



SHELTER-BUILDING BEHAVIOR OF *Erionota torus* Evans (Lepidoptera: Hesperiiidae) CATERPILLARS FEEDING ON BANANA LEAVES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Shelter construction by different larval instars of *Erionota torus* Evans (Lepidoptera: Hesperiiidae) is most crucial for ensuring proper nutrition, improving microclimatic conditions needed for the normal larval development, and for avoiding the attack of predators and parasitoids. The present study focus on detailed description of the life cycle and shelter building mechanism of different larval instars of banana skipper *Erionota torus*, a rapidly invading foliophagus insect pest in the banana plantations of Malabar region of Kerala. With detailed observations carried out both in the field and on the laboratory populations of *Erionota* larvae, we illustrate the types of shelters constructed during the larval ontogeny and also all the activities performed during the process of shelter-building. Further the paper evaluates the factors as well as threats that influence the shelter making behavior. The location of shelters on the host plant and various aspects of larval feeding behavior are also described. First and second instar larvae of *E. torus* build and inhabit in their own shelters by successively abandoning the shelters and constructing new ones. The fourth and fifth instars larvae use the shelter made by the third instars. However the leaf area used to construct the shelter of successive instars increases as the larva increases in size. The larvae of *E. torus* produce shelters in two distinct styles which change as the larvae grow. These changes in the style of shelter building are likely to be correlated with larval size, needs, biotic and abiotic factors. The average area of leaf used to construct the shelters of first, second, third, fourth and fifth instar larvae were $3.62 \pm 0.78 \text{ cm}^2$, $17.3 \pm 0.61 \text{ cm}^2$, $102 \pm 0.81 \text{ cm}^2$, $162.8 \pm 1.34 \text{ cm}^2$, $275 \pm 1.41 \text{ cm}^2$ respectively. Average area of leaf consumed by first, second, third, fourth and fifth instars were $0.67 \pm 0.07 \text{ cm}^2$, $5.78 \pm 0.15 \text{ cm}^2$, $27.93 \pm 1.37 \text{ cm}^2$, $104 \pm 0.51 \text{ cm}^2$ and $114.2 \pm 0.081 \text{ cm}^2$ respectively. Average length of the leaf shelter of first, second, third, fourth and fifth instars were $1 \pm 0.22 \text{ cm}$, $4.64 \pm 0.61 \text{ cm}$, $13 \pm 0.75 \text{ cm}$, $15 \pm 1 \text{ cm}$, $15 \pm 3 \text{ cm}$ respectively.

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1. INTRODUCTION

Based on their mode of feeding insect defoliators fall into three main groups: leaf miners, skeletonizers, and whole leaf feeders. Leaf miners feed upon the succulent interior leaf tissues as they tunnel between the upper and lower epidermis of the leaf. Skeletonizers eat all the leaf except the vascular portions, thus skeletonizing the leaf. The majority of the leaf-feeding insects, however, are whole-leaf feeders, which eat the leaf tissues completely. Switching from one mode of feeding to other is also frequently seen in some insects. Thus some defoliators are miners during a part of their developmental period and skeletonizers at a later stage. Still others are skeletonizers during their early stages and act as whole-leaf feeders during their later stages. The immature stages of some whole-leaf feeders, particularly butterflies and moths, may spin silk, which is used to twist or distort the leaf. The insects feed, rest, develop, and pupate within this protective web of silk and leaves. Defoliating insects possess a pair of powerful tooth-like mandibles or true jaws which are adapted for cutting and crushing the leaf during the course of their feeding. They are referred to as having mandibulate or chewing mouthparts [1]. One of the most complex and exciting areas of insect biology is that of nest architecture and construction behavior. The four major groups of insect nest builders belong to the orders Isoptera, Hymenoptera, Lepidoptera and Trichoptera [2]. The shelters thus constructed assume several forms and are used by arthropod builders in at least one part of their life. When abandoned, these constructed shelters might persist on the plants and may be later used as shelter by other groups of animals, including herbivores and predators. Shelters vary among species and also across ontogenetic stages within a species [3]. Many larval instars construct simple structures externally on host plants by covering, tying, folding, cut-and-folding, or rolling plant leaves with silk [4]. Shelter construction may be initiated at the leaf margin or in the center of the leaf and may involve a small portion of a leaflet, an entire leaf, or multiple leaves ensuring nutrition, amelioration of microclimatic conditions, as well as protection from natural enemies [3]. These shelter-building herbivores can be considered as "microhabitat manipulators" [5]. Severe infestation of hesperiid butterfly, banana leaf roller *Erionota torus* Evans was reported for the first time on banana in the cultivating areas of Malabar region of Kerala, a part of southern Western Ghats [6]. The various larval instars of *E. torus* feed on banana leaves. Leaves are rolled by the larvae and tied with silk thread, as they construct their nests. After

reviewing about 190 studies Cock [7] stated that a careful observation and behavioral studies of how the larvae construct their shelters on different food plants may provide additional diagnostic characters of the species. For making the shelter the *Erionota* larvae make two adjacent incisions from the edge of the leaf towards the midrib at a distance of 8–10 cm from each other, and then proceed to roll this piece of the leaf up like a cigar, the larva living in the cylindrical hollow within this roll [8,7]. The larva is snow-white, covered with a very short wax-like pubescence, and with a black head. It is easily discovered, as it gnaws out of the gigantic banana leaves, by two parallel-cuts a longitudinal piece which it rolls up like a cigar, in the centre of which it lives [8,7]. Detailed descriptions of the leaf shelter building behavior are not available [7]. In the available literature however there is only very little information about the mechanism of shelter construction, current severity of damage and the economic losses caused by the invasion of *E. torus* in the state of Kerala, India. Thus the aim of the study was to characterize the shelter-building behavior and its role in the life cycle of *Erionota torus*. The ontogenetic requirements connected with caterpillars' choice of leaves for building shelters were also investigated. This study aims to describe the feeding behavior of different larval instars which will be helpful to design different management strategies to control this pest. Since the shelter construction behavior follows a regular pattern and is specific to the group the pattern of shelter construction is much helpful in the phylogenetic analysis and the correct species identification.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted during the period between June 2015 and May 2016 in a banana cultivating plot at Payyannur, Kannur district of Kerala, India (FAL; 15°55' S and 47°55' W). Environmental factors like temperature, relative humidity and rainfall data during the study period were also recorded.

2.2 Shelter Making Behavior

In each banana cultivating plot, all the host plants were counted and inspected for the presence of eggs, larvae and pupae of *E. torus*. 40 plants of banana which had larvae of different instars in shelters were tagged in the field. Records on the behavior of these specimens on the host plant in the field were made in every two days. Simultaneously all the stages were also taken to the Research Laboratory of Department of Zoology

Government college Kasaragod, where they were subsequently reared in individual plastic containers at temperature of 28 ± 2 and RH 80 ± 5 . The larvae were fed on fresh leaves of the banana. Every two days, the rearing containers were inspected cleaned and fresh leaves were added. The hatching of the egg, changes of the larval instars, feeding of different larval instars, the construction of new shelters, pupation, and adult emergence were also continuously monitored and recorded.

The length of different larval instars, diameter of head capsules, shape of the nest, area of leaf used to construct the nest, average area of leaf consumed and average length of shelter constructed were also studied. Measurements are presented as minimum and maximum values. Immature stages and adult voucher specimens were deposited in the Department of Zoology, Government college Kasaragod.

3. RESULTS AND DISCUSSION

The adult female laid eggs individually or in patches on the lower surface of the leaf. Each cluster consists of 26 ± 2 eggs ($n=30$). Isolated patches of 2 to five eggs were also noted. Soon after hatching, the first instar larvae consume the part of the chorion of the egg and then move towards the margin of the leaves in random at a speed of about 2-4 cm/minute. It then starts to construct its first shelter by making single incision in the margin of the leaf followed by further cutting towards the upper side in a straight, slightly inclined or L shaped manner. The first shelter constructed by the newly hatched larvae of *E. torus* is a single-cut shelter (Shelter Type 6 of Group II, following Greeney [9]), in which the larvae make a cut at of 0.2–.5cm in length at an angle of 30 to 45 degree from the margin towards the middle region. It constructs three types of shelters, namely conical, C shaped and cylindrical shelters, by simple folding of leaf (Fig. 1). The larvae spin silk so that the cut portion will be securely attached to the leaf surface in the form of a fold. These shelters are usually folded onto the lower surface of the leaves, but in a few cases, they are folded onto the upper surface. The first instar larvae usually build a shelter on the same leaf where the eggs are released. Usually they construct leaf nest either on single margin or on both margins of the leaf. Some larvae however may accidentally reach other leaves also, where they construct new nest. The shelters are very closely placed to each other on the leaf margin or may be placed at random if isolated eggs are hatched (Fig. 2). A maximum of 28 first instar larval shelters were noticed in one margin of single leaf blade with a space of 2-5 cm between the successive shelters (Fig. 3). The larvae eat the portion of the leaf on the incising region, feeding is followed

by spinning the threads by moving the mouth in to and fro fashion. The force required to pull the leaf surfaces close together is achieved by stretching the silk strands during the course of spinning. The process of spinning and tightening continues till the shelter is sealed except at its distal end. At the end of one day the fold like shelter turns into small roll of about 2 cm length. First instar larva undergoes moulting from this shelter and soon after moulting the second instar larvae leaves the shelter and constructs new nest in the same leaf or neighboring leaves of the same plant. The length of the leaf roll of the second instar is 4.64 ± 0.61 and is cylindrical in nature. Conical shelters were also noted in the field (Fig. 4). The shape of third, fourth and fifth instar shelters are cylindrical but varies in its diameter, length and number of layers (Figs. 5, 6 and 7). Larvae feed on the inner margin of the leaf roll, which is closed at the inner end and open at the distal end, where fecal debris may accumulate. The formation of large cylindrical roll during later stages is aided by the tightening of the silk strands in the innermost layer. The diameter of the opening of the leaf roll is lesser than the larval body. The upper end of the nest is usually sealed in a semicircular manner by pasting the margin of leaf roll using silk threads with the leaf surface. Another interesting observation about the fifth instar larva is that they frequently touch its mouth region to the newly formed black fecal body just as in coprophagy (Fig. 8). During the later stage of fifth instar, the larva turns upside down and makes one or two horizontal thick silk strands of 1.5-2 cm length by weaving silk by frequent to and fro movement of the mouth. On these horizontal strands the tail end of the last instar attaches with a white waxy material, during pupation (Fig. 9). From the second instar onwards the larva becomes covered with an increasingly thick layer of white waxy powder, so that the body appears white except the black head region which is partially covered by white waxy powder. The amount of waxy material varies amongst larvae and is also dependent on the variety of banana leaves used. Many erect pale hairy setae found around the dorso lateral side of the larval body is also covered with fine white waxy powder. This powder some times forms coagulated mesh works. Yellowing of leaf roll is noticed in the inner layer of prepupal leaf roll. Pupal leaf roll then completely turns yellow and gradually becomes dry and necrotic (Fig. 10). These dry leaf roll either remains in the leaf itself or may fall off as dry rolls. The adult insect then emerges through the slit at the cephalic end of the pupa. Shifting from old shelter and construction of new shelter is common in first, second and is rare in later age larvae. This shifting is due to nature, size and thickness of leaves, presence of water and predators like ants, spiders, slugs etc. Plant to plant migration of early instar larvae were also often

noticed. Leaf fragments and small spherical fecal debris can be seen on the open end of the leaf roll. Occasionally these debris close the mouth of the shelter (Fig. 11). Larval stage, shape of the nest ,average area of leaf used to construct the shelter

in cm^2 , average area of leaf consumed in cm^2 and average length of leaf shelter in cm are given in the Table 1. Duration of larval instar in days, the length of the larva in cm, the head capsule diameter in cm of different larval instars are given in Table 2.



Fig. 1. C- shaped and cylindrical shelters, by simple folding of leaf



Fig. 2. Arrangement of leaf shelters of early larval stages



Fig. 3. Arrangement of leaf shelters on one margin of single leaf blade



Fig. 4. Conical type leaf shelters of *E. torus*



Fig. 5. Leaf shelters of third instar larva *E. torus*



Fig. 6. Leaf shelters of fourth instar larva *E. torus*



Fig. 7. Leaf shelters of fifth instar larva *E. torus*

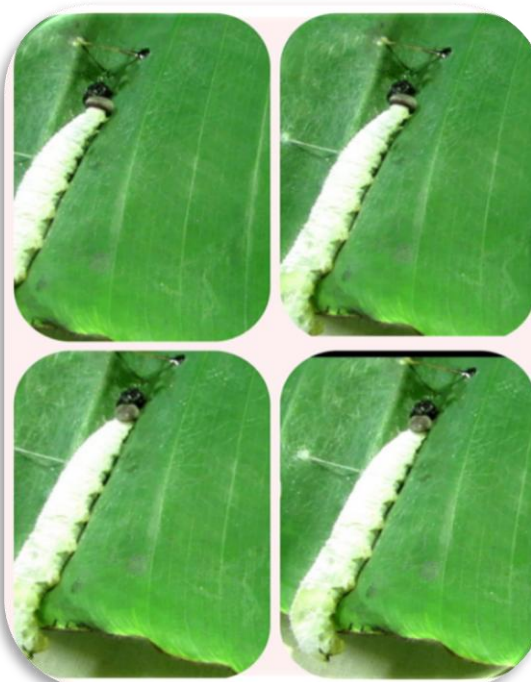


Fig. 8. Feeding of faecal pellets by fifth instar larva of *E. torus*



Fig. 9. Horizontal strands the tail end of the last instar



Fig. 10. Pupa of *E. torus* inside the leaf shelter

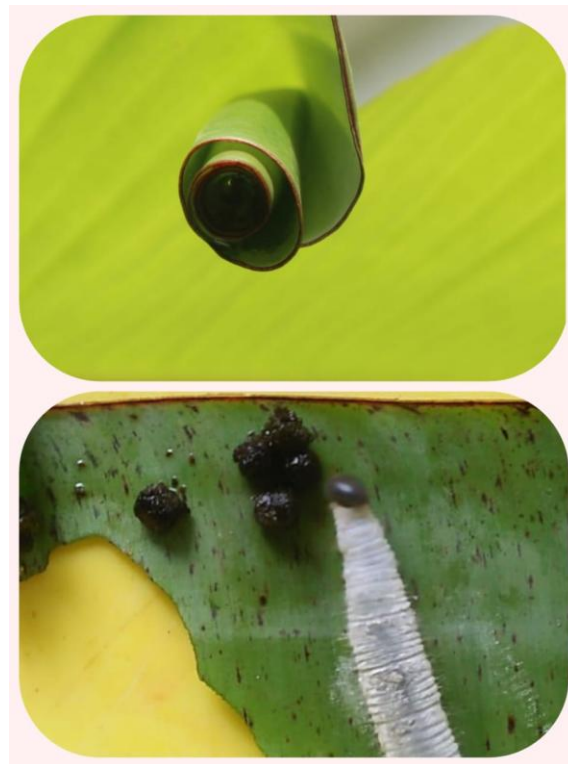


Fig. 11. Open end of the leaf roll cut open

Table 1. Table showing larval stage, shape of the nest, average area of leaf used to construct the shelter in cm^2 , average area of leaf consumed in cm^2 and average length of leaf shelter in cm

| Larval stage | Shape of the nest | Average area of leaf used to construct the shelter(in cm^2) | Average area of leaf consumed (cm^2) | Average Length of leaf shelter in cm |
|--------------|---|--|---|--------------------------------------|
| Instar -1 | Cone shaped, C-shaped or cylindrical. | 3.62 ± 0.78 | 0.67 ± 0.07 | 2.1 ± 0.22 |
| Instar -2 | Cylindrical or conical open at one end. | 17.3 ± 0.61 | 5.78 ± 0.15 | 4.64 ± 0.61 |
| Instar -3 | Cylindrical open at one end. | 102 ± 0.81 | 27.93 ± 1.37 | 13 ± 0.75 |
| Instar -4 | Cylindrical open at one end. | 162.8 ± 1.34 | 104 ± 0.51 | 15 ± 1 |
| Instar -5 | Cylindrical open at one end. | 275 ± 1.41 | 114.2 ± 0.081 | 15 ± 3 |

Table 2. Table showing the stages, duration in days, length in cm, head capsule diameter in cm

| Stages | Duration in days | Length in cm | Head capsule diameter in cm |
|------------|------------------|--------------|-----------------------------|
| I Instar | 3.5±0.5 | 0.35 ±0.05 | 0.09± 0.008 |
| II Instar | 3.5 ±0.5 | 1.5±0.45 | 0.14 ±0.008 |
| III Instar | 4.5±0.5 | 2.42 ±0.09 | 0.18±0.014 |
| IV Instar | 4 | 3.3±0.18 | 0.285±0.011 |
| V Instar | 6±1 | 5.3±0.2 | 0.50 ± 0.02 |

Insect larvae as a protective mechanism make a variety of structural retreats by rolling, folding, tying, or webbing the leaves to protect its most vulnerable stage, as the larval stage is more prone to the attack of the parasitoids and predators. The family Hesperidae comprises over 3,000 species of skipper butterflies and likely contains the greatest diversity of larval shelters within any lepidopteran family [10]. The variety of shelter types constructed by the lepidopterans mostly remain unstudied. While natural history studies on immature skipper stages devote much attention to physical characteristics of the larvae, they often fail to describe accurately the larval shelters and thus shelter construction may prove to be the most useful information in resolving the hesperiid phylogenies [11]. Skipper butterflies construct shelters throughout larval development and show a large amount of inter-specific and ontogenetic variation, which may be phylogenetically informative within this group [11,12]. While Neotropical skipper larvae are known for building shelters, Greeney [13], the details of shelter architecture are available for only a few species [13]. Thus, like larval morphology, shelter architectural details remain unavailable for phylogenetic analyses for nearly all species of Hesperidae [13]. The banana skipper *Erionota torus* (Lepidoptera:Hesperidae) is a foliophagus banana pest recently reported from Malabar region of Kerala, India and is often confused with *E. thrax* in most of the publications. Thus shelter building and the behavior of the butterfly larvae may give additional diagnostic characters of this group. After reviewing about 191 literature about *Erionota* spp Cock [7] suggested that additional diagnostic characters may be discovered through the careful observation and behavioral studies of how larvae build shelters on various food plants. The larva of *E. torus* initially makes a cut in the banana leaf, and rolls the cut portion of the leaf, and as the larva feed and grows by remaining inside the roll it further extends the cut across the leaf and enlarges the roll. No evidence of eating can be seen outside the shelter, and when the tube is unrolled, the unrolled leaf does not refill the space it originally occupied [9]. As the larva remains in the shelter and feeds by eating the inner folds of the constructed shelter, the dangers of the larvae being attacked by parasitoid and predators are reduced considerably. As the caterpillar feeds by eating the

inner layers of the tube by avoiding the dangers inherent in emerging from its shelter. One effect of this is that the inside diameter of the tube becomes much greater than is usual in Hesperinae tube shelters [9]. The bottom end of the shelter is loosely sealed with silk, however it often retain the frass, thus hindering access by predators and other natural enemies. However the top of the shelter is almost closed with silk, but when the larva start to enlarge the roll it opens it and further extends the roll by enlarging the cut. Old strands of silk are cut and remain visible along the cut on the section not incorporated into the shelter [9].

Study from western Malaysia reveals that the silk strands used to form the shelter are attached to parts of the leaf that are subsequently incorporated into the shelter. Further observations are needed to clarify whether these are local or individual variation in behavior [7]. In the present study we found a horizontal thick fibre on which the tail of pupae were attached. From an early age, the larva is covered with an increasingly thick layer of white powder, so that the body appears white and the erect pale setae become partially covered with the powder, and the uniformly dark head is partially obscured with the white powder. Pupation takes place in the final leaf roll shelter which is closed at the distal end by a thin mesh, which is easily broken by the emerging adult [14,7]. However such type of mesh was not observed in the present study as this end is covered by white waxy powder and rarely with faecal debris. More over all the shelters except the shelters built by first instar form almost invariant in pattern, size, shape, and orientation, suggesting a more or less stereotypical process of shelter building and location. Larvae of the banana skipper, *Erionota torus* (Hesperidae), construct cylindrical or conical shelter that change predictably over larval ontogeny. Thus the knowledge about the nature of shelter building, its orientation location etc can help in easy identification of the pest and thereby implementing immediate measures for the successful management the pest. In *Erionota torus* the nest construction and their location plays a significant role in ensuring feeding, enhancement of microclimatic conditions and for the protection from natural enemies including parasitoids, predators and protection from different climatic factors.

4. CONCLUSION

The present study on the nest building behaviour of *Erionota torus* is the first time detailed report of nest building in this species. In *E. torus* the method of nest construction and their location plays most crucial role in ensuring the proper microclimatic conditions for the growing larva and pupa thereby ensuring the proper feeding and for the protection of its most vulnerable stages from the attack of natural enemies including parasitoids, predators and also from the different adverse climatic factors. The method of shelter building also help us in analysing the phylogenetics and the correct identification of the species. Understanding the life history, habitat variability, and nest making behavior also aid in identifying the potential regions of susceptibility and thus to design better pest management strategies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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