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"Life History And Larval Performance Of The Nymphalid Butterfly, Junonia Iphita Cramer From India"

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Abstract:
We describe, for the first time, the life history of the Chocolate pansy butterfly, Junonia iphita Cramer and larval performance in terms of food consumption and utilization, and the length of life cycle on its host plant Dipteracanthus prostratus (Poir.) Nees. Our study was conducted throughout 2009 in the Andhra University campus and the Zoo Park area, 5 km away from the campus, at Visakhapatnam (17° 42' N and 82° 18' E), South India. Junonia iphita completes its life cycle in 26.00 ± 1.87 days (eggs 3, larvae, 15-17, pupae 6-8 days). The values of nutritional indices across the instars were AD (Approximate Digestibility) 33.15-96.48%; ECD (Efficiency of Conversion of Digested food) 0.88-38.67%; ECI (Efficiency of Conversion of Ingested food) 0.85-19.14%, measured at the temperature of 28 ± 2 °C and RH of 80 ± 10% in the laboratory. These relatively high values, at least partially explain ecological success of J. iphita in the urban environment.

Key words: *captive rearing, immature stages, Dipteracanthus prostratus, food utilization indices*

1.Introduction

Among insects butterflies are a charismatic group and are regarded as model organisms in studies of evolution, behaviour, ecology and biogeography (Settele & Ku¨hn 2009). Due to worldwide pressures on natural biomes, butterflies have already been shown to be highly sensitive indicators of climate change (Sparks et al. 2005, 2007, Hambler et al. 2011), biotope fragmentation (Warren et al. 2001) and urbanization (Jana et al. 2006, Kadlec et al., 2008, Van Dyck et al. 2009). The biodiversity crisis currently seems to appear more critical among butterfly species than other species (Thomas 1991, Thomas et al. 2004). Butterflies being highly diversified in their habits require specific ecological conditions for their survival. Knowledge of butterfly habitat (Dennis et al. 2003, 2006a,b, Dennis 2010) and larval host plants is a prerequisite for any butterfly conservation program. Therefore, it is necessary to know the exact needs of the immature stages to make conservation successful (New et al. 1995).

It is often suggested that captive rearing/breeding and releasing of butterflies in the wild will help restock at-risk populations and serve as a means of conservation (Nicholls & Pullin 2000, Mathew 2001, Crone et al. 2007, Schultz et al. 2008). For example, the American Zoo and Aquarium Association recently launched the butterfly conservation initiative, which reflects the mandate of 53 Zoos and associated organizations to engage in local (North American) conservation efforts by supporting the recovery of 22 butterfly species, largely with captive propagation programs (BFCI 2010). Similarly, for 10 of 25 at-risk British butterfly species with a Species Action Plan, reintroduction, often implemented with captivatingly propagated stock, is a priority (Butterfly Conservation 2010). The basic protocol is to collect eggs from wild-mated female, rear larvae to adult butterflies in captive propagation facilities, and release adults/pupae back into wild populations (Crone et al. 2007). For the development of effective breeding/rearing programs and conservation management of butterflies, information on the life history and exact habitat requirements is essential. Further, immature stages of butterflies are of increasing importance as sources of systematic characters, and often give important clues as to the placement of species in major groups (DeVries et al. 1985, Igarashi & Fukuda 1997, Freitas et al. 2002). Such knowledge in most cases of Indian butterflies is seriously inadequate (Gay et al. 1992, Gunathilagaraj et al. 1998). In this sense the present study furnished the necessary information about immature stages, larval performance on its host plant Dipteracanthus prostratus (Poir.)

Nees., and the length of life cycle from egg to adult eclosion for the butterfly species, Junonia iphita Cramer. This species is found from Sri Lanka and India to China, and through the Malay archipelago to Bali and the Lesser Sunda Isles.

2.Material & Methods

The present study was carried out in Visakhapatnam during the calendar year 2009. Visakhapatnam (17^o 42' N latitude and 82 (18' E longitude) is located on the east coast of India in the State of Andhra Pradesh. It is a fast growing industrial city declared as the Greater Visakha Municipal Corporation. Consequently, the vacant places and the sub-urban areas with beautiful patches of vegetation are disappearing. Even the hill slopes are not spared; they are being occupied by the people, thus causing a threat to the vegetation on the hills also. We chose two sites for our study viz. Andhra University campus and the Zoo Park area, 5 km away from the campus. Both these sites can be regarded as "islands' within an urbanized landscape (Brown & Freitas 2002) of Visakhapatnam city, because a variety of herbs including grasses and sedges and shrubs put on luxuriant growth in the rainy season. The seasonal annuals that come up during the rainy season dry up and disappear with the onset of winter. Some of these may reappear when cyclonic rains provide enough moisture, and thrive through the summer. With the advent of summer the deciduous trees begin to shed their foliage and prepare to bloom. The whole area is subject to human disturbance giving rise to secondary growth of vegetation.

Both sites were regularly searched for the reproductive activity of the Chocolate Pansy butterfly, Junonia iphita. Once located detailed observations were made in order to observe the period of copulation and oviposition, following which fresh eggs were collected, without causing any damage, to study the life history and the duration of early stages. After oviposition, the leaf with eggs was collected in Petri dishes (15 cm × 2.5 cm depth) and brought to the laboratory. The piece of the leaf with the eggs was then placed in a smaller Petri dish (10 cm × 1.5 cm depth), the inside of which lined with moistened blotter to prevent the leaf from drying. Such Petri dishes were kept in a clean, roomy cage fitted with wire gauge. Since ants were never detected, no special protection device was tried to avoid predation of eggs. They were examined regularly at 6 h intervals for recording the time of hatching. Each of the freshly emerged larvae was transferred to a clean Petri dish inside of which lined with moistened blotter with the help of a camel hair brush. The larvae were supplied daily with a weighed quantity of tender leaf pieces of the host plant. The faeces and the leftover of the food was collected and weighed each day (24 h). The growing larvae were observed regularly to note the instar change and characters including length and weight measurements. As the larvae grew, they needed more space. Increased space was provided by transferring the growing larvae to bigger Petri dishes (15 cm × 2.5 cm depth). Larval performance in terms of food utilization indices were calculated as described by Waldbauer (1968) as:

- GR: Growth Rate

$$GR = \frac{\text{Weight gained by the instar}}{\text{Mean weight of instar} \times \text{Number of feeding days}}$$

- CI: Consumption Index

$$CI = \frac{\text{Weight of food ingested}}{\text{Mean weight of instar} \times \text{Number of feeding days}}$$

- AD: Approximate digestibility (also called Assimilation Efficiency)

$$AD = \frac{\text{Weight of food ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$$

- ECD: Efficiency of Conversion of Digested food also called Net Conversion Efficiency

$$ECD = \frac{\text{Weight gained by the instar}}{\text{Weight of food ingested} - \text{Weight of faeces}} \times 100$$

- ECI: Efficiency of Conversion of Ingested food also called 'Gross Conversion Efficiency'

$$ECI = \frac{\text{Weight gained by the instar}}{\text{Weight of food ingested}} \times 100$$

Fresh weight measurements were used for the purpose. Five replications were maintained for the study of all parameters. The preparation of full grown larvae to pupate and particulars of pupae including color, shape, size, weight and the time of adult eclosion were also recorded. Millimeter graph paper was used for taking measurements. The laboratory temperature was 28 ± 2^0 C and relative

humidity 80 ± 10% with normal indirect sunlight conditions that varied in duration between 12 h during November/ January and 14 h during June/July. In describing the details of adult characters, the butterflies that have emerged from the pupae in the laboratory, and those caught in the wild were used.

3.Results

3.1.Adult stage (Figure 1a):
Upperside of both wings is chocolate brown. Forewing with two small ocelli and hindwing with a row of minute ocelli. Apex of forewing and tornus of hindwing slightly produced. Underside thick brown with indistinct ocelli. Wingspan is between 55-80 mm. It flies both at ground level and at upper canopy level. It frequently settles on the ground and on the leaves for basking with their wings fully opened. It kept its wings full spread while foraging at flowers for nectar. In the study area, the nectar host plants included Catheranthus roseus (L.) G. Don, Duranta repens L., Lantana camara L., Antigonon letopus Hook & Arn., Anacardium occidentale L., and Santalum alba L.

3.2.Egg stage (Figure 1b):
Mating and oviposition took place during 0900-1300 h. The gravid female laid eggs singly on the underside of the young and mature leaves of Diptheracanthus prostratus (Poir.) Nees. About 6-10 eggs were laid at a time but on different leaves. For egg laying this butterfly mainly prefers shaded places. It also lays eggs on the leaves of plants that are growing intermingled with its host plant (indirect egg laying). The eggs were creamy, spherical with longitudinal ridges and shining. Measured 0.85 – 1.00 (0.91± 0.06) mm in height. They hatched in three days of incubation. Immediately after hatching, the larva ate its egg-shell. It passed through 6 distinct instars over a period of 15 – 17 (16.00 ± 1.00) days.

3.3.Laval stage (Figure 1c-h):
Instar I lasted for 2 – 4 days. On the first day of hatching, the instar measured 2.00 (2.00 ± 0.00) mm in length. It grew to 2.30 – 3.00 (2.70 ± 0.29) mm in length and 0.90 – 1.00 (0.94 ± 0.05) mm in width. Head black in color and measured 0.70 – 0.80 (0.78 ± 0.04) mm in diameter. The body was black in color and not clearly segmented. Body was fully covered with hairs. Instar II lasted for 2 – 3 days. The larva attained a length of 3.30 – 4.70 (3.92 ± 0.51) mm and a width of 1.00 (1.00 ± 0.00) mm. Head measured 1.00 (1.00 ± 0.00) mm in diameter and black. Color of the body turned into thick chocolate color. There was a white creamy streak along the mid-dorsal surface. Segmentation was clear. Hairs were more clearly distinct at this stage. Just behind the head (neck region) a region of white creamy band was found. Instar III lasted for 2 days. The larva attained a length of 5.30 – 6.50 (5.72 ± 0.47) mm and a width of 1.00 – 1.10 (1.06 ± 0.05) mm. Head measured 1.40 – 2.00 (1.16 ± 0.22) mm in diameter. Hairs black in color and arranged in 6 longitudinal rows along lengthwise and 12 horizontal rows across the body. Hairs were singly branched. On the ventral side of the body it was light chocolate in color. Instar IV also lasted for 2 days. The larva attained a length of 7.00 – 10.30 (8.92 ± 1.39) mm and a width of 1.30 – 1.60 (1.42 ± 0.16) mm. Head measured 2.00 – 3.00 (2.50 ± 0.41) mm in diameter. The color of the mid-dorsal streak turned into light chocolate in color. Larva was inactive. Other characters are same as previous instars. Instar V lasted for 2 – 3 days. The larva attained a length of 15.30 – 17.00 (16.40 ± 0.74) mm and a width of 2.60 – 2.90 (2.68 ± 0.13) mm. Head measured 4.30 – 5.00 (4.56 ± 0.26) mm in diameter. The mid-dorsal streak that found in the previous instars disappeared. Head was covered with minute hairs. Instar VI lasted for 3 – 4 days. The larva attained a length of 29.00 – 35.70 (31.64 ± 2.71) mm and a width of 4.60 – 5.50 (5.02 ± 0.33) mm. Head measured 8.00 – 8.50 (8.30 ± 0.27) mm in diameter. Head was thick brownish at tips and remaining part of the head was black in color. Body was black and hairs turned light grey colored. Larva stop feeding and body contracted before pupation.

3.4.Pupal stage (Figure 1i):
This stage lasted for 6 – 8 days. It was 17.00 – 19.00 (17.70 ± 0.84) mm in length and 6.00 – 6.70 (6.14 ± 0.31) mm in width at its broadest point. The pupa resembles color of the dry leaves. On the dorsal side there were tiny spiny projections. These were arranged in six rows. On the wing cases and near the head region it was chocolate colored. Pupa was rough to touch. Average pupal weight was 411.40 – 530.80 (451.10 ±46.99) mg.

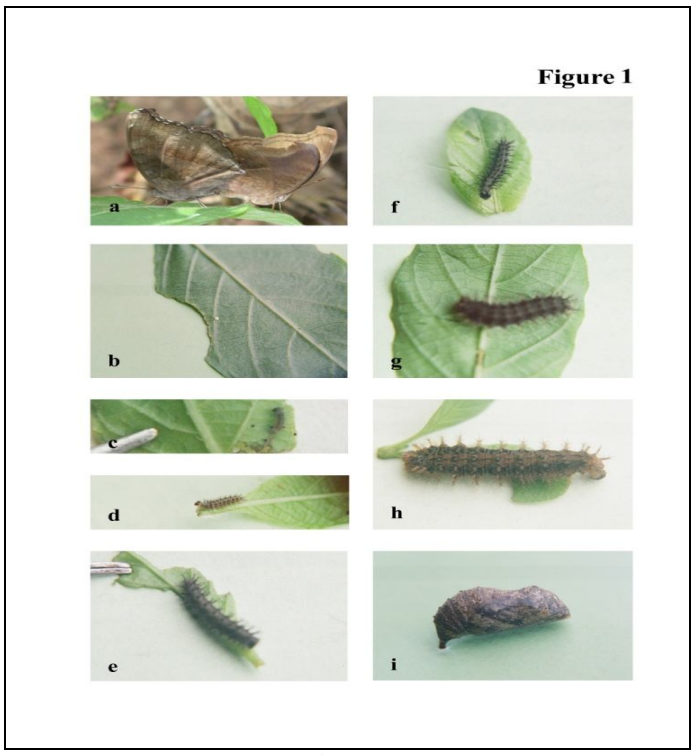

Figure 1

4.Duration Of Life Cycle

The total development time from egg to adult eclosion ranged between 24 – 28 (26.00 ± 1.87) days. [Egg: 3; Larva: 15 – 17; Pupa: 6 – 8 days).

5.Food Consumption, Growth And Utilization

The data on the amount of food consumed by each of the six instars and the corresponding data on weight gained by different instars are given in Table 1. Of the total amount of food consumed, the percentage shares of the successive instars were 0.66, 0.90, 2.13, 4.58, 19.44 and 72.29% and the proportions of weight gained in relation to total weight gained by the successive instars were 0.04, 0.18, 0.79, 6.83, 19.88 and 72.27%. Thus, there was over 91% of the total food consumption and 92% of total weight gained in the fifth and sixth instars together. There was a direct relationship between food consumption and growth across the five instars (Figure 2). The values of growth rate (GR) increased from first to fourth instar and then decreased to final instar. The values of consumption index (CI) decreased from first to last instar. The values of GR varied between 0.11 – 0.49 mg/day/mg and those of CI between 1.54 – 13.34 mg/day/mg. Table 1 also included the data on Approximate Digestibility (AD), Efficiency of Conversion of Digested food (ECD), and Efficiency of Conversion of Ingested food (ECI). The values of AD from instar to instar decreased from a high of 96.48% in first instar to a low of 33.15% in the last instar. The values of ECD showed irregular pattern from the first instar to the last instar and varied from 0.88 – 38.67%. The values of ECI increased from first to fourth instar and decreased to final instar and ranged between 0.85 – 19.14%.

Instar number	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR (mg/day/mg)	CI (mg/day/mg)	AD (%)	ECD (%)	ECI (%)
I	30.73 ± 07.12	1.08 ± 00.28	0.26 ± 00.04	0.11	13.34	96.48	0.88	0.85
II	41.52 ± 06.54	2.36 ± 00.39	1.10 ± 00.27	0.31	11.65	94.31	2.81	2.65

Instar number	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR (mg/day/mg)	CI (mg/day/mg)	AD (%)	ECD (%)	ECI (%)
III	98.56 ± 10.56	5.88 ± 01.23	4.66 ± 00.95	0.36	7.6	94.03	5.03	4.73
IV	211.48 ± 23.28	17.72 ± 02.53	40.48 ± 05.48	0.49	2.56	91.62	20.89	19.14
V	898.12 ± 56.22	132.54 ± 12.69	117.84 ± 10.19	0.29	2.27	85.24	15.39	13.12
VI	3340.12 ± 95.25	2232.74 ± 88.96	428.28 ± 22.14	0.2	1.54	33.15	38.67	12.82

Table 1: Food Consumption, Growth And Food Utilization Efficiencies Of Junonia Iphita Larva Fed With Dipteracanthus Prostratus Leaves

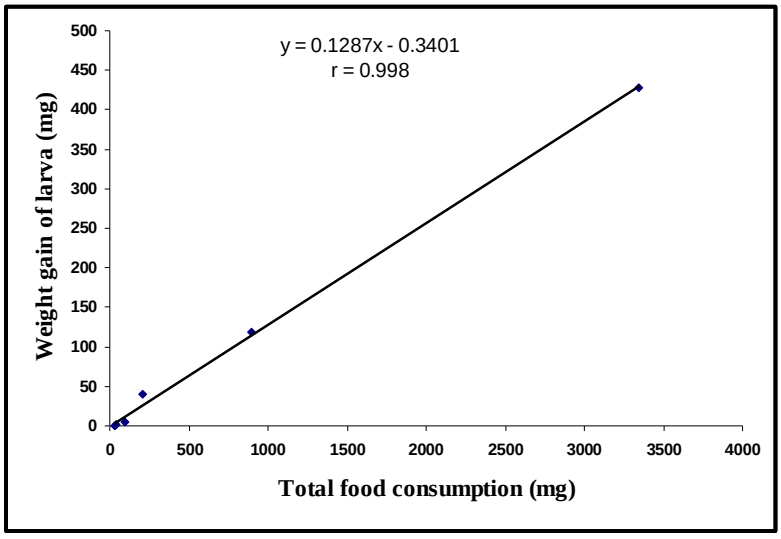

Figure 2: Relationship Between Food Consumption And Growth In Junonia Iphita On Dipteracanthus Prostrates.

6.Discussion

Floral nectar is main source of food energy in the adult stage of butterflies (Ezzeddine & Matter 2008). Junonia iphita visits flowers frequently and imbibes nectar and nectar intake may increase its longevity and egg production (Fischer & Fiedler 2001, Grill et al. 2013). This butterfly mostly visits the flowers of Catheranthus roseus, Duranta repens, Lantana camara, Antigonon letopus, Anacardium occidentale, and Santalum alba.

The total development time from egg laying to adult eclosion was determined as 26 ± 1.87 days at about 28 ± 2^0 C. This behavior is in line with the expectations of short life cycles in tropical butterflies (Owen 1971). Since temperature influences instar duration and the overall development time (Mathavan & Pandian 1975, Palanichamy et al. 1982, Pathak & Pizvi 2003, Braby 2003), the duration of life cycle may vary from our records depending on the prevailing temperatures. As no temperature extremities occur at Visakhapatnam, the duration of life cycle did not vary much over the overlapping seasons.

Over the entire period of its growth, a larva consumed on average over 4.62 g of leaf material, increasing consumption in the last two instars. This tendency of greater consumption by the last two instars has been reported in lepidopterous larva in general (Waldbauer 1968, Mathavan & Pandian 1975, Scriber & Slansky 1981, Palanichamy et al. 1982, Selvasundaram 1992, Gosh & Gonchaudhuri 1996, Lorenz et al. 2006), and it compensates the energy expenditure of non-feeding pupal stage (Pandian, 1973). The values of CI are near to the range (0.27 – 6.90) predicted for forb foliage chewers (Slansky & Scriber 1985). Food consumption rate depends on the conversion efficiency of ingested food to biomass (ECI), the rate increasing as the conversion efficiency decreases or vice versa (Slansky & Scriber 1985). In this sense, the high CI value (13.34) of instar I is probably due to low conversion efficiency and this character is reflected in the low values of ECI for instar I compared to other successive instars. Higher growth rates occur with penultimate than with final instars (Scriber & Feeny 1979). The GRs of penultimate and final instars of Junonia iphita are in line with the above decreasing trend.

The values of AD that were obtained in this study are on the higher side of the range 19 – 81% given for 60 species of lepidopteran larvae by Pandian and Marian (1986), 28.70 - 84.60% for Pericallia ricini (Ghosh & Gonchaudhuri 1996), and 53.91 – 96.85% (Gopala Swamy et al. 2010) & 59.70 – 85.80 (Naseri et al. 2010) for Helicoverpa armigera. The average AD percentage is over 82.47% and this high AD substantiate the statement of Slansky & Scriber (1985) that foliage chewers often attain high AD values. Such high AD values also are expected when a food item is rich in nitrogen (and also water) (Pandian & Marian 1986). As such the larvae feeding on such foliage are expected to assimilate food more efficiently which naturally resulted in high values of AD. In addition, the foliage might be having a minimum of recalcitrant, indigestible fibre and noxious secondary compounds to permit high AD values (Mattson 1980). Similar results were repeated with Pieris brassicae (Yadava et al. 1979), Ariadne merione merione (Atluri et al. 2009) and Bhupathi Rayalu et al. (2012). The declining trend in AD as the larvae grow older is related to the changes in the composition of food selected by the successive instars. Evans (1939) suggested that the first instar larvae eat from between small veins which later instars eat almost the whole leaf. That way the larvae ingest a larger proportion of indigestible crude fibre as they become aged which influences AD values to decrease along the successive instars (Waldbauer 1968, Bailey & Mukerji 1976, Slansky & Scriber 1985, Kogan 1986). The changes in feeding behaviour of the growing larvae might be correlatable with changes in mandibular morphology. First instar has been found to feed on the upper surface of the leaf and skeletonize it with fork-like mandibles whereas later instars ingest the entire leaf. The mandibles of final instar have a thin-ridged cutting edge and an inner roughened surface.

The values of ECD increase from early to last instars (Slansky & Scriber 1985). Such trend is observed with the ECDs of Junonia iphita, with the lowest value in instar I and the highest in instar VI. The ECDs obtained are low compared to the ADs and such low values are not unusual (Waldbauer 1968, Naseri et al. 2010). This is indicative of low efficiency of conversion of digested food to body tissues. This poor utilization of food is often attributed to deficiency in some essential nutrient in food (Bailey & Mukerji 1976) or a factor causing an increase in energy expenditure on metabolism (Muthukrishnan 1990). The pattern of ECI values followed closely the pattern of ECD. The values (0.85 – 19.14) obtained are comparable with the range of values expected for forb foliage chewers (1 – 78%) (Slansky & Scriber 1985). The values of ECD and ECI, particularly those of the last three instars, are also relatively high (20.89, 15.39, 38.67; 19.14, 13.12, 12.82), thus respectively indicating tissue growth efficiency and ecological growth efficiency, which enabled Junonia iphita to thrive successfully in the urban environment.

Thus, the present study provides information on the oviposition larval host, Diptheracanthus prostratus and larval performance in terms of food consumption, growth and utilization, and the length of life cycle from egg to adult eclosion of the Chocolate pansy butterfly, Junonia iphita. The present data may be profitably utilized in the successful conservation management of this butterfly species either in parks, Zoos and butterfly houses or in the field. Butterfly houses are popular exhibits in Zoos and have an immense educational (Veltman 2009) and conservational potential (Mathew 2001, Veltman 2009). The present study also indicted that captive rearing the larvae at about 28 ± 2^0C permits enough stock of adults for restocking the areas poor in populations of the Chocolate Pansy butterfly.

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