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Olfactory conditioning in *Goniozus nephantidis* (Musebeck) a parasitoid of coconut black headed caterpillar, *Opisina arenosella* Walker

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ABSTRACT: Egg maturation and behavioral response to imaginal conditioning was studied on bethylid, *Goniozus nephantidis,* a parasite of coconut black-headed caterpillar, *Opisina arenosella.* Age and feeding had influence on *G. nephantidis* reared on *O. arenosella.* Eclosed females of 0–24 h had no mature eggs. Maximum numbers of mature eggs in the abdomen were observed 10 days after eclosion. Parasitoids that emerged from *Corcyra cephalonica* when offered a choice to parasitize *C. cephalonica* and *O. arenosella* showed preference to *C. cephalonica,* the host on which it was reared (Unconditioned response). Though unconditioned, they had 32 per cent response to *O. arenosella* and this is termed innate response. But when they were conditioned to odour of *O. arenosella* and then provided a choice, they preferred to parasitize *O. arenosella* as compared to *C. cephalonica.* Hence, it is clear that attraction to odours in imago that develops after eclosion is partly induced and partly constitutive.

KEYWORDS: Olfactory conditioning, *Goniozus nephantidis, Opisina arenosella*

Goniozus nephantidis (Musebeck) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid of larvae of the coconut black headed caterpillar, *Opisina arenosella* Walker (Oecophoridae: Lepidoptera). The larva of *O. arenosella* is a major pest on coconut. They make galleries of silk and frass on the undersurface of the coconut leaves on which they feed. Adult *G. nephantidis* females sting and permanently paralyze *O. arenosella* larvae and then lay eggs within 1–3 days (Hardy *et al.*, 1992). The interval between paralysis and oviposition is 'host guarding period' and the period when the female stays with the brood until pupation is 'brood guarding period' (Sundaramurthy and Santhanakrishnan, 1978; Hardy and Blackburn, 1991). Females of *G. nephantidis* have very few reproductive opportunities due to the long time spent in host and brood guarding (Cock and Perera, 1987; Hardy *et al.*, 1999). *G. nephantidis* females engage in direct contest for possession of host (Peterson and Hardy, 1996). Ownership status

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is linked to difference in egg load between the contestant females. Egg loads of *G. nephantidis* when exposed to rice moth, *Corcyra cephalonica* were influenced by age, body size and ownership status (Stokkebo and Hardy, 2000). Currently, *G. nephantidis* is mass multiplied on *C. cephalonica* (Ramadevi *et al.*, 1981) and wax moth, *Galleria melanollela* (Venkatesan *et al.*, 2003). The laboratory multiplied parasitoids were then released in the field to target *O. arenosella* for which they had no previous experience with the larvae in the recent past.

The insect species, which breed on two or more hosts, tend to become split into biological races. The biological characters of such races are germinally fixed and in many cases, the group of individuals may become restricted to certain host species not by germinal change but by a kind of conditioning. As a result, the adult female is attracted for oviposition to a particular species on which she has fed as larvae and this is called the 'host selection principle' by Walsh (Thorpe, 1939).

Understanding the physiology and behavior of the parasitoid is critical to achieve a successful bio suppression of the pest. Although Stokkebo and Hardy (2000) had studied the egg development in *G. nephantidis* when reared on *C. cephalonica*, an attempt was made to study the development of egg load in *G. nephantidis* when reared on *O. arenosella* and the parasitoid's behavioral response to imaginal conditioning with the host that it had to target when released in the field.

The study was conducted at Central Plantation Crops Research Institute, Kasaragod, India. *G. nephantidis* culture maintained on *C. cephalonica* (Lepidoptera: Pyralidae) and *O. arenosella* was used for the study. The parasitoids were cultured by placing the adult female wasp and larvae of *C. cephalonica* and *O. arenosella* in a vial of 2.5 × 10 cm plugged with cotton. Drops of 50 % honey (in water) placed on the wax coated paper served as food. They were starved when the effect of feeding on egg load was to be investigated. All the cultures were maintained at 25 °C and 65–70% RH with a constant dim light illumination.

G. nephantidis on emergence were collected in a vial. The male and female parasitoids were kept together for 12 hours with food (50% honey). The mated females (n = 30) were dissected on 2, 4, 6, 8 and 10 days after eclosion. The number of mature eggs in their abdomen was counted. The dissection was made with the micro forceps (INOX 5 – A. Dumont & Fils, Switzerland). In order to assess the effect of feeding on egg development in adult females (n = 30) after mating they were kept in the vial under starvation and dissected after the periods mentioned above.

In order to assess if the egg maturation improved with the presence of suitable host, forty 24 hours old female wasps that emerged from *C. cephalonica* larvae after mating were collected and placed individually in separate vials (length 5 cm internal diameter 1 cm) containing the hosts (*C. cephalonica* and *O. arenosella*, both n = 20) for a day. On the following day, when the hosts were paralyzed and the eggs not laid, the parasitoids were removed and kept separately in individual tubes. The parasitoids were dissected at varied intervals (2, 4, 8 and 10 days after eclosion) to observe the number of mature unlaid eggs.

In order to assess the learning in *G. nephantidis,* twenty adult females (5 days after eclosion) reared on *C. cephalonica* larvae were placed on a conditioning apparatus developed in our Entomology laboratory. The apparatus consisted of two glass tubes (Borosil, length 10 cm, internal diameter 2 cm) fitted tightly together by a polycarbonate tube (length 3 cm, internal diameter 2.2 cm). The contents in the glass tubes were separated by a nylon mesh (1 mm = 40 mesh) with a gap in the middle between the two meshes. Two adjustable screw holes were provided to permit the flow of air. The parasitoids were kept in the tube on one side, and *O. arenosella* along with damaged leaflets and frass were placed in the opposite side. The adult wasps were conditioned for 72 hours. They were provided with 50% honey as food during the conditioning period. Response of *G. nephantidis* conditioned to the odour of *O. arenosella* was assessed by exposing the parasitoid to either host by choice method in a 35 mm sterile Petri dish. Observations were made three hours after the release of the parasitoid and the host (*C. cephalonica* and *O. arenosella*) in petridish. Two sets of experiments were carried out. In one set, the unconditioned *G. nephantidis* (emerged from *C. cephalonica* but not exposed to *O. arenosella*) were used and in the other, the conditioned parasitoids (emerged from *C. cephalonica* but exposed to odour of *O. arenosella*) were used. The conditioned and unconditioned parasitoids were assayed for their learning ability by exposing them to both the hosts kept in 35 mm petridishes (Tarsons India ltd). Care was taken to use clean larvae of the same size. Each experiment had 10 pairs of larvae with five replications. In order to assess if two sets of tables (conditioned and unconditioned) were significant the chi square tests were performed.

Age and feeding treatment of female *G. nephantidis* had influence on the egg load. The eclosed female of 0–24 hours had no mature eggs. The parasitoids started to harbour mature eggs from 24 hours after eclosion. The egg load increased from day 2 to 10 days after eclosion and thereafter declined with the increase in age. Maximum egg load was observed 10 days after eclosion (Fig. 1).

G. nephantidis is synovigenic i.e. adults eclose without full complement of mature eggs and oogenesis occurs during adult stage. Synovigeny is reported from other *Goniozus* sp. (Luft, 1993). Females that were fed with honey had higher egg load as compared to unfed females (Fig. 1). Stokkebo and Hardy (2000) reported that female *G. nephantidis* reared on *C. cephalonica* when fed, had higher egg load than unfed females. The decline in the egg load among the older *G. nephantidis* females is possibly due to resorption of eggs in order to reallocate nutrients from reproduction to survival. Partially resorbed eggs in older females were seen in *G. hanoiensis* (Gordh *et al.*, 1993).

Weight of the female adults had influence on number of mature eggs in the abdomen. Females weighing 1.2 mg harbor 13 mature eggs as compared to 7 matured eggs in females that were 0.6 mg in weight. Positive correlations between body size and egg load have been observed in *Goniozus ligneri* (O'Neill and Skinners, 1990). The reproductive success of an individual female is strongly influenced by its size. The egg load in *G. nephantidis* plays a vital role as females with higher egg load had a better chance for the possession of host as compared to its intruder (Stokkebo and Hardy, 2000).

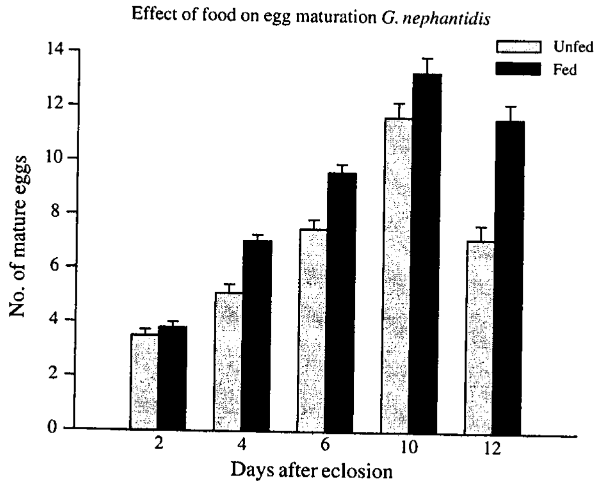

FIGURE 1. Effect of feeding on egg maturation in *G. nephantidis*.

Females *G. nephantidis* when presented with *O. arenosella* had higher egg load as compared to ones that had been presented with *C. cephalonica*. When permitted to lay eggs, maximum eggs (12 Nos) were laid on *O. arenosella*. The development period was also host dependent. The eggs laid on *O. arenosella* had a short period for development. The larval period when fed on *O. arenosella* was 3 days as compared to 5 days in *C. cephalonica*. Cues from *O. arenosella* causes *G. nephantidis* to decide on larger clutch size. Clutch size is known to vary with host size in the number of gregarious parasitoids (Godfray, 1987). Ramachandra Rao and Cherian (1928) and Antony and Kurian (1960) reported that more females were produced per brood when reared on *O. arenosella* than on *C. cephalonica*. Apart from the host suitability for egg development, higher male mortality of *G. nephantidis* was reported when they were reared on *C. cephalonica*. The reduction in male population led to high proportion of virginity among the females that had an undesirable effect on augmentation of natural enemies (Kapadia and Mittal, 1993).

Higher egg loads in *G. nephantidis* increased their ability to lay more quickly optimum number of eggs on the host (Peterson and Hardy, 1996) and this egg load (Physiological state) affects the parasitoid behaviour (Stokkebo and Hardy, 2000). Hardy *et al.* (1992) reported that the female bethylids when presented with the host, laid more eggs and lived longer as against the parasitoids which had no exposure to host. Higher male mortality of parasitoids when developing on *C. cephalonica* than on *O. arenosella* would lead to usually high proportion of virginity (Kapadia and Mittal, 1993).

The *G. nephantidis* adult females reared on *C. cephalonica* when given a choice to parasitize between *C. cephalonica* and *O. arenosella*, were strongly attracted to the host, *C. cephalonica* (68 per cent) on which it was reared as compared to *O. arenosella* (32 per cent) for which it had no previous exposure.

The parasitoids that emerged from *C. cephalonica* were conditioned by exposing to the odour of *O. arenosella* without being allowed any physical contact between them by placing them in a conditioning apparatus. The parasitoids conditioned in this manner for three days when offered a choice for parasitism, preferred *O. arenosella* (64%) as against the host (36%) on which they were reared. In the case of unconditioned parasitoids, the 32 per cent parasitisation on *O. arenosella* is an innate response of the parasitoid while it was 68% on *C. cephalonica*. Navarajan Paul *et al.* (1979) reported that *G. nephantidis* when continuously reared on *C. cephalonica* did not develop host adaptability and it was able to parasitize the *O. arenosella* larvae readily when available.

Since chi square is 10.25 ($P < 0.01$) (d.f. 49), the difference between the two sets (conditioned and unconditioned) is significant this justifies the imaginal conditioning in *G. nephantidis*. Conditioned parasitoids do not show any diminution in their ability to parasitize *C. cephalonica*, but there is a significant reduction in the proportion that parasitize *C. cephalonica* when they were exposed to both *O. arenosella* and *C. cephalonica*. The innate response (32 per cent) in the unconditioned parasitoids was increased by 64 per cent due to conditioning effect. This indicates that attraction to odours in the imago that develops after eclosion (conditioning) is partly induced and partly constitutive. The induced response is specific to the conditioning odour and this proves that the *G. nephantidis* has the ability to learn. Adult *Nemaeritis* that emerged from the natural host *Ephestia* were confined in an apparatus through which air was pumped from source containing living *Meliphora* larvae. Olfactory assay of *Nemeretis* showed that it had positive response to *Meliphora* larvae to which it had no previous experience (Thorpe, 1939).

Improvement of the physiological state and the behaviour can enhance the *G. nephantidis* efficacy in managing the *O. arenosella* in the field. Though *C. cephalonica* is an ideal host for mass multiplication of the parasitoid in the laboratory, continuous laboratory rearing of *G. nephantidis* causes inherited response to host from which it had emerged but their preference can be altered by mere exposure to the odour cues of *O. arenosella* without any physical contact. This would help the parasitoids to search for the host and parasitize it in the field with ease. Though the conditioned *G. nephantidis* shows the ability to learn, the duration of conditioning required for effective learning and the decay rate of the memory in *G. nephantidis* needs to be studied.

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