Growth of the Teratocytes of *Microplitis rufiventris* Kok. Parasitoid in its Preferable Host Instars of the Cotton Leaf worm *Spodoptera littoralis* (Boisd.) Larvae

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(Received, January 10, 2005; Accepted, February 12, 2005)

ABSTRACT

Seven age classes from late first to fourth instars of *Spodoptera littoralis* larvae were singly parasitized by *Microplitis rufiventris* Kok. to study changes in diameter of maturing teratocytes at the completion of parasitoid development. The largest cell diameter was observed when hosts were parasitized in their fourth instar compared with parasitization on younger hosts. The diameter of mature teratocyte cell was instar-dependent. The results suggest that the teratocytes may be involved in active absorption of some host material(s) from the surrounding haemolymph of the parasitoid.

Key Words: *Microplitis rufiventris*, teratocytes, *Spodoptera littoralis*.

INTRODUCTION

Microplitis rufiventris Kok. is a solitary endoparasitoid. It oviposits and develops in many noctuid caterpillars including the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Kokujev, 1914; Hammad *et al.*, 1965; Gerling, 1971), the lesser cotton worm *Spodoptera exigua* Hbn. (Meier, 1929), *Spodoptera latebrosa* Lederer (Hammad *et al.*, 1965) and American bollworm *Heliothis armigera* Hb. (Meier, 1929; Ibrahim and Tawfik, 1975). When the egg of *M. rufiventris* hatches in its host larva *S. littoralis*, the cells which make up one of the egg membranes dissociate, giving rise to numerous cells which are freed into the haemolymph. Approximately 400 cells are liberated from the parasitoid egg (Khafagi, 1997).

These cells were assigned various names that reflected a proposed function and/or embryological source. They are known as "giant cells" (Gerling and Orion, 1973), "trophic cells" (Sluss, 1968), "trophamnion cells" (Tremblay, 1966), "teratocytes" (Salt, 1968; Vinson and Lewis, 1973) and "trophserosa cells" (Jackson, 1928).

The teratocytes are most commonly found in the haemolymph of hosts attacked by braconids (Tawfik, 1961; Kitano, 1962; Sluss, 1968; Vinson and Lewis, 1973) but have been reported in the Trichogrammatidae (Voegele *et al.*, 1974), Scelionidae (Gerling and Orion, 1973) and Platygasteridae (Hill and Emery, 1937). Teratocytes persist during the course of parasitoid larval development, but their role in parasitism is unclear. Some studies suggest teratocytes serve atrophic function (Sluss, 1968; Okuda and Kadono-Okuda, 1995) whereas others reported that they affect the development and immune response of hosts (Salt, 1968; Joiner *et al.*, 1973; Strand *et al.*, 1986; Zhang and Dahlman, 1989; Tanaka and Wago, 1990).

Interest in no protein secretory products from teratocytes has emphasized production of juvenile hormone (JH). Joiner *et al.* (1973) provided clear evidence of JH activity in extracts from *Cardiochiles nigriceps* Viereck teratocytes. They did not know whether the JH was stored or synthesized, although extracts from older teratocytes had greater JH activity in *Galleria* bioassay than extracts from younger teratocytes. Grossniklaus-Bürgin and Lanzrein (1990) suggest that teratocytes from a *Chelonus* species release JH.

This is the first report to describe the effects of host instars and ages within each instar on growth pattern of teratocytes derived from eggs of its *M. rufiventris* parasitoid.

MATERIALS AND METHODS

Cultures of the parasitoid *M. rufiventris* were reared on larvae of *S. littoralis* at 27±1°C, 65±5% R.H. and a photoperiod of LD: 14:10 h. Both populations were reared following the methods developed in the Department of Economic Entomology in Alexandria (Hegazi *et al.*, 1977; Hegazi and El-Minshawy, 1979). Infusions of field-collected insects were made for both cultures.

Mating in *M. rufiventris* wasps occurs as soon as both sexes are put in the presence of one another (Hegazi, 1977), thus couples of newly emerged females held together in glass vials (25x100mm) for 48h were presumed mated. Also, they were provided daily with fine droplets of honey to ensure maximum reproductive success.

The following experimental procedures were used to determine whether the host instar and ages within instar have an influence on the growth pattern of *M. rufiventris* teratocytes. The host larvae were grouped into instars and ages within the instar; *i.e.*, early second, third and fourth instar (determined by the presence of a molted head capsule) and late first, second, third and fourth instar (determined by their color and weight). For each group of host larvae, 5-6 2-days old mated female parasitoids which had no previous contact w ith host larvae were used singly and each served as a replicate. Oviposition by the female was induced by placing the female wasp in a glass vial (7.0x1.8 cm) and tapping the female into contact with the host larvae (10 larvae/female) which was removed immediately after a single oviposition (used to enhance precision in the procedure). Larvae that were accidentally parasitized more than once were discarded. The stung larvae were observed daily.

At the completion of parasitoid development (confirmed by dissection of the host larvae after bleeding) the host larvae were first warmed up to 60°C for 1 min to inhibit melanization. Preliminary tests proved that this

procedure had no effects on the teratocytes at that temperature. Then the larvae were bled through one of the first two abdominal legs. The first one or two drops of haemolymph were collected on a depression slide. In all cases, 50 teratocytes per host larvae were selected at random (5 parasitized larvae/female/host age) and their diameters were measured using a compound microscope equipped with an optical micrometer. For elliptical cells both length and width diameters were measured and the average was used to estimate the cell diameter.

After haemolymph removal, the host larva was re-investigated and dissected under a binocular microscope in order to ascertain the presence or absence of the parasitoid larvae. In all experiments, data of host larva containing more than one parasitoid were not used.

Data were subjected to analysis of variance for determination of differences between means. Where significant differences occurred, Duncan's multiple range test was applied for mean separation.

RESULTS AND DISCUSSION

The parasitoid *M. rufiventris* attacks and can develop on earlier instars of *S. littoralis* larvae (late first to fourth). However, third instars are preferred (Hegazi *et al.*, 1977). The parasitoid oviposits a single egg per host and the ontogeny includes three instars which feed on the host's haemolymph (Hegazi and Führer, 1985). When the egg of *M. rufiventris* hatches in its host larva *S. littoralis*, the cells which make up one of the egg membranes dissociate giving rise to 390.7±22.0 cells per egg in hosts parasitized once (Hegazi *et al.*, 1998). The initial diameter of liberated cells averaged 14.0±0.4μm (Hegazi and Khafagi, 1999). These cells showed a fast growth up to day 7 after exclusion and a slow growth prior to parasitoid emergence. To determine if the size of host larvae at the time of parasitization has an influence on the growth of *M. rufiventris* teratocytes, seven age classes (late first to fourth) were each singly parasitized. Changes in diameter of maturing *M. rufiventris* teratocytes at the completion of parasitoid development; *i.e.*, just before parasitoid egression are illustrated in Fig. (1). Means of teratocyte diameters (μm) prior parasitoid development significantly differed (P = 0.01). The smallest cells were observed in host larvae parasitized at their late first instar (64.1±2.2μm). The average mean diameter of mature *M. rufiventris* teratocytes observed in hosts parasitized as early and late second instars reached to 72.1±2.2 and 83±2.4μm, respectively. These averages increased to 87.1±2.4 and 103.3±3.7 μm for hosts parasitized as early and late third instars, respectively. However, the largest cells were observed when parasitization occurred on the fourth instar of *S. littoralis* larvae. The average diameter of mature cells in hosts parasitized as early and late fourth reached to 117.4±3.4 and 137.0±7.2μm, respectively. The results suggest that the teratocytes may be involved in active absorption of some host material(s) from the surrounding haemolymph of the parasitoid. The dense coat of microvilli and cellular out pocketing observed on teratocytes could be interpreted as adaptations for absorption of nutrients from the host's haemolymph (Dahlman, 1990). It was reported by Vinson and Iwantsch (1980) that teratocytes rapidly took up ^{14}C-labeled amino acids and synthesized proteins and secreted some of them back into the medium. It was observed that some of singly parasitized *S. littoralis* larvae appeared and behaved as non-parasitized larvae. Dissection of these hosts (unsuccessfully parasitized hosts) revealed that some were teratocytes-free and with dead parasitoid eggs for unknown factors. Thus, it seems that the teratocytes may have an immune-suppressive role. Dahlman (1990) mentioned that when teratocytes were injected into *Helicoverpa virescens*, they produce many of the same developmental abnormalities when host larvae are truly parasitized.

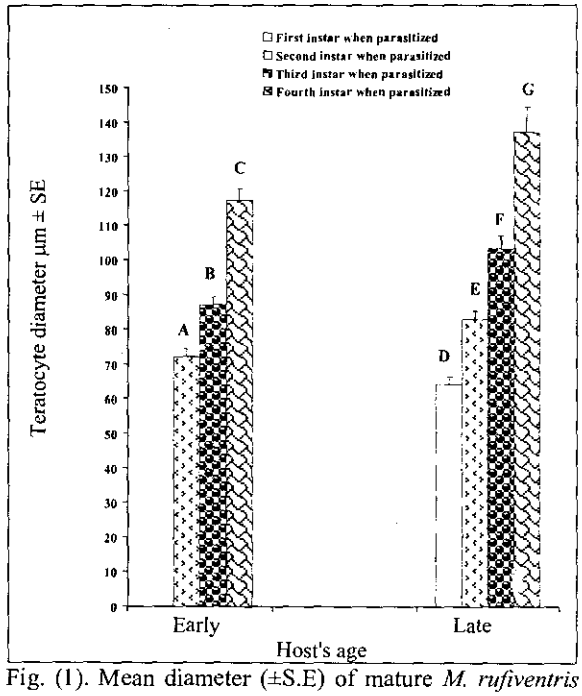

Fig. (1). Mean diameter (±S.E) of mature *M. rufiventris* teratocytes in *S. littoralis* larvae.
In each set, bars with the same letter are not differed by Duncan's multiple range test.

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