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Oviposition behavior of an ectopupal parasitoid *Nesolynx thymus* (Hymenoptera: Eulophidae): A biocontrol agent of the Uzi fly *Exorista bombycis* (Diptera: Tachinidae)

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Abstract
Nesolynx thymus Girault (Hymenoptera: Eulophidae) is an endo-pupal, gregarious parasitoid of the uzifly, *Exorista bombycis*, a major endo-larval parasitoid of mulberry silkworm *Bombyx mori* L. (Lepidoptera: Saturnidae). The estimated loss due to E. bombycis is to the tune of 40-45%. Owing to possession of desirable attributes of a biocontrol agent *viz.*, short life cycle, amenability to mass production under laboratory conditions, high parasitisation potential, efficient host searching ability, good adoptability to survive under field conditions, ability to synchronize its life cycle with that of host, *N. thymus* has been recommended as a biocontrol agent of *E. bombycis* apart from including in IPM programme. Some of the biological aspects and mass production of the parasitoids have been studied so far. No reports are available on the oviposition behaviour of the parasitoid. The present study was conducted to study the oviposition behaviour of *N. thymus*. When parasitoid was allowed to parasitize the host pupa, it exhibited a series of distinct behavioural responses that culminated in oviposition. These responses included host recognition, drumming, tapping, drilling and oviposition.

Keywords: Host recognition, drumming, tapping, drilling, grooming

Introduction

There is an increasing awareness among the insect pest control specialists that parasitoids, predators, and pathogens have the potential to keep the insect pest populations in check. They live alongside the pests and their abundance and activity fluctuate in relation to those of pests. Use of these organisms as biological control agents of insect pests is realized to be environment-friendly, cost-effective, self-perpetuating, and long lasting. Among them, parasitoids offer the highest potential as the success rate obtained by them is of the order of 82% in comparison to 17% and 1% by predators and pathogens, respectively [1]. Among parasitoids, the parasitic Hymenoptera constitutes a large number of species (65,000). However, the number of species that have been used in the biological control programmes the worldwide is negligible (765 species) [2]. In view of prevalence of a vast number of parasitic Hymenoptera, there is an ample opportunity for identifying many more species of parasitic Hymenoptera that have high inherent fecundity, prolonged survival, and excellent host searching ability. Obviously, such an effort precedes in-depth investigations on the biology and behaviour and, more so, the developmental dynamics of parasitoids. Knowledge on these aspects is highly essential for mass culturing of parasitoids in the laboratory with due emphasis on the most important parameters such as female quality (fitness) and parasitoid sex ratio.

Keeping in view the desirable attributes of bio-agents, a few of them, especially the parasitoids and predators, have already been exploited in mulberry sericulture and have shown great promise in the pest suppression [3-7]. They include the parasitoids such as *N. thymus* against *E. bombycis*, *Tetrastichus howardii* (Olliff) against the leaf roller, *Diaphania pulverulentalis* (Hampson), *Trichogramma chilonis* Ishii against the Bihar hairy caterpillar, *Spilosoma* (= *Diacrasia*) *obliqua* Walker and *D. pulverulentalis* and predators like the Australian ladybird beetle *Cryptolaemus montrouzieri* Mulsant against the mulberry mealy bug, *Meconellicocccus hirsutus* (Green), and *Brumus suturalis* against the white fly, *Dialeuropora decempuncta* Quaintance & Baker. Simultaneously, their inclusion as biocontrol component of Integrated Pest Management (IPM) packages has led to elevated pest suppression with a concomitant appreciable increase in yields of mulberry and silkworm.

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Nesolynx thymus (Girault) (Hymenoptera: Eulophidae) is one among 20 hymenopteran parasitoids [8] of the uzi fly, *E. bombycis,* which is a serious endo-larval parasitoid (pest) of *B. mori,* causing 10 – 20% damage to silkworm cocoon crops in the premier silk producing states of India *viz*., Karnataka, Andhra Pradesh, and Tamil Nadu. Owing to possession of majority of the desirable attributes of a biocontrol agent like, short life cycle, amenability to mass production under laboratory conditions, high parasitisation potential, efficient host searching ability, good adoptability to survive under field conditions, ability to synchronize its life cycle with that of host (*E. bombycis*), etc. [3], *N. thymus* has been recommended as a biocontrol agent of *E. bombycis*, apart from exploiting it as one of the components of an IPM package developed by the Central Sericultural Research and Training Institute (CSR & TI), Mysore, against uzi fly.

Though *N. thymus* has been exploited as a biocontrol agent of *E. bombycis*, studies pertaining to only a few of its biological aspects and mass production were undertaken previously [9, 3]. However, basic investigations such as developmental dynamics of the parasitoid, super-parasitism, multiple-parasitism, Oviposition behavior, etc. are scanty. In the backdrop of the above, the present investigation was taken up to study the oviposition behaviour of *N. thymus.*

2. Material and Methods
The experimental insect *Nesolynx thymus* was cultured on the pupae of *Exorista bombycis* in the laboratory of Department of Sericulture, University of Mysore at 25±3 °C temperature and &0% RH. The *N. thymus* females were fed on 50% aqueous honey solution. To study the oviposition behavior, 3 day old, 3 – 5 pupae of *E. bombycis* were offered to 2 day old *Nesolynx thymus* female in a petridish (6 cm diameter). Oviposition behavior was observed under stereo microscope (x 10). The experiment consisted 10 replications.

3. Results
3.1 Oviposition behaviour of *N. thymus*
When a gravid female of *N. thymus* was allowed to parasitize host pupae (*E. bombycis)*, it exhibited a series of distinct behavioural responses that culminated in oviposition. These responses included host recognition, drumming, tapping, drilling and oviposition that are mostly performed in that sequence. Rarely, an additional event namely host feeding too was revealed by the parasitoid by way of feeding the host body fluid (haemolymph) that oozed from the feeding tube created in the hole drilled by the parasitoid on the host, especially by those females that were not fed carbohydrate diet prior to allowing for oviposition. Sometime, an incomplete behavioural response was observed leading to host Rejection (Fig. 1). The detailed description of each of the behavioural responses is furnished below.

3.2 Host recognition
When a parasitoid (*N. thymus*) female was provided host pupae, it walked in the oviposition container for host recognition. Sometimes, even after the host recognition, the parasitoid moves away from the host only to reestablish the contact with the host. At other times, the parasitoid failed to establish host contact only to abandon (reject) the host.

3.3 Drumming and Tapping
On recognizing the host, the parasitoid female mounted on the host, inspected it by repeated contact of the host surface with antennae that moved upward and downward rigorously. This was followed by tapping of the area, which was subjected to drumming in the preceding process, by the abdominal tip that bent vertically downward and was observed to be beset with sensory hairs. For this, the parasitoid moved forward to exactly coincide its abdominal tip with the drummed area. Tapping activity was found to be more frequent than drumming activity. Occasionally these activities were found overlapping.

3.4 Drilling and Oviposition
The parasitoid after completing the process of drumming withdrew its ovipositor (which originated from the antero-ventral region of the abdomen and held back in position parallel to the entire length of the abdomen on the ventro-median line) perpendicular to the abdomen and positioned it against the identified (tapped) area. At this time, the female parasitoid took a firm grip on the host surface and became motionless in order to give sufficient reinforcement to the ovipositor (to hold it straight) for drilling. The process of drilling started slowly by making clockwise and anticlockwise movement of the ovipositor to facilitate drilling. During this period, the rear part of the abdomen remained bent straight downward, the wings held parallel to abdomen dorsally, and anterior half antennae were bent downward to give an appearance of inverted V. As the drilling continued, occasionally, the parasitoid worked its ovipositor back and forth and finally succeeded in drilling a hole through the puparial shell. Once drilling was made, the entire ovipositor was sunk into the host to initiate oviposition on the pupa. At this time too, the parasitoid remained immobile, its abdomen appeared triangular (as a result of drawing it downward by the ovipositor) ventrally in the middle and the rear part remained straight. The antennae and wings continued to be in the same posture they were at drilling. After oviposition, the parasitoid withdrew the ovipositor from the host and resorted to grooming that involved cleaning of antennae and dorsal and lateral thoracic regions with anterior pair of legs, while cleaning of wings with hind pair of legs. At this time, the parasitoid became hyperactive, raised the wings often, and left the host, which was followed by walking briskly. Further, it continued grooming several times before initiating the oviposition behaviour for the next clutch.
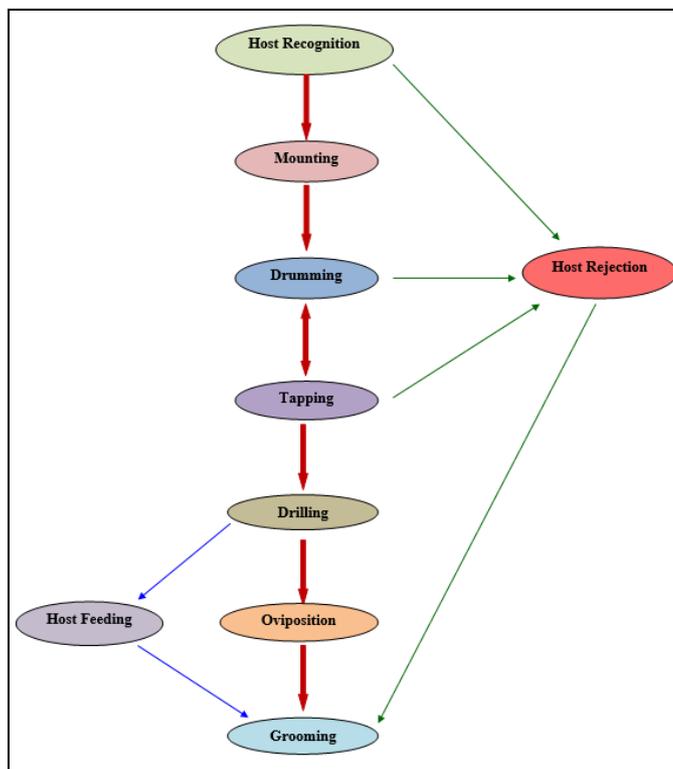

Host recognition　　　　　　**Drumming**

Tapping　　　　　　　　**Drilling**
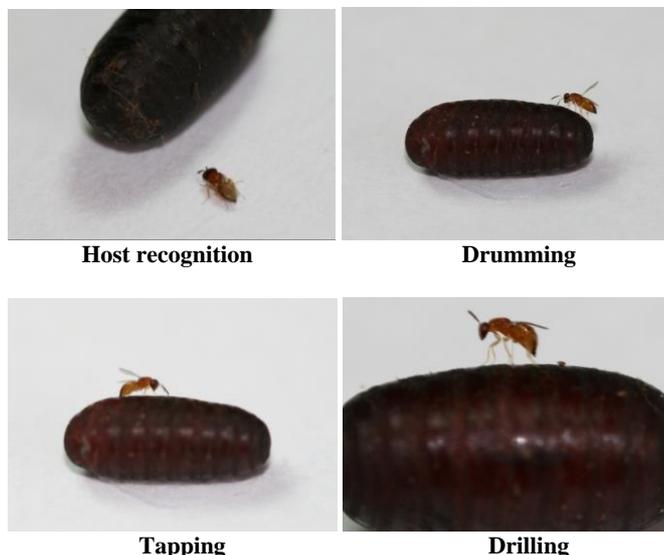
Fig 1: Ethogram of ovipositional behaviour of *Nesolynx thymus* on *Exorista bombycis* pupa

4. Discussion

Limited studies are available with regard to oviposition behaviour of parasitoids. Oviposition behaviour in parasitic Hymenoptera involves a series of pre-oviposition, oviposition and post-oviposition events that are performed in certain sequences. Sadoyama [10] using *Eumicrosoma blissae* (Maki) (an egg parasitoid) and Rivers [11] employing *Nasonia vitripennis* and *Muscidifurax zaraptor* (ecto-pupal parasitoids) presented an explicit account on the oviposition behaviour of the respective parasitoids. In *E. blissae*, it included a sequential events *viz*., host encounter, drumming, extension of ovipositor sheath, probing, drilling, insertion of ovipositor, withdrawal of ovipositor sheath, marking, and retraction of ovipositor sheath. Oviposition was found to ensue when this type of cycle was exhibited. The parasitoid was also observed to display another set of sequential oviposition behavioural phases like, host encounter, drumming, extension of ovipositor sheath, probing, drilling, and withdrawal of ovipositor sheath, resulting in failure of oviposition. With reference to *Nasonia vitripennis*, the sequential behavioural events associated with oviposition comprised touching the puparium, mounting the puparium, drumming, tapping, drilling, lubricant release, puparium penetration, probing, venom injection and / or oviposition / host feeding followed by grooming. In the second sequence of oviposition behavioural events, in addition to those from touching the puparium to puparium penetration, the latter phase was followed by probing and host rejection or by probing, venom injection, host feeding, probing and host rejection or by probing, venom injection, oviposition, host feeding, probing and host rejection. Some intermediary events also were observed to be carried out by the parasitoid such as leaving puparium and stopping drilling which were associated with the activities between touching the puparium and drilling. *M. zaraptor* was found to exhibit relatively simpler oviposition behaviour than *N. vitripennis*. It consisted of touching the

puparium, mounting the puparium, drumming, tapping, drilling, puparium penetration, probing, venom injection, and oviposition followed by grooming. In the second cycle, in addition to maintaining all the events from touching the puparium to puparium penetration, the parasitoid exhibited probing and host rejection events. The intermediary events observed for both the parasitoids were similar.

Wiedemann *et al.* [12] observed five distinct ovipositional events in *G. gallardoi viz*., drumming, ovipositior insertion, egg marking, walking, and resting, apart from recording the time allocated for a few events like drumming and ovipositor insertion. In *A. fuscipennis*, Manzano *et al.* [13] reported the occurrence of walking, host encountering, drumming, and probing as the distinct oviposition behavioural events. In *Psyllaephagus pistaciae*, Mehrnejad and Copland [14] reported oviposition behaviour, which included host searching, host encounter, antennation, ovipositor tapping and probing, drilling, and ovipositing in that sequence. A deviation in the behaviour from drilling activity, chiefly for host feeding, culminated in resting via continued drilling, host feeding, repeated drilling and host feeding, and preening has also been reported. A second deviation from the main ovipositional sequence has been observed at the level of antennation leading to host rejection. The third deviation occurred at ovipositor tapping and probing level leading to host rejection. Based on the ovipositional behaviour in *Xanthopimpla stemmator*, Fischer *et al.* [15] reported that the parasitoid displayed the following events: walking, antennation (drumming), probing, insertion of ovipositor, and pausing (grooming) in that sequence. However, the authors did not record some of the important behavioural events like the oviposition and the withdrawal of the ovipositor.

In *Oomyzus sokolwski*, Nakamura and Noda [16] reported only three distinct oviposition behavioural events such as 1) drumming, 2) ovipositor penetration and oviposition and 3) host feeding. Further, they mentioned that ovipositor penetration and oviposition were not behaviourally distinguishable. However, withdrawal of ovipositor after oviposition has not been considered as a distinct event as reported by many other investigators. The authors also attempted to record the time allocation by *O. sokolowski* for each of the events indicated by them against host age (stadium). Irrespective of the host age, the parasitoid allocated significantly longer duration for ovipositor insertion and oviposition and host feeding compared to drumming.

In our efforts to study the ovipositional behaviour of *N. thymus*, the important oviposition behavioural events comprised host recognition, drumming, tapping, drilling, insertion of ovipositor, oviposition, and withdrawal of ovipositor that were mostly performed in that sequence. In addition, the parasitoid also exhibited a few minor events like walking that preceded host recognition, mounting the puparium, and grooming that generally succeeded oviposition. Occasionally, an incomplete behavioural response would be revealed where one or more of the events preceeding oviposition was missing. Such an incomplete display of oviposition behavioural events would be leading to failure of oviposition / host rejection. The ovipositional events recorded for *N. thymus*, in the present investigation included a series of distinct behavioural responses *viz*., host recognition, drumming, tapping, drilling and oviposition which is in agreement with those found in *N. vitripennis* and *M. zaraptor*. Further, *N. thymus* was found to differ from these parasitoids in not displaying the event like probing.

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