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The effects of host age and superparasitism by the parasitoid, *Microplitis rufiventris* on the cellular and humoral immune response of *Spodoptera littoralis* larvae

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Abstract

To study the dynamics of stage-dependent immune responses in *Spodoptera littoralis* (Boisd.) larvae (Lepidoptera: Noctuidae), single and superparasitism experiments were carried out using the parasitoid *Microplitis rufiventris* Kok. (Braconidae: Hymenoptera). Compared to younger (preferred) host larvae, the older (non-preferred) host larvae displayed a vigorous humoral response that often damaged and destroyed the single wasp egg or larva. Superparasitism and host age altered both the cellular and humoral immune responses. Younger host larvae showed a stronger encapsulation response compared to older host larvae. Moreover encapsulation rates in younger hosts (e.g., second instar) decreased with increasing numbers of parasitoid eggs deposited/larvae. In older larvae, the encapsulation rate was low in fourth, less in fifth and absent in sixth instar hosts. Conversely, the order and magnitude of the cellular immune response in *S. littoralis* hosts were highest in second instar larvae with the first instar larvae being a little lower. The immune response steadily decreased from the third through to the fifth instar and was least obvious in the sixth instar. In contrast, the general humoral immune response was most pronounced in sixth instar larvae and diminished towards younger stages. The results suggest that both cellular and humoral responses are stage-dependent. Wasp offspring in younger superparasitized host larvae fought for host supremacy with only one wasp surviving, while supernumerary wasp larvae generally survived in older superparasitized larvae, but were unable to complete development. Older instars seem to have a method for immobilizing/killing wasp larvae that is not operating in the younger instars.

Keywords: *Spodoptera littoralis*; *Microplitis rufiventris*; Superparasitism; Insect immunity

1. Introduction

Many species of parasitoid Hymenoptera develop inside the body of another insect species, where the developing parasitoid consumes and eventually kills the host. Superparasitism exists as a possible reproductive strategy and occurs, where a host contains more parasitoid eggs than are able to successfully complete development. If multiple eggs are present the emerging parasitoid larvae must compete for possession of the host (Van Alphen and Visser, 1990).

A number of mechanisms have been proposed that affect the fight for supremacy inside the host larva. These include both physiological suppression and physical combat (MacKauer, 1990). The physiological suppression refers to changes within the hemolymph of the parasitized host organism that exert some effect on subsequent parasitism, inhibiting younger larvae by oxygen or nutrient depletion (Roberts et al., 2004). Physical combat involves strong mandibulated first instar wasp larvae that are highly mobile, assaulting older less mobile larvae with comparatively soft mandibles (Dijkerman and Koenders, 1988).

Encapsulation is a multicellular immune reaction mounted by insect hosts towards endoparasitoids (Lavine and Strand, 2002). It is a multi-step process involving rec-

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ognition by insect immune cells, opsonization of the foreign object and the formation of multi-layered hemocyte capsule that engulfs the foreign object (Ratcliffe, 1993). The process may require special phenoloxidase-rich haemocytes (Sugumaran and Kanost, 1993).

The earlier host instars are often less effective in encapsulating foreign objects than later instars (Strand and Pech, 1995; Thoetkiattikul et al., 2005). Despite the fact that we know much about the basic mechanisms of immune defence, there is a great deal still to investigate (Khafagi and Hegazi, 2001; Cotter and Wilson, 2002; Dani et al., 2003; Kanost et al., 2004; Schmid-Hemple, 2005). One approach is to determine how insect parasitoids deal with the defense systems of their hosts.

Microplitis rufiventris Kokujev is a sexually reproducing endoparasitoid of some noctuid pests including the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Hammad et al., 1965). In the field, this parasitoid prefers to attack earlier instars of *S. littoralis* when they still live in clusters near the place of egg deposition, though third instars are preferred. Fourth instars disperse from these clusters and so are less suitable and less easily accessed than earlier instars (Hegazi, 1977). No literature exists to suggest that *M. rufiventris* females normally attack fifth or sixth instar hosts in the field. The parasitoid ovipositions a single egg per host and the larvae develop through three instars feeding on the host hemolymph before emerging from the host to pupate (Hegazi and Führer, 1985). *M. rufiventris* larvae are killers (i.e., siblicide) such that when more eggs are deposited into a host, elimination of supernumerary larvae takes place (Hegazi and Khafagi, 2005). The study represents an artificial protocol using *M. rufiventris* wasp as a model for investigation the defenses of *S. littoralis* larvae. The present study was undertaken to determine: (1) how the immunocompetence of the host changes with host age, and (2) whether the number of eggs laid by *M. rufiventris* wasps influences the degree of hemocytic defense reactions by the host. We predict that older hosts would be more immunocompetent than younger hosts, which could explain the preference of *M. rufiventris* for 1–3rd instars. Furthermore, we predict that the encapsulation response will be more robust with larger numbers of eggs due to the stimulation of the immune system by numerous combating larvae.

2. Materials and methods

2.1. Insects

Cultures of the cotton leafworm, *S. littoralis* and the parasitoid *M. rufiventris* were obtained from a laboratory colony established at Alexandria University. The colony of *S. littoralis* and *M. rufiventris* originated from field crops, including cotton at Alexandria, Egypt. Field-collected individuals were added to the colonies twice a year to maintain genetic diversity. Larval *S. littoralis* were reared on artificial diet (Hegazi et al., 1977) at 27 ± 1 °C, 60–65% RH and a 14:10 photoperiod. The *M. rufiventris* colony was maintained using third instar *S. littoralis* as hosts according to methods described by Hegazi and El-Minshawy (1979).

Mating in *M. rufiventris* wasps occurs as soon as both sexes are present, thus male and female wasps held together in glass vials (25 × 100 mm) for 24 h were presumed mated. Groups of presumed mated females (hereafter referred to as mated females) were identified and held together with accompanying males throughout the test period. The wasps were daily provided with fine droplets of honey and distilled water (1:1) to ensure maximum reproductive success.

2.2. Experimental protocol

To determine the effect of host instar on the ability to encapsulate multiple *M. rufiventris* larvae, the six host stages: late first, early second, third (preferred hosts), fourth, fifth and sixth instars (non-preferred hosts) of *S. littoralis* larvae were used. These hosts are henceforth referred to as L_1, L_2, L_3, L_4, L_5 and L_6. *S. littoralis* larvae of a given instar were determined by color and weight for L_1 and the presence of newly molted exuviae and a lightly colored head capsule that darkens to light brown within 3 h after the molting for L_2–L_6. Larvae of a given instar differed from each other by a range of 0.0–3 h from their ecdysis to the next instar. Surprisingly, the wasp females readily parasitized the newly molted sixth instar (0- to 3-h old). However, the females were reluctant to attack 24-h old sixth instar larvae (Hegazi and Khafagi, 2005). Female wasps were willing to attack hosts that had been newly parasitized by themselves or by conspecifics. Wasps were also willing to attack large hosts, despite the fact that larger larvae were more likely to fight back during oviposition. Rejection of parasitized *S. littoralis* hosts starts to occur about 24 h after the initial attack and the discriminatory ability of the wasp female increases with the age of developing parasite (Hegazi et al., 1990). Thus, it is easy to study the impact of variable ranges of superparasitization by *M. rufiventris* on host defense in preferred and non-preferred host larvae.

Spodoptera littoralis larvae of a selected instar were divided into three groups (each 16–20 larvae/replicate) for exposure to parasitoids. Oviposition was observed individually by mated parasitoid females (1-d old) in 15 × 60 mm Petri dishes (5–7 females/dish) and only 1, 3 or 5 ovipositions were allowed per host larva of the 1st, 2nd and 3rd groups of larvae, respectively. In superparasitized hosts, the time interval between ovipositions ranged from few seconds to less than 5 min. If oviposition was not observed within 2 min, the females were replaced. Each experiment was replicated at least 5 times for each level of parasitization. In each test, larvae that accidentally received an undesired number of ovipositional thrusts were discarded. Parasitized larvae were then placed individually in Petri dishes (1.5 × 3.5 cm) and provided daily with fresh larval diet ad libitum.