Available online at www.sciencedirect.com
ScienceDirect
Journal of Insect Physiology 52 (2006) 1137–1142
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Physiological suppression of the larval parasitoid *Glyptapanteles pallipes* by the polyembryonic parasitoid *Copidosoma floridanum*

Daisuke Uka, Tsuyoshi Hiraoka, Kikuo Iwabuchi*

Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Received 6 April 2006; received in revised form 1 August 2006; accepted 1 August 2006

Abstract

Precocious larvae, clonally produced together with reproductive siblings in the polyembryonic parasitoid *Copidosoma floridanum*, are known to physically attack competitors in multiparasitized hosts. In this study, we show that physiological suppression by *C. floridanum*, as well as precocious larval activity, causes death of the larval parasitoid *Glyptapanteles pallipes*. Approximately 70% of the hosts multiparasitized by *C. floridanum* and *G. pallipes* produced *C. floridanum* offspring, irrespective of the interval of multiparasitism. *G. pallipes* eggs or larvae died even in multiparasitized hosts that did not contain precocious larvae of *C. floridanum*. An injection of *C. floridanum*-parasitized or multiparasitized-host hemolymph into *G. pallipes* singly-parasitized hosts paralyzed almost all *G. pallipes* larvae within 70 h. In vitro analysis showed that the hemolymph factor toxic to *G. pallipes* eggs and larvae was present in *C. floridanum*-parasitized hosts through their larval stages. Heating or proteinase treatment reduced its toxicity, suggesting that the factor is a protein.

Keywords: *Copidosoma floridanum*; Polyembryo; *Glyptapantelles pallipes*; Physiological suppression; Interspecific competition

1. Introduction

Multiparasitism occurs when a single host is parasitized by more than one parasitoid species. Since each host supports the complete development of only one species, interspecific conflict arises among the parasitoids. Several mechanisms, including physical attack and physiological suppression have been reported to provide competitive advantages to parasitoid wasps that are involved in multiparasitism (Fisher, 1971; Vinson and Iwantsch, 1980; Mackauer, 1990). The elimination of competitors by physical attack has been observed mainly in solitary parasitoids (Vinson, 1972; Chow and Mackauer, 1984, 1986; McBrien and Mackauer, 1990), and is rare in gregarious species. Physiological suppression, which occurs in both solitary and gregarious species, involves multiple mechanisms, including the elimination of competitors by toxic factors, anoxia induction and nutrient removal (Muesebeck, 1918; Spencer, 1926; Fisher, 1963; Strand and Vinson, 1984; Hagver, 1988; Vinson and Hegazi, 1998). Although there are many such reports on multiparasitism, the mechanism of physiological suppression remains obscure in most cases.

Some polyembryonic Encyrtid parasitoids produce two larval morphs (Cruz, 1981). One is a reproductive larva, which grows as a polyembryo until the host develops to the final instar, when the embryo pupates and eventually develops into the adult. The other is a precocious larva, which is present through the larval period of the host, does not molt and dies without pupation. The number of precocious larvae increases as the host grows (Grbic et al., 1992). During interspecific competition between encyrtid parasitoids and solitary parasitoids, precocious larvae act as a soldier caste and physically attack the competitor (Cruz, 1981, 1986; Harvey et al., 2000; Giron et al., 2004).

Copidosoma floridanum (Hymenoptera: Encyrtidae) is a polyembryonic egg-larval parasitoid of the plusiine Lepidoptera. A single egg of *C. floridanum* develops into more than 1000 progeny (Strand, 1989; Utsunomiya and Iwabuchi, 2002). It was reported that *C. floridanum* precocious larvae function as soldiers (Grbic et al., 1992; Harvey et al., 2000; Giron et al,. 2004).

*Corresponding author. Tel./fax: +81 42 3675693.
E-mail address: kikkuo@cc.tuat.ac.jp (K. Iwabuchi).

0022-1910/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2006.08.002

In our preliminary study, we showed that in some cases of multiparasitism between *C. floridanum* and the gregarious endoparasitoid *Glyptapanteles pallipes* (Hymenoptera: Braconidae), the latter parasitoid died even in hosts that did not contain precocious larvae of *C. floridanum*. *G. pallipes* can parasitize their larval host *Acanthoplusia agnata* from the first to the fourth instars. Since reproductive larvae of *C. floridanum* never appear before the host's final instar, when multiparasitism between *C. floridanum* and *G. pallipes* occurs, only polyembryos and precocious larvae can confront their competitors. In the present study, we examined whether the interval between multiparasitism affects the competitive outcome and determined the factors that kill *G. pallipes* eggs and larvae.

2. Materials and methods

2.1. Insect

The host, *A. agnata* larvae, the encyrtid parasitoid *C. floridanum* and braconid parasitoid *G. pallipes* were maintained as described by Utsunomiya and Iwabuchi (2002). *A. agnata* eggs (24 h post-oviposition) were used for *C. floridanum* parasitism. One-day-old third instars (L3D1) were used for *G. pallipes*. Multiparasitism was accomplished as follows. First, 24-h old eggs of *A. agnata* were parasitized by *C. floridanum*. After hatching, the parasitized hosts were maintained on an artificial diet until L3D1, at which time they were presented to *G. pallipes*. Parasitism was carefully observed to ensure that all wasps oviposited only once. Since sex determination in hymenopteran parasitoids occurs by haplodiploidy, unmated females of *C. floridanum* produce male-only broods. We examined multiparasitism between *C. floridanum* male broods and *G. pallipes*.

To determine whether the interval of multiparasitism affects the competitive outcome, hosts were multiparasitized at L1D2, L2D1, L3D1 and L4D1. The host L1D2 is the earliest stage that can be used for *G. pallipes* parasitism. The parasitized hosts were held until parasitoid emergence, host pupation or host death. Fifty larvae were used for each experiment.

2.2. G. pallipes eggs in the hosts that lacked precocious larvae of C. floridanum

Our preliminary experiment showed that when using 24-h- and 72-h-old *A. agnata* eggs as hosts, both *C. floridanum* male and female broods produced precocious larvae from the first instar of the host. However, the production of precocious larvae was reduced in the hosts parasitized by 72-h-old host eggs. The number of precocious larvae was approximately 0.7, 0.6, 11.4, 27.0 and 110 for L2, L3, L4, L5 and L6, respectively when 72-h-old host eggs were used, while that for L2, L3, L4, L5 and L6 when using 24-h-old host eggs was 1.5, 3.0, 15.4, 32.6 and 116.5, respectively. So, for this experiment only, 72-h-old host eggs were used for *C. floridanum*. After hatching, the parasitized hosts were maintained until L1D2, at which time they were presented to *G. pallipes*. Two days after multiparasitism, the hosts were dissected in Carlson's solution (Carlson, 1946), and the number of precocious larvae was counted under a phase-contrast microscope. Multiparasitized hosts were classified according to the presence or absence of *C. floridanum* precocious larvae, and the mortality of *G. pallipes* eggs was calculated within each host. The mortality status of *G. pallipes* eggs was judged based on necrosis, shrinkage or comparison of the developmental stage with typical *G. pallipes* development.

2.3. Characterization of the toxic factor(s) in the hemolymph

Seven days after multiparasitism, host larvae reached their final instar (L6). Larvae were surface-sterilized with 70% ethanol solution, and hemolymph was collected from a pierced proleg of each larva into a centrifuge tube on ice. To prevent melanization of the hemolymph, phenylthiourea was added to a final concentration of 0.01%. Hemocytes were removed by centrifugation at 1000*g* for 20 min at 4 °C. The supernatant was stored at −40 °C until use. Hemolymph was also collected from hosts that were unparasitized and hosts that were parasitized either *C. floridanum* or *G. pallipes*. For one experiment, hemolymph was also collected from *C. floridanum* parasitized as fourth and fifth instar host larvae.

We injected 6 μl of the collected hemolymph (unparasitized, *G. pallipes* parasitized, multiparasitized or *C. floridanum* parasitized) or Carlson's solution into the proleg of a new host containing 3-day-old *G. pallipes* eggs. After injection, the new hosts were dissected in Carlson's solution every 24 h for 96 h. Four days after oviposition by *G. pallipes*, i.e. 24 h after the injection of hemolymph, almost all *G. pallipes* eggs had hatched into the first instar.

The in vitro experiment was performed as follows. L3D1 host larvae were parasitized by *G. pallipes*, as above. Seven days after parasitism, host larvae were surface-sterilized in 70% ethanol solution and dissected in MGM450 medium (Mitsuhashi and Inoue, 1988). *G. pallipes* larvae collected from the host were washed four times in the medium, and each larva was transferred into a 20 μl drop of MGM450 medium in a 35 mm plastic Petri dish. The Petri dishes were maintained at 25 °C under a 16L:8D photoperiod for 7 days. *G. pallipes* larvae were observed daily, and dead larvae were counted.

Each hemolymph sample, collected from the hosts that were either unparasitized, *C. floridanum* parasitized or multiparasitized, was added to the MGM450 medium to make a final concentration of 10%, and sterilized by 0.22 μm filtration. The effect of hemolymph collected from L4 and L5 host larvae was checked using the same procedure.

Hemolymph collected from *C. floridanum*-parasitized hosts was dipped in hot water at 60, 63, 65 or 70 °C for