposed cavities. In Papilio, which lacks the 7th segmental discs, all components of the EGS
Fig. 2. WM (whole mount) of the genital histoblasts in the 8th and 9th abdominal segments of the 5th instar larva. x 60

Fig. 3 and 4. Sections through the anterior (Fig. 3) and posterior (Fig. 4) histoblasts showing their lumens and external openings underlaid by the old larval cuticle. x 50

Fig. 5. WM of the parent body in the process of formation by fusion of the histoblasts in the early prepupa. x 100

Fig. 6 WM of the parent body of 12 hr pupa with its rudiments and split in the LOD rudiment. x 100

Fig. 7 Sagittal section showing formation of the ostium bursae and ductus bursae with ovipore at the end of the parent body in 24 hr pupa. x 50

Fig. 8 WM of the EGS in 36 hr pupa showing union of the bursal and ductus rudiments with the attachment to the parent body still continuing. x 100

Fig. 9 WM of the EGS in 48 hr pupa showing detachment of the bursa copulatrix from the parent body (COD part) with the future seminal duct region still attached. x 25

Fig. 10 The fully developed EGS in a 4 day old pupa. x 16

Abbreviations used in the figures
AG accessory glands
AGR accessory gland rudiment
BC bursa copulatrix
BCR bursa copulatrix rudiment
COD common oviduct
CODR common oviduct rudiment
DB ductus bursae
GHA anterior genital histoblasts
GHP posterior genital histoblasts
ISM intersegmental membrane
LAG lagena
LC old larval cuticle
LPB lumen of the parent body
LOD lateral oviduct (ectodermal part)
OB ostium bursae
ODR oviduct rudiment
OVP ovipore
PB parent body
RD reservoir duct
RDR reservoir duct rudiment
RES reservoir
SD seminal duct
SDR seminal duct rudiment
SL split line
SPG spermathecal gland
SP spermathecal rudiment
UT utriculus
VG vagina
VGR vaginal rudiment
including the COD are derived from the 8th and 9th segmental discs albeit after their fusion into a parent body that does not permit identification of the specific discs of origin, as in *Solenobia*. Another feature in which *Solenobia* differs is in possessing a purely mesodermal LOD which in *Papilio* are partly ectodermal (basal parts) and partly mesodermal (distal parts).

More striking differences in the development of the EGS, however, were reported by Leclercq-Smekens (1976) in *Euproctos chrysorrhea*. The imaginal discs in this insect were not only shown to differ morphologically in the 8th and 9th abdominal segments, but also in the right and left discs of the 8th segment itself. In *Papilio* the discs are all spherical and identical. Further, the components of the EGS in *Euproctis* arise from the imaginal discs themselves prior to their fusion, while in *Papilio* this occurs after all the discs have fused to form a common parent body. Furthermore, a thickened genital area, producing the precursor to the oviduct, and located between the 8th and 9th abdominal segments in *Euproctis*, is lacking in *Papilio*. A feature about which most workers seem to differ is the origin of the two genital pores, the ovipore and the ostium bursae. While Srivastava and Srivastava (1959) note that in *Leucinodes* these structures exist from the very beginning of the larval stage, Brunold (1957) in *Solenobia* and Leclercq-Smekens (1976) in *Euproctis* describe their formation by partition of a single genital passage and opening. In *Papilio*, however, the external opening of the parent body constitutes the ovipore with the ostium bursae formed *de novo* from an invagination occurring on the 8th abdominal sternum, meeting the rudiment of the bursa copulatrix above most likely by mutual growth of the two toward each other. In the process the ductus bursae is formed (see Figs. 7 and 9). Photographic evidence of the developmental events of EGS, as presented in this paper, have not been presented heretofore.

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Literature Cited


