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Life history and larval performance of the Joker butterfly, *Byblia ilithyia* (Lep.: Nymphalidae)

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Abstract

We describe, for the first time, the life history of the Joker butterfly, *Byblia ilithyia* Drury and larval performance in terms of food consumption and utilization, and the length of life cycle on its host plant *Tragia plukenetii* A. R. Smith. Our study was conducted throughout 2007 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. *Byblia ilithyia* completes its life cycle in 19.20 ± 1.30 days (eggs 3, larvae, 9-12, pupa 5-6 days). The values of nutritional indices across the instars were AD (Approximate Digestibility) 55.77-94.98%; ECD (Efficiency of Conversion of Digested food) 1.93-25.26; ECI (Efficiency of Conversion of Ingested food) 1.83-14.09, 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 *B. ilithyia* in the urban environment.

Key words: life history, *Byblia ilithyia*, captive rearing, immature stages, *Tragia plukenetii*, food utilization indices

چکیده

دوره‌ی نشو و نمای پروانه‌ی *Byblia ilithyia* Drury، تأثیر نوع تغذیه روی ویژگی‌های لاروی و طول چرخه‌ی زندگی آن روی گیاه میزبان *Tragia plukenetii* A. R. Smith برای اولین بار شرح داده می‌شود. این مطالعه طی سال ۲۰۰۷ در پردیس دانشگاه Andhra و ناحیه‌ی Zoo Park واقع در ۵ کیلومتری پردیس، در شهر Visakhapatnam (۴۲' °۱۷ N، ۱۸' °۸۲ E) در جنوب هندوستان انجام شد. طول چرخه‌ی زندگی پروانه‌ی مذکور ۱/۳۰ ± ۱۹/۲۰ روز بود (تخم ۳، لارو ۱۲-۹، شفیره ۶-۵ روز). مقادیر شاخص‌های تغذیه‌ای شامل AD (قابلیت هضم تقریبی)، ECD (کارآیی تبدیل غذای هضم‌شده) و ECI (کارآیی تبدیل غذای خورده‌شده) برای سنین مختلف لاروی در شرایط آزمایشگاهی (دمای ۲ ± ۲۸ درجه‌ی سیلسیوس و رطوبت نسبی ۱۰ ± ۸۰ درصد) به‌ترتیب ۹۴/۹۸-۵۵/۷۷، ۲۵/۲۶-۱/۹۳ و ۱۴/۰۹-۱/۸۳ درصد محاسبه شد. این مقادیر به‌نسبت بالا، تا اندازه‌ای بیانگر موفقیت اکولوژیکی پروانه‌ی *B. ilithyia* در محیط شهری است.

واژگان کلیدی: دوره‌ی نشو و نما، *Byblia ilithyia*، پرورش در ظروف، مراحل نابالغ، *Tragia plukenetii*، شاخص‌های مصرف غذا

Introduction

Insects and plants are two of the most diverse groups and the interactions between them are considered to be largely responsible for the high abundance and diversification of both groups. In insect-plant interactions, the phytophagous insects eat plants and convert plant carbohydrates to animal protein. This interaction served as the dominant factor in plant evolution (Ehrlich & Raven, 1964). Among insects, butterflies provide economic and ecological benefits to the human society by virtue of their incontestable beauty and their ability to accomplish pollination, a key ecological process in natural sustainability throughout the world (Venkata Ramana *et al.*, 2003; Lomov *et al.*, 2006). Being dependant on vegetation both as adults and larvae, they involve themselves in complex feeding relationships with green plants. As adults, they require a succession of adequate nectar resources and as larvae, they are typically host specific (Venkata Ramana *et al.*, 2003; Lomov *et al.*, 2006). As such, the butterflies provide the rapid indication of habitat quality and are also sensitive indicators of climate change (Brown, 1991, 1996a, 1996b, 1997; Erhardt & Thomas, 1991; Kremen, 1992; New *et al.*, 1995; New, 1997; Lomov *et al*., 2006; Nelson, 2007). However, due to large scale loss, fragmentation and degradation of natural habitats, many species are in the verge of extinction (Schultz & Chang, 1998) and urgent measures are required for conserving them from extinction. "Butterfly Trade" is also implicated as one of the major factors for the decline of butterfly populations (Atluri *et al.*, 2002).

In urban areas plants and animals have frequently experienced local extinction due to extensive habitat destruction (Wilcove *et al*., 1998; McKinney, 2002; Miller & Hobbs, 2002). Remnant habitats associated with urbanized areas are becoming increasingly important 'islands' within a sea of urbanized landscape (Brown & Freitas, 2002). These can take the form of parks and gardens, roadside vegetation, recreation parks and nature reserves, conservation parks and national parks (Nelson & Nelson, 2001; Pryke & Samways 2003). Such urban habitat fragments are becoming particularly important for maintaining butterfly diversity (Brown & Freitas, 2002; Horner-Devine *et al*., 2003; Takami *et al*., 2004), and may become habitat refuges providing suitable host plant and nectar plant sources for native butterflies (Shapiro, 2002; Koh & Sodhi, 2004). The existing situation of conservation of butterflies is lamentable, but there is an urgent need to take up proper measures for the conservation of these small, fragile, and highly mobile organisms (Venkata Ramana *et al.*, 2003).

In the past few decades, butterfly populations in India have declined (Grewal, 1996), and 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 (Varshney, 1986; Herms *et al*., 1996; Nicholls & Pullin, 2000; Mathew, 2001; Crone *et al*., 2007; Schultz *et al*., 2008). Several zoos and other facilities currently engaged in captive rearing programs for protected butterfly species. 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 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; Freitas *et al.*, 2002). Haribal (1992) noted that such information is lacking for 70% of the Indian butterflies. In this sense the present study furnished the necessary information about immature stages, larval performance on its host plant, *Tragia plukenetii* A. R. Smith, and the length of life cycle from egg to adult eclosion for the Joker butterfly, *Byblia ilithyia* Drury. There are only two species of *Byblia* Hubner in the world, of which one (*ilithyia* Drury) occur in India. This species is distributed in India, Sri Lanka, Pakistan, Myanmar, Southeast Arabia and East Africa. Surprisingly, in spite of such wide distribution there is no geographical subspeciation or variation in this butterfly (Gay *et al*., 1992).

Materials and methods

The present study was carried out at Visakhapatnam during the calendar year 2007. Visakhapatnam (17 °42' N latitude and 82 °18' E longitude) is located on the east coast of India in the State of Andhra Pradesh. 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, 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 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 during 0800 to 1500 h for the reproductive activity of the Joker butterfly, *B. ilithyia.* Adult butterflies were seen mostly near the larval host plant, *Tragia plukenetii* A. R. Smith. Once adult butterflies were located detailed observations were made in order to observe the period of copulation and oviposition. After detecting ovipositions, the leaf with eggs was collected in Petri dishes (15 × 2.5 cm) and brought to the laboratory. The leaf piece with eggs was then placed in a smaller Petri dish (10 × 1.5 cm), that was lined with moistened blotter to prevent leaf 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 interval 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 hairbrush. The larvae were supplied daily with 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 × 2.5 cm). Larval performance in terms of food utilization indices were calculated as described by Waldbauer (1968) as:

Growth rate (GR) = weight gained by the instar / (mean weight of instar × number of feeding days)

Consumption index (CI) = weight of food ingested / (mean weight of instar × number of feeding days)

Approximate digestibility (AD) (also called "assimilation efficiency") = ((weight of food ingested – weight of faeces) / weight of food ingested) × 100

Efficiency of conversion of digested food (ECD) (also called "net conversion efficiency") = (weight gained by the instar / (weight of food ingested – weight of faeces)) × 100

Efficiency of conversion of ingested food (ECI) (also called "gross conversion efficiency") = (weight gained by the instar / weight of food ingested) × 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, particulars of pupae including colour, shape, size, weight and the time of adult eclosion were also recorded. Millimetre graph paper was used for taking measurements. The laboratory temperature was 28 ± 2 °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.

For 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.

Results

Different life stages

Adult stage — Both male and female were nearly identical. The upper side of both wings are thick orange colored with black spots and the underside is light orange with brown coloured bands on the edges. Mating and oviposition took place during 0900-1300 h (fig. 1a).

The gravid female laid eggs singly on the under surface of the leaves of its larval host plant, *T. plukenetii* and also on the fruits. Females closed their wings during egg laying and deposited 6-8 eggs in a single bout. There was no bias for the age of the leaf. Adults were found probing for nectar on the *Tridax procumbens* L. and *Croton bonplandianum* Bail.

Egg stage — The eggs were creamy white, dome shaped, 0.90-1.00 (0.94 ± 0.05) mm in diameter. When first laid the eggs appeared soft in texture, but within 6-10 seconds they became hairy (fig. 1b). They hatched in three days of incubation. Soon after hatching the larvae ate its egg-shells. Each larva passed through five distinct instars over a period of 18-21 (19.20 ± 1.30) days.

Larval stage — Instar I lasted for 2 (2.00 ± 0.00) days. On the first day of hatching, the larvae measured 2.00-2.50 (2.22 ± 0.19) mm in length. They grew to 2.40-2.80 (2.60 ± 0.16) mm in length, and 0.75-0.85 (0.82 ± 0.04) mm in width. Head capsule measured 0.60-0.70 (0.68 ± 0.04) mm in diameter. Body was cream coloured, with three distinct black bands that are equally distanced. At this stage the body was also fully covered with black hairs and the head was black coloured without any horn like structures. Instar II lasted for 1-2 (1.60 ± 0.55) days. It measured 4.00-5.50 (4.62 ± 0.63) mm in length and 1.00 mm (1.00 ± 0.00) in width. Head capsule measured 1.40-1.80 (1.62 ± 0.20) mm in diameter. The body was light orange and covered with black bands resulting in alternate bands of light orange and black coloured bands. The body was also covered with hairs and the colour of the hair corresponds with the colour of the band. Instar III lasted for only one (1.00 ± 0.00) day. Developing to a length of 7.00-7.50 (7.30 ± 0.27) mm and a width of 1.00-1.10 (1.02 ± 0.04) mm. Head capsule measured 2.20-2.40 (2.26 ± 0.09) mm in diameter. The head was black with a pair of light orange horns. These horns possess a row of hairs in pairs on them on both sides. The body is orange coloured and with the branched black hairs. Instar IV lasted for 2 (2.00 ± 0.00) days and attained a length of 10.50-12.50 (11.78 ± 0.78) mm and a width of 1.50-1.70 (1.62 ± 0.08) mm. Head capsule measured 2.80-4.00 (3.28 ± 0.50) mm in diameter. Hairs are arranged in six longitudinal rows in anterior-posterior direction, and 12 rows in horizontal wise. The last two longitudinal rows on both sides of the body were white in colour. These white hairs turned to black by the next day. There appears a thick orange streak on the mid-dorsal surface of the body. Instar V lasted for 3-5 (4.00 ± 0.71) days. When fully grown the larva was 20.00-23.60 (22.18 ± 1.50) mm in length and 2.70-3.10 (2.92 ± 0.18) mm in width. Head capsule measured 6.70-7.00 (6.88 ± 0.13) mm in diameter. On both sides of the mid-dorsal orange coloured streak black longitudinal lines were found. Again these were followed by orange coloured streaks. On the ventral side the body was light orange coloured. By the last day in the middle of the mid-dorsal streak there appears a black longitudinal line. This orange coloured mid-dorsal line was interrupted by light orange spots at ridges. Larva stopped feeding and body contracted before pupation (fig. 1c-g).

Pupal stage — Pupal stage lasted for 5-6 (5.60 ± 0.55) days. It was 17.00-19.00 (17.80 ± 0.76) mm in length and 4.00-4.60 (4.28 ± 0.28) mm in width at its broadest end. The pupae exhibit dimorphism in their colour pattern, in such a way that they are either green or grey. On the ventral surface there are two longitudinal markings. On dorsal view there were two ridges. Average pupal weight was 221.70 ± 29.00 mg (fig. 1h-i).

Duration of life cycle the total development time from egg to adult eclosion ranged between 18-21 (19.20 ± 1.30) [egg: 3, larva: 9-12, pupa: 5-6] days.

Food consumption, growth and utilization

The data on the amount of food consumed by each of the five 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 1.02, 2.20, 2.43, 15.60, 78.75% and the proportions of weight gained in relation to total weight gained by the successive instars were 0.14, 0.54, 1.38, 13.77, 84.16 %. Thus, there was over 94% of the total food consumption and 97% of total weight gained in the fourth and fifth instars together. There was a direct relationship between food consumption and growth across the five instars

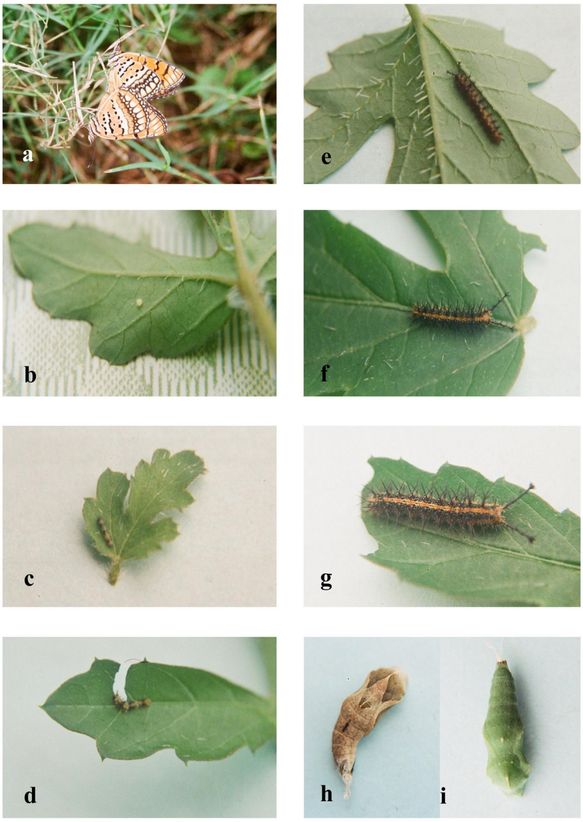

Figure 1. Life stages of *Byblia ilithyia*. a) adult pairing; b) egg; c) instar I; d) instar II; e) instar III; f) instar IV; g) instar V; h-i) pupae showing colour dimorphism.

(fig. 2). The values of CI decreased from first to final instar, while the GR was increased from first to third instar and again decreased to the final instar. Values of CI ranged between 1.49-13.28 mg/day/mg and those of GR between 0.21-0.62 mg/day/mg. Table 1 also included the data on AD, ECD, and ECI. The values of AD from instar to instar decreased from a high of 94.98% in first instar to a low of 55.77% in the last instar. The values of ECD and ECI increased progressively from the first instar to the last instar. The values of ECD varied from 1.93-25.26% and those of ECI from 1.83-14.09%. Thus there was an inverse relationship between the values of AD and those of ECD and ECI.

Table 1. Food consumption, growth and food utilization efficiencies of *Byblia ilithyia* larva fed on *Tragia plukenetii* leaves.

Instar	Weight of food ingested (mg)	Weight of faeces (mg)	Weight gained by larva (mg)	GR (mg/day/mg)	CI (mg/day/mg)	AD (%)	ECD (%)	ECI (%)
I	20.72 ± 04.22	1.04 ± 00.41	0.38 ± 0.04	0.24	13.28	94.98	01.93	01.83
II	44.52 ± 04.52	3.44 ± 00.25	1.44 ± 0.42	0.40	12.53	92.27	03.50	03.23
III	49.23 ± 06.25	3.74 ± 00.89	3.70 ± 0.51	0.62	08.31	92.40	08.13	07.51
IV	316.00 ± 10.88	26.08 ± 2.54	36.78 ± 2.24	0.43	03.70	91.75	12.67	11.64
V	1594.92 ± 76.12	705.36 ± 26.22	224.72 ± 5.58	0.21	01.49	55.77	25.26	14.09

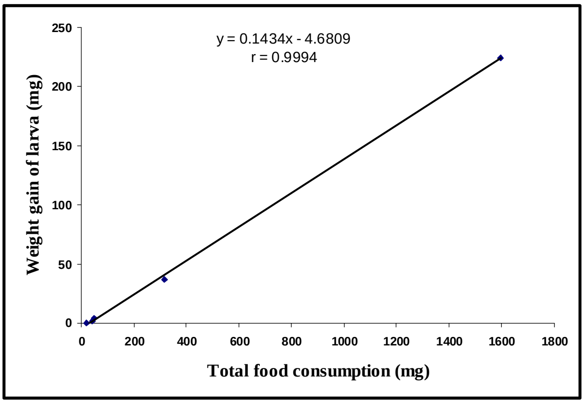

Figure 2. Relationship between food consumption and growth in *Byblia ilithyia* on *Tragia plukenetii*.

Discussion

Floral nectar is an important food resource for butterflies (Boggs, 1987). *Byblia ilithyia* visits flowers frequently and imbibes nectar and nectar intake may increase its longevity and egg production (Stern & Smith, 1960; Murphy *et al*., 1983). This butterfly mostly visits the flowers of *T. procumbens* and *C. bonplandianum*.

The total development time from egg laying to adult eclosion was determined as 19.20 ± 1.30 days at about 28 ± 2 ^{0}C. This behaviour 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; Braby, 2003; Pathak & Pizvi, 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 2.02 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), 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 (13.28) 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 *B. ilithyia* are in line with the above decreasing trend.

The values of AD that were obtained in this study are comparable with the range of AD values (19-81%) for lepidopterous larvae (Pandian& Marian, 1986). The average AD percentage is over 85.43% 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 food item is rich in nitrogen (and also water) (Pandian & Marian 1986). Similar results were repeated with *Pieris brassicae* (L.) (Yadava *et al*., 1979), *Euploea core* (Cramer) (Venkata Ramana *et al*., 2001) and *Ariadne merione merione* (Cramer) (Atluri *et al*., 2009).

The values of ECD increase from early to last instars (Slansky & Scriber, 1985). Such trend is observed with the ECDs of *Byblia ilithyia*, with the lowest value in instar I and the highest in instar V. The ECDs obtained are low compared to the ADs and such low values are not unusual (Waldbauer, 1968). 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 (1.83-14.09) 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 two instars, are also relatively high (12.67, 25.26; 11.64, 14.09), thus respectively indicating tissue growth efficiency and ecological growth efficiency, which enabled *B. ilithyia* to thrive successfully in the urban environment.

Thus, the present study provides information on the oviposition larval host, *T. plukenetii* and larval performance in terms of food consumption, growth and utilization, and the length of life cycle from egg to adult eclosion of the Joker butterfly, *B. ilithyia.* 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 fields. 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 °C permits enough stock of adults for restocking the areas poor in populations of the Joker butterfly.

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