MORPHOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM OF PHANEROTOMA (PHANEROTOMA) OCULARIS KOHL (HYMENOPTERA: BRACONIDAE)

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Received 10 Mars 1992; accepted 7 September 1992

ABSTRACT: The female internal reproductive system of the hymenopteran species Phanerotoma (Phanerotoma) ocularis Kohl consists of 2 pairs of elongated ovarioles. The distal portion of the ovariole or germanium is enlarged and contains a number of reproductive cells. The medial portion contains the ovocytes and nurse cells. The proximal portion of the egg tube is enlarged and contains 20-30 mature ova. The calyx is also enlarged and filled with a fluid. The venom apparatus is Type II modified; the reservoir is located at the distal end of the only gland filament.

KEY WORDS: Hymenoptera, Braconidae, Phanerotoma (Phanerotoma) ocularis, female reproductive system, ovarioles, calyx, reproductive cells, nurse cells, venom apparatus.

INTRODUCTION

Phanerotoma (Phanerotoma) ocularis Kohl (Hymenoptera, Braconidae) is an important egg-larval parasitoid of lepidopterous pests. It has been reported as a parasitoid of Ectomyelois ceratoniae Zeller, Paramyelois transitella (Walker), Ephestia kuehniella Zeller (Lep., Pyralidae) and Prays citri Milliere (Lep., Yponomeutidae) (Biliotti & Daumal, 1969; Caltagirone, Shea & Finney, 1964; Hawlitzky, 1978; Moreno, 1991; Moreno, Hawlitzky & Jiménez, 1991).

The first and second larval instar of P. (P.) ocularis are endoparasitic and feed exclusively on haemolymph; the third larval instar emerges from the host larva and completes development as an ectoparasitoid feeding as a sarcophag. (Biliotti & Daumal, 1969; Hawlitzky, 1978; Moreno, 1991).

After studies developed in this laboratory to determine the effects of parasitization by this parasitoid on the morphology and physiology of its host, it was not found that the number of larval instars or time of development was different from controls. However, a reduction of cephalic capsule and in the size and weight of the parasitized larvae did occur. In addition, a compression and displacement of the internal organs of the parasitized larvae were observed (Hawlitzky, 1978; Moreno, 1991; Moreno, Hawlitzky & Jiménez, 1991).

The action of parasitoids on their host larva has been reported to induce a modification in the immune response and, consequently, in the inhibition of the encapsulation process. Several authors have reported the importance of the female calyx and venom fluids in the modification of the immune response observed in hosts (Edson et al., 1981; Ferran & Daumal, 1973; Ferran & Laforge, 1974; Le Masurier, 1987; Norton, Vinson & Stoltz, 1975; Vinson, 1972, 1974, 1977 a, b; Salt, 1973; Shaw, 1981; Stoltz & Vinson, 1977, 1979; Stoltz, Vinson & Mackinnon, 1976). Laboratory studies were conducted as a first step to determine the source of the observed effects on the host. In this paper we examine the morphology of the internal reproductive system of the female of P. (P.) ocularis.

MATERIAL AND METHODS

The parasites used in this study were reared from Ephestia kuehniella (Lepidoptera, Pyralidae) maintained according to the methodology described by Biliotti & Daumal (1969). Female parasites were dissected, just after emergence, in Ringer’s saline and examined microscopically. The reproductive system was isolated on a slide, washed with Ringer’s saline and manipulated into the correct position for observation. The isolate system was fixed for 10 minutes in Dubosq Brasil fixer (alcohol 80%; formal 40%; picric acid; 150 cm$^2$; 60 cm$^2$; 1 g) to which 10% of acetic acid was added. The fixer was removed and the tissues were dehydrated with a decreasing alcohol series. The fixed reproductive system was fixed to the slide with collodion (2 minutes). The reproductive system was rehydrated and stained with Feulgen’s (Schiff-Light Green) method. Photographs were taken by a Leitz Orthoplan microscope fitted with a Leitz Orthoplan Automat camera.

RESULTS AND DISCUSSION

The ovary is of a typical polytrophic meristic type consisting of two pairs of elongated ovarioles (Fig. 1A). The two ovarioles are similar but separate, being joined at the proximal region, as reported for Cardiochiles nigriceps Viereck (Hymenoptera: Braconidae) by Vinson (1969) (Fig. 1B). Each ovariole becomes progressively slender toward the distal portion. The distal portion of the ovariole is swollen and forms an elliptical germanium, where proovocytes occur (Fig. 1B).

The egg tube is polytrophic and shows a high number of reproductive cells that can be observed with microscope after Feulgen’s staining. These cells are the ovaric
Fig. 1.— Female reproductive system of P. (P.) ocularis: A) general aspect: ovariole (Ol), uteruslike chamber (Utc), calyx (Cx), lateral oviduct (Odl), common oviduct (Odc), spermatheca (Spt), venom apparatus (Va), reservoir (R), gland filament (Glf), ovipositor (Ov); B) detail of the ovariole distal portion and germanium (Gr); C) detail of the mature oocytes (Ovym) and the trophic cells (Tcd) in degeneration process; D) calyx (Cx) and lateral oviduct (Odl); one mature ovum can be observed embedded in the calyx fluid. Scale bars: A, B: 400 µm; C: 140 µm; D: 80 µm.
Female reproductive system of *Phanerotoma (Ph.) ocularis*

Follicles, oocytes and «nurse» cells, more or less evolved in function of their location along the ovariole (Fig. 1C). Each oocyte is found associated with a group of small cells, the trophic or «nurse» cells all along the length of the ovariole (Fig. 1C). The nurse cells degenerate as the oocytes mature. The trophic cells are not associated with the oocytes in the uterluslike chamber. *Vinson* (1969) suggests that the nurse cells could be secreted together with the calyx fluid. This author reports that the number of these small calyx secretory cells increases with the parasitoid female age.

The proximal portion of each ovariole or uterluslike chamber (*Vinson*, 1969) is swollen, and is billed with mature oocytes, even in newly emerged females (Fig. 1A). *Biliotti & Daumal* (1969) indicate that the eggs remain in non-degenerating females. Moreover about 20 to 30 mature oocytes can be observed in the basal portion of each ovariole of the just emerged females.

The uterluslike chambers of the paired ovariole open into an enlarged calyx, forming a cup-shaped structure, where generally only one mature egg can be observed embedded in a Schiff's stained fluid (Fig. 1D). This fluid seems very similar to the fluid suggested by *Salt* (1973), *Vinson* (1969, 1972 b) or *Shaw* (1981) as responsible for the protection of the parasite egg against the host's cellular defense.

Each calyx of *P. (P) ocularis* gives rise to a short lateral oviduct that is fused with the other to form a short, broad, common oviduct that opens into the ovipositor (Fig. 1A).

The spermatheca (Fig. 1A) is very small and is situated near the joining of the lateral oviducts to form the common oviduct.

The ovary of *P. (P) ocularis* is similar to the ovary of *Protopops* Wesmael (Hymenoptera, Braconidae) described by *Iwata* (1959) but differs from the ovary described by *Iwata* (1959) in that it has an enlarged calyx. Similar to the reproductive system of *Cardiochiles nigriceps* Viereck (Hymenoptera, Braconidae) (*Vinson*, 1969), it has a clearly differentiated well developed germanium and each ovariole has no contact with the other one of the pair all along its length.

There is one accessory structure associated with the ovary; this structure is partially responsible for the changes observed in the parasitized host (*Shaw*, 1981; *Vinson*, 1977 b). The venom apparatus of *P. (P) ocularis* opens into the common oviduct (Fig. 1A). The venom apparatus of this species is Type II (*Edson & Vinson*, 1979) consisting of a thin-walled reservoir surrounded by relatively few muscles. The Type II venom apparatus also typically presents two gland filaments of one cell in thickness surrounding a central lumen (*Edson & Vinson*, 1979) and the venom duct extends from the base of the reservoir to the ovipositor.

In *P. (P) ocularis* a variation of the Type II gland occurs: the reservoir is placed at the distal extreme of the only gland filament. Moreover the venom gland duct extends from the base of the gland filament to the common oviduct. This modified Type II venom apparatus has been found in other genera of Cheloninae (*Edson & Vinson*, 1979), which are egg-larval parasitoids.

The function of the venom apparatus secretion in females which do not paralyze their host is unknown. Probably this function, like the calyx fluid, is related to the observed effects on the morphology and physiology of the host and, particularly, to the reduction of the host immune response after parasitization. At present we are studying the ultrastructural morphology of the internal reproductive system in *P. (P) ocularis* in order to obtain further information with regard to the function of the venom apparatus in those female which do not paralyze their hosts.

**ACKNOWLEDGEMENTS**

This work was supported by financial assistance provided by the C.I.C.Y.T. Department of Spanish Government (Ministry of Education and Science) (Project PB90-0419). We are grateful to Dr. C. Boulay (INRA-CRA Versailles, France) for her aid in the histological aspects, to Dr. N. Hawlitzky (INRA-CRA Versailles, France) for her critical comments, and to Dr. C. Frenoy and Ch. Brachet (INRA-CRA Versailles, France) for their technical support and help in the laboratory.

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