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RESEARCH

Adult Behavior of *Tirumala limniace* (Lepidoptera: Danaidae)

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ABSTRACT. *Tirumala limniace* Cramer as an ornamental butterfly is utilized in butterfly garden, in this article we study their adult activities include flight, foraging, courtship, mating, and oviposition. We found that males spent 22.1% of its time flying, 14.1% foraging, 63.8% in courtship and mating. And females spent 30.8% of its time flying, 10.1% foraging, 57.1% in courtship and mating, and 2% ovipositing. Adults emerged from pupae when temperatures were above 23.5°C and eclosion took only ∼1 min, typically followed by a small amount of flight on the first day. Flight activity peaked from the ninth to the thirteenth day after eclosion, and there were two daily peak flight times: 10:00–13:00 and 15:00–18:00. The peak of flower-visiting activity was from the eighth to the thirteenth day after eclosion, and there were two daily peak foraging times: 11:00–12:00 and 16:00–17:00. Flight and foraging frequency and time were positively correlated and both were closely related to temperature, with very little flight or foraging below 18°C and an increase at temperatures above 22°C. Courtship and mating took place on the sixth day after eclosion, while oviposition occurred the following day. Oviposition occurred over 8 d, and the shortest time of a single oviposition was 2 s. The average life expectancy of males was 16.5 d, while that of females was 15 d.

Key Words: *Tirumala limniace* (Danaidae), flight, flower-visiting, adult activity patterns, adult behavior

Tirumala limniace Cramer (Lepidoptera: Danaidae) is distributed throughout China, India, Sri Lanka, Pakistan, Bangladesh, Myanmar, and Thailand (Ambrose and Raj 2005). Its host plants include *Dregea volubilis* Apocynaceae and several species *Heterostemma* and *Hoya* (Chen et al. 2008). Previous studies have investigated the adult behavior of some species of butterflies: Brussard and Ehrlich (1970) described the daily activity, flight, courtship, mating, and predator avoidance of *Erebia epipsodea* Satyrinae, while Scott (1973a) compared the adult behavior of two sympatric butterflies, *Neominois ridingsii* Edwards and *Amblyscirtes simius* Edwards, including feeding, oviposition, and thermoregulation. Other research on the adult behavior of various butterflies has examined *Parnassius phoebus* F. (Scott 1973b), *Danaus plexippus* L. (McCord and Davis 2010), and *Placidula euryanassa* Felder&Felder (Freitas 1993). Prior studies of *T. limniace* have mainly focused on life stages (Naidu and Ramana 2010; Naidu et al. 2011), diagnostic morphological features, habitat range, larval and adult food sources (Ambrose and Raj 2005), consumption index (CI), growth rate (GR), approximate digestibility (AD) of food in each instar (Naidu et al. 2011), adult habitat preferences (Mathew and Rahamathulla 1993), migration, aggregation, and mud-puddling behaviors (Wang and Emmel 1990; Mathew and Binoy 2002), visual and olfactory responses during foraging (Tang et al. 2013), the major components of hairpencil secretions (Komae et al. 1982), and classification based on data from morphology and DNA sequencing (Smith et al. 2005). However, few studies have reported on behavior of adults of *T. limniace.*

T. limniace is a butterfly commonly found in gardens that has increasingly been bred for release in butterfly parks. This study, by improving our understanding of the behavioral characteristics of emergence, flight, foraging, courtship, mating, and oviposition of *T. limniace*, increases our understanding of the butterfly's biology, and will inform the protection and artificial breeding of this species.

Materials and Methods

No specific permits were required for the described field studies.

Research Site and Experimental Arena. Experiments took place at the experimental station (25° 13′ N, 102° 12′ E) of Research Institute of Resource Insects in Lufeng County, Yunnan Province, China in August 2013. The study area was at an altitude of 1,843 m, with an average annual temperature of 19°C, an average annual rainfall of 1,000 mm and annual relative humidity (RH) of 70–80%. *T. limniace* bred artificially in the experimental station were observed in a net house (8 m long, 4 m wide and 4 m tall) with evenly scattered sunlight in which was equally distributed five host plants *D. volubilis* and five nectar-producing plants *Asclepias curassavica* L.

Experimental Animals. Eggs were obtained in the laboratory from three fertile females in 2013. Larvae were raised on young leaves of *D. volubilis* in a climate room at 28°C with a 13:11 (L:D) cycle, and 50% RH.

Behavioral Observations. Fifty pupae of *T. limniace* produced by artificial breeding were placed in the net house upon emergence. The time and temperature at eclosion were recorded.

Twenty newly emerged butterflies (with a sex ratio of 1:1, day 1) were marked on their hind wings with a pen to distinguish gender. The activity of four pairs (♀:♂ = 1:1) of these marked butterflies was tracked daily from 8:00 to 19:00, and all activities of their flight (the period between start flying and landing), flower-visiting, courtship, mating, and oviposition were recorded until death. Video footage was used to assist these observations.

Statistics Analysis. The duration of the observed activities were expressed as means ± standard error. Microsoft Excel 2007 was used to draw charts, and the data was analyzed by SPSS 18.0. A Wilcoxon test was used to determine significant differences in the daily flight (foraging) frequency and duration between males and females. Spearman correlation analysis was used to examine the correlation between the daily average number of flights and the daily average temperature, the daily flight (foraging) frequency and flight (foraging) duration for both males and females, daily flight and foraging frequency, daily flight and foraging durations for both males and females.

Results

Eclosion

The ambient temperature ranged from 18 to 35°C in the net house from 8:00 to 19:00. Most adults emerged (Fig. 1A) from 11:30 to 14:00, with males emerging earlier than females, and during this period the temperature ranged from 23.5 to 26°C (Fig. 2).

Flight. The time from emergence to first flight averaged 6.4 ± 9.3 h (range 1.5–20.3 h). Both flight frequency and duration were lower during the first 3 d after emergence. From the beginning of the fourth day, flight frequency and duration showed an increasing wave trend until the 13th day. Peak of male flight frequency and duration occurred between days 8 and 13 after eclosion, whereas females peaked between days 9 and 13, and flight patterns were nearly identical between the sexes (Figs. 3 and 4). On sunny days, butterflies exhibited more active flight behavior, beginning as early as 8:20 and continuing as late as 18:50, while on rainy days flight behavior was less active, with the earliest flight at 9:00 and the last concluding at 18:10.

There were two peaks of flight activity: the first from 10:00 to 13:00 (35.2% of total flight time) and the second from 15:00 to 18:00 (37.5%). Males were more active in the morning, with an activity peak from 10:00 to 11:00 (14.9%), while females tended to be more active in the afternoon, with a peak from 16:00 to 17:00 (22.0%) (Fig. 5).

The flight activity of *T. limniace* was closely related to temperature. Flying began when the ambient temperature was higher than 18°C and flight behavior slowed at temperatures higher than 30°C (Fig. 5). Moreover, the trends of the daily average number of flights and of the daily average temperature were consistent (Fig. 6). Correlation analysis showed that the daily average number of flights and the daily average temperature had a significant positive correlation ($P < 0.05$, $r = 0.633$). When the daily average temperature was > 22°C, 66.7% of males, and 53.3% of females flew over 40 times during the day. For 8 d after emergence, the daily average number of flights was higher in males than females, but this trend reversed in days 12–15 (Fig. 6).

Over the 16 d from emergence to death, males averaged a total of 752.5 flights with an average total duration of 176.6 min. Daily, the average number of flights was 47 and the average flight duration in total was 11.0 min. For females the average lifetime total number of flights was 713 with an average total time in flight of 276.1 min. Daily, females averaged 47.5 flights and flights in total averaged 18.4 min. Although we found no significant differences in the daily flight frequency and duration between males and females ($P > 0.05$), we did find significant positive correlations between the daily flight frequency and flight duration for both males ($P < 0.01$, $r = 0.768$) and females ($P < 0.01$, $r = 0.931$).

Foraging. The longest duration from emergence to first forage was 100.6 h, whereas the shortest was 29.7 h, with an average length of 68.8 ± 36 h. No butterfly visited flowers on the day of emergence, and flower-visiting (Fig. 1B) gradually appeared from the second day on.

Flight and flower-visiting activities rose from the beginning of supplementing nutrition, and declined until the postspawning stage.

Fig. 1. Four kinds of adult behavior of *Tirumala limniace.* (**A**) Eclosion. (**B**) Foraging. (**C**) Mating. (**D**) Oviposition.

Butterfly foraging frequency and duration showed an up and down trend in the days after eclosion and peaked from days 8–13. In general, foraging frequency and duration were greater on sunny days (days 8, 9 and 11) and lower on rainy days (days 7 and 10) (Figs. 7 and 8). Foraging had two daily peaks, the first from 11:00 to 12:00 (14.5%) and the second from 16:00 to 17:00 (16.9%). Both males and females had the same foraging behavior patterns (Fig. 9).

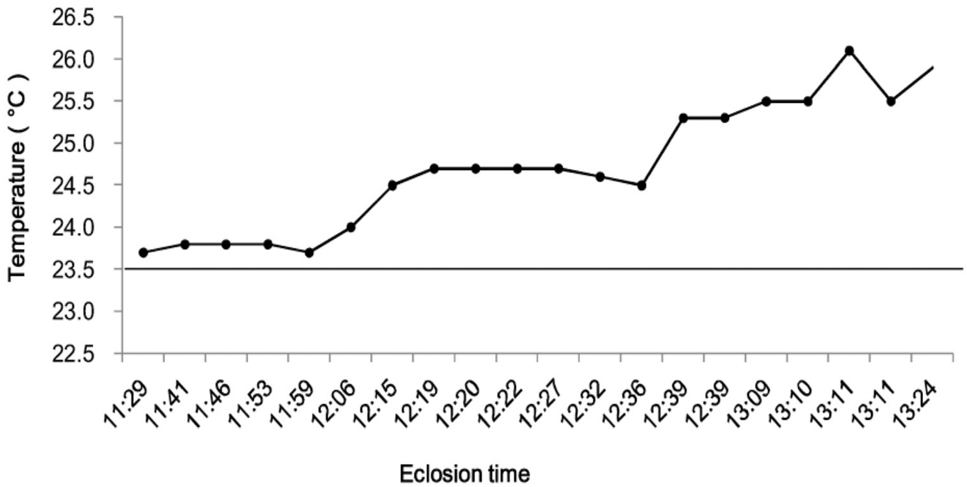

Fig. 2. Eclosion time of *T. limniace* in 1 d.

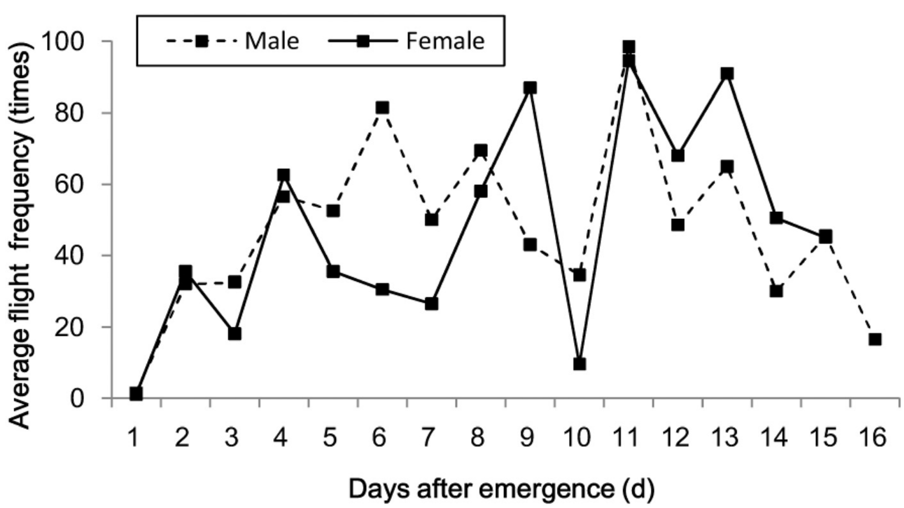

Fig. 3. Daily flight frequency of *T. limniace*. The y-axis title means the mean of the frequency all males or females were flying in total at the given day.

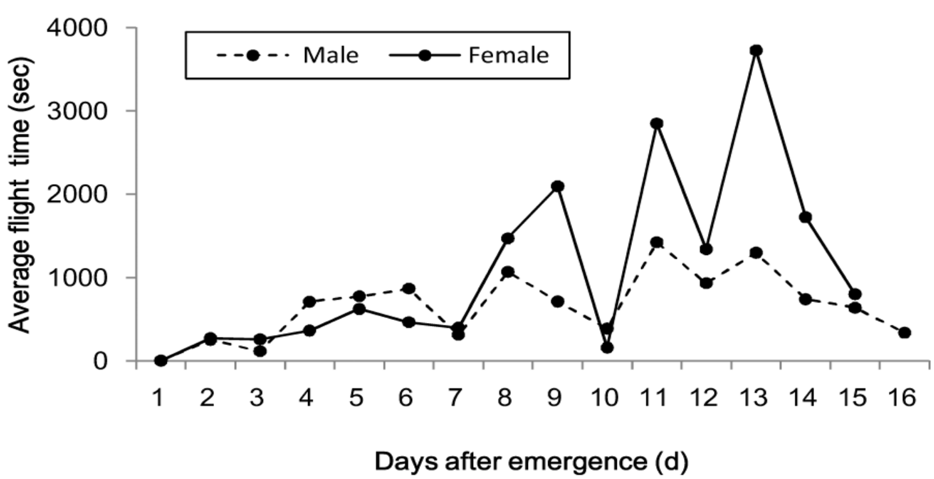

Fig. 4. Daily flight duration of *T. limniace*. The y-axis title means the mean of the time all males or females were flying in total at the given day.

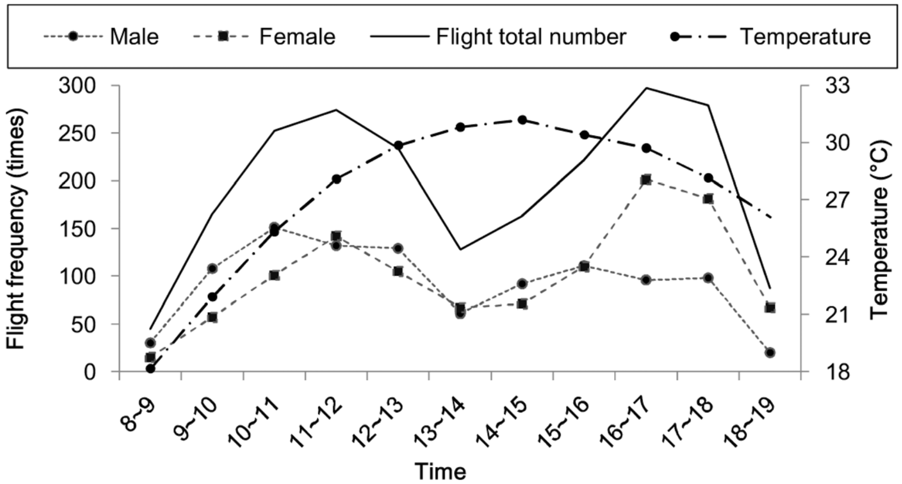

Fig. 5. Daily flight rhythm of *T. limniace.* The temperature profile means the average temperature of 16 d at the given time. Flight frequency means the average frequency of 16 days at the given time.

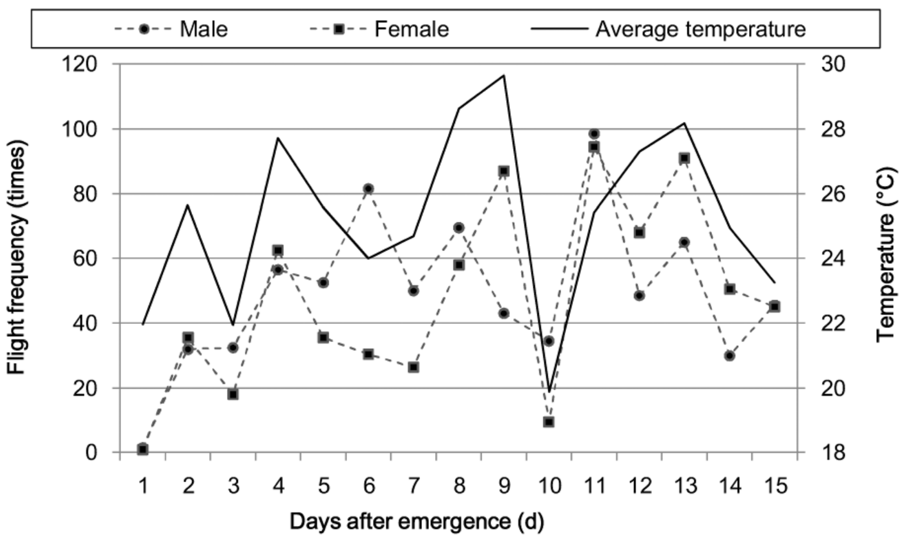

Fig. 6. The relationship between temperature and daily flight frequency of *T. limniace.* The temperature profile means the average temperature of 16 days at the given day.

Over the course of their adult life, males visited an average of 51 flowers for a total of 112.9 min, or 3.2 flowers visited for 7.1 min each day. The average length of a single flower visit by males was 1.8–3.4 min. Females visited a total of 37.5 flowers on average, for a total of 90.1 min or 2.5 flowers for 6.0 min per day. The average length of a single flower visit by females was 1.4–3.0 min. Although there were no significant differences between male and female daily foraging frequency and duration ($P > 0.05$), there was a significant positive correlation between daily foraging frequency and duration for both males ($P < 0.01$, $r = 0.891$) and females ($P < 0.01$, $r = 0.836$).

For both males and females, there were also significant positive correlations between daily flight and foraging frequency ($P < 0.01$, $r = 0.889$) and daily flight and foraging durations ($P < 0.01$, $r = 0.793$). With increasing flight frequency and duration, foraging frequency and duration also increased, accounting for 5–9% of flights (Fig. 10).

Mating. In general, males waited several days after emergence before displaying courtship behavior, typically on the sixth day after eclosion from 10:00 to 17:30. When the females approached, males began to chase them, and sometimes other males joined in, forming a chain of up to 3 to 4 adults in the net room. During the chasing behavior, the female stopped on the screens, and then the male would fly around the female slowly seeking to mate. Often, most of the males would give up the pursuit and fly away when the females stopped. Females could mate more than once.

Oviposition. Oviposition (Fig. 1D) occurred on the seventh day after eclosion (the day after mating), and there was two peaks, the first from 10:00 to 14:00 (40.8%) and the second from 15:00 to 17:00 (43.7%). Oviposition lasted for 8 days, and the longest time for a single bout of oviposition was 44 s, while the shortest was 2 s. Although male life expectancy was 16.5 d, that of females was only 15 d.

Females laid only one egg each time, and after laying an egg females would often fly around the host plant and lay another. One female laid on average 21 ± 2.35 eggs on a single host plant in one day. Most eggs were laid on the back of host plant leaves, but a very small number of eggs were laid on plant stems and flowerpots. Eggs on the leaves were laid in an irregular arrangement.

Discussion

From a behavioral point of view, the life of this butterfly could be divided into four stages: the precourtship adult stage (from the 1st to the 8th days after emergence), the nectar-feeding stage, which promotes egg development (from the 2nd to the 16th days), courtship and mating (from the 6th to the 13th days), and oviposition (from the 7th to the

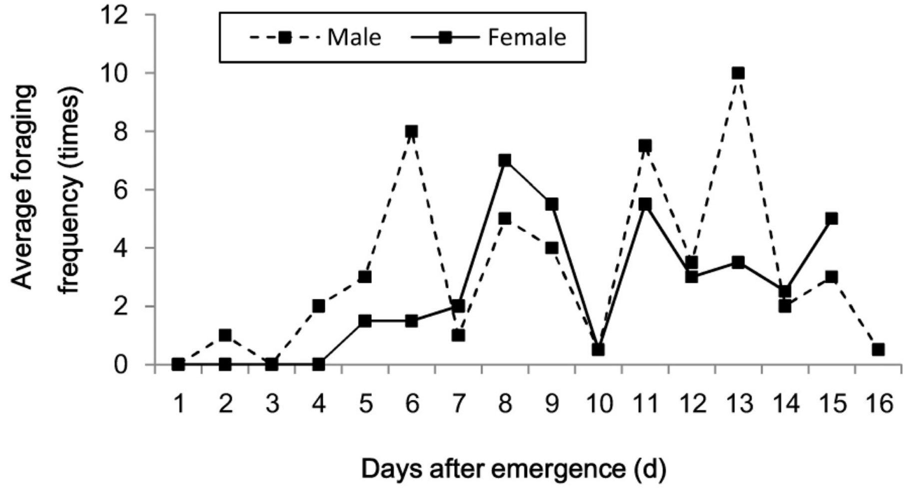

Fig. 7. Daily foraging frequency of *T. limniace.* The y-axis title means the average frequency of all males or females were foraging in total per day.

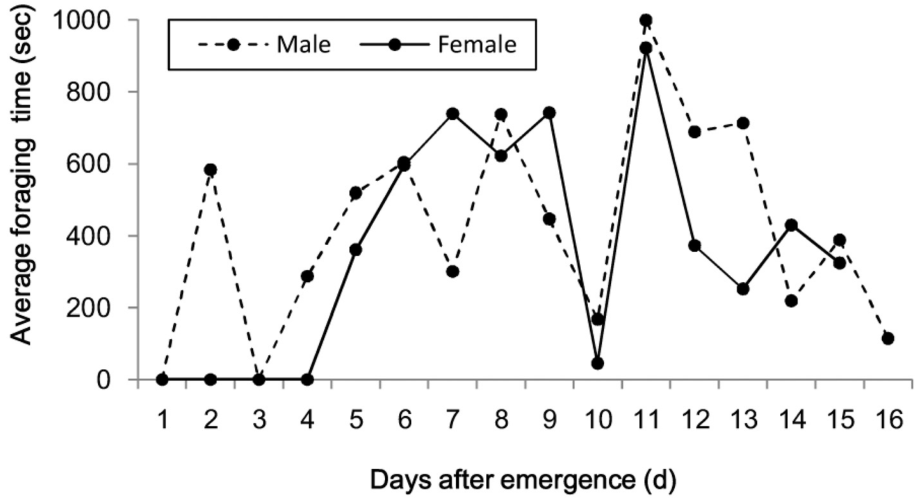

Fig. 8. Daily foraging duration of *T. limniace.* The y-axis title means the average duration of all males or females were foraging in total per day.

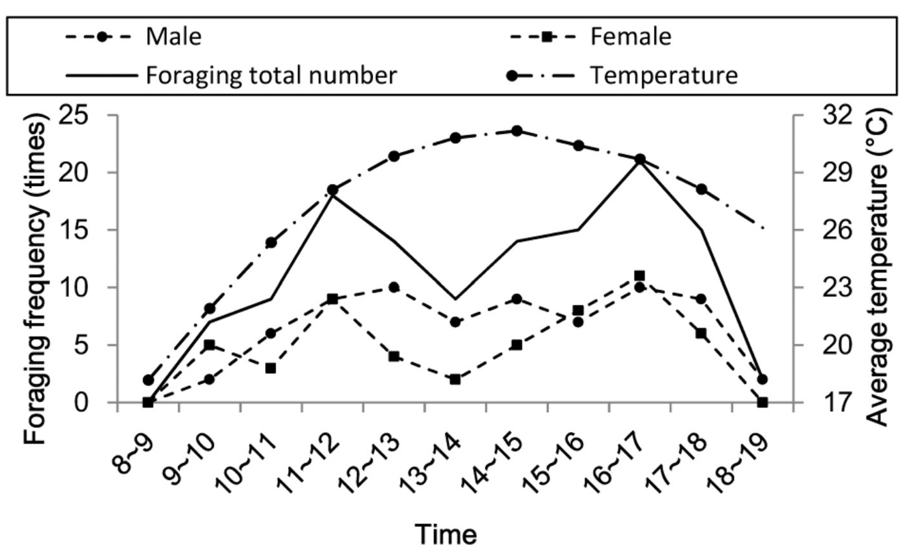

Fig. 9. The foraging frequency of *T. limniace* over the course of one day. The temperature profile means the average temperature of 16 days at the given time.

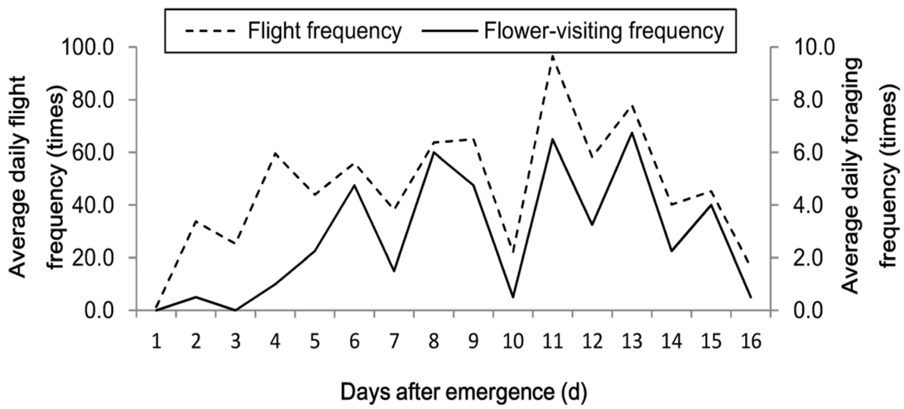

Fig. 10. Daily flight and foraging frequency of *T. limniace.*

Table 1. The duration and proportionate importance of different behaviors of *Tirumala limniace*

Behavior	Male (♂)		Female (♀)	
	Time (min)	%	Time (min)	%
Flight	176.6	22.1	276.1	30.8
foraging	112.9	14.1	90.1	10.1
Courtship	0.4	0.1	1.7	0.2
Mating	510	63.7	510	56.9
Oviposition	–	–	17.9	2.0
$\sum$	799.9	100	895.8	100

15th days). Overall, adult flight accounted for 22.1% (♂) or 30.8% (♀) of the time spent in recorded behaviors, while visiting flowers accounted for 14.1% (♂) or 10.1% (♀), courtship and mating 63.8% (♂) or 57.1% (♀), and oviposition 2% (♀) (Table 1).

Precourtship Adult Stage

Before its first flight, a butterfly would exhibit high frequency, low-amplitude wing vibration, repeated over and over. This may be exercising the stretching function of its flight muscles, thereby increasing its ability to fly (Fernanda and Carminda 2010). Moreover, such low-amplitude wing vibrations could rapidly warm the butterfly's thorax, allowing it to fly at lower temperatures (Kammer 1970). Very little flight and no flower-visiting took place on the day of emergence, perhaps because newly emerged adults needed refection and because wing muscles were not yet mature (Luo 1996; Wang et al. 2013).

The majority of moth courtship and mating takes place in days 6–7 after eclosion (Wang and Zhang 2001). Luo (1996) analyzed the flight muscle ultrastructure of *Mythimna separate* Walker, and found that flight muscle development continued for seven days after eclosion. In this study, the courtship and mating of *T. limniace* took place on days 6–7 after eclosion, suggesting that adults promoted reproductive maturity by first flying and foraging for this period of time.

Nectar-Feeding Stage. Nectar-feeding was a common behavior in all four of the adult life stage periods, beginning with a small amount of foraging on the day after emergence. Flight and foraging behavior increased on the fourth day, and foraging frequency and duration increased for a time thereafter. This behavior both was made possible by and allowed for nutritional supplement, thereby promoting egg development (Karlsson and Wickman 1990; Rankin and Burchsted 1992). Most lepidopteran insects need some nutrition in the adult stage, usually nectar. Carbohydrate-rich nectar provides energy not only for flying (Baker and Baker 1973), but also for oocyte synthesis, increasing fecundity and prolonging the oviposition period, making nectar acquisition important for butterfly reproduction (Boggs 1986; Karlsson and Wickman 1990).

Courtship and Mating Stage. Courtship and mating began on the 6th day after emergence, and remained at a high level from the 7th to the 11th days, while butterflies continued to supplement their nutrition even after beginning to mate. Several studies have found that the coincidence of flower-visiting and mating not only promotes the development and maturation of reproductive organs (Wheeler 1996), but also increases fecundity (Boggs 1986). Wang et al. (2013) studied the effects of mating on the flight muscle development of *Agrotis ypsilon* Rottemberg, and found that mating can reduce flight muscle energy use and promote apoptosis of flight muscle cells. Stjernholm and Karlsson (2008) studied *Pieris napi* L., and found that flight muscle breakdown is a general phenomenon in butterflies, whose most prominent feature is a decrease in thoracic flight muscles. *P. napi* showed reduced thoracic flight muscles at mating, after which flight muscle began to degrade. At this stage, flight muscles of the female begin to degrade, and nitrogenous resources from the thorax were used for egg production (Karlsson 1998; Stjernholm and Karlsson 2008), suggesting that after the start of mating, in addition to energy gained from flight muscle degradation, butterflies still need to eat to promote egg maturity while maintaining normal activities.

Oviposition Stage. The oviposition stage was very short. Females laid eggs one by one, flying around the host plant before laying another egg. Only when females laid a lot of eggs, they would then fly to nectar plants for supplemental nutrition. Most of the eggs were laid on the back of host leaves, which has been considered a strategy for escaping egg predation (Baguette and Schtickzelle 2003).

Of the observed adult behaviors, mating occupied the most time, over half of the butterfly's lifespan. Flying was the next most common behavior, not surprising in that it promoted the development of reproductive organs and made foraging, mating, and oviposition possible. Foraging was the third most common behavior, and these three activities together accounted for >97% of the butterfly's lifespan. Oviposition behavior was very brief, indicating an economy of energy and possibly a strategy for avoiding predators while vulnerable laying eggs.

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