LABORATORY BIONOMICS OF *TRIPTEROIDES ARANOIDES*

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ABSTRACT. *Tripteroides aranoides* was colonized in the laboratory. Total duration of the immature stages was ca. 3 weeks at 28°C, L:D=15.5:8.5 with an ample food supply. Retardation of 4th instar development was observed in larvae fed on insufficient food. Females were autogenous for the first clutch of eggs and required a blood meal for maturation of the second clutch. Mating was initiated in flight and copulation occurred on the cage wall. Gravid females hovered in small oblique loops above water in bamboo cups, whereupon a white egg appeared at the abdominal tip, which was propelled by the swing of the abdomen towards water surface. The females propelled eggs in the same manner into small apertures (11 × 4 mm) bored in bamboo.

INTRODUCTION

Tripteroides is one of the largest genera of the tribe Sabethini but the biology and behavior of the species in this genus have scarcely been studied. Miyagi (1973) observed the durations of the developmental stages and ovipositional behavior of *Tp. bambusa* (Yamada) in the laboratory. Mori (1976) described autogeny in this species. Beaver (1979) made observations on the biology of 3 *Tripteroides* species in pitcher plants. For the rest of this genus, bionomic information is virtually confined to descriptions of the breeding sites and observations on the attraction to humans at the time of collection (Mattingly 1981).

In this study we established a laboratory colony of *Tp. aranoides* (Theobald) and observed the length of the developmental stages, blood-feeding, fecundity and mating and ovipositional behavior.

MATERIALS AND METHODS

COLONY MAINTENANCE. The laboratory colony was established with ca. 60 larvae collected from bamboo stumps in San Pa-Tong, south of Chiang Mai, Thailand. Gravid females were provided with a bamboo cup (ca. 10 cm diam and ca. 8 cm height) containing 200 ml tap water for oviposition. Hatched larvae were transferred to a white plastic tray (20 × 30 × 5 cm) containing 1,000 ml tap water, and powdered mouse pellets plus dry yeast. Adults were maintained in screened cages (20 × 20 × 30 cm) and provided with cotton balls soaked with a 2% sugar solution. The colony was maintained and the following observations made in an insectary at 28°C, 75–85% RH and L:D=15.5:8.5, unless otherwise stated.

LENGTH OF THE DEVELOPMENTAL STAGES AND REPRODUCTIVE PHYSIOLOGY. Three hundred larvae per tray were reared under 2 different conditions: one group was given 0.02 gm/day/tray of food throughout larval life, the other 0.2 gm before and 0.5 gm/day/tray after the appearance of 4th instar larvae. The number and stage of larvae were recorded daily and pupae were checked for adult emergence. For larvae, the median time for development was determined graphically from the 50% molting time. Cumulative percentages of stages from egg to pupa were plotted on graph paper daily. Points of each developmental stage were connected and the point at which the line crossed the 50% level indicated 50% molting time. One half of the adults emerging from each tray were given a 5% sugar solution and the other half, a 0.1% sugar solution. Females were dissected and examined for mature eggs 6 days after emergence.

Premating period for females was determined from insemination rates. Ten to 20 newly-emerged, 24, 48 or 72 hr-old females were kept in the 20 × 20 × 30 cm cages for 24 hr with the same number of mature males. All females were dissected and their spermathecae were examined for insemination. The insemination ability of males was determined from insemination rates of mature females kept for 24 hr with newly-emerged, 24- or 48 hr-old males.

The prefeeding periods of two groups of females were determined by the incidence of engorgement among females offered a hairless mouse for 1 hour every day after emergence. One group was provided with a bamboo oviposition cup soon after emergence, while the other 3 weeks afterwards.

To examine the possibility of maturing a second egg clutch without a blood meal, females fed on an ample food diet during larval life were allowed to lay eggs of the first clutch in a bamboo cup. All the eggs laid were removed

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and counted daily. Five days after the last eggs were laid, parous females were divided into two groups. Females which engorged on a hairless mouse were provided access to a 0.1% sugar solution. The other, a blood-starved group, was given a 5% sugar solution. Six days later, all females were dissected to examine their ovaries.

EGG RESISTANCE TO DESICCATION. About 2,000 eggs laid on the same day were collected on a filter paper and divided into 10 batches of 152–292 eggs. One control batch was immediately transferred to tap water and kept at 28°C and L:D=15.5:8.5. Nine experimental batches were taken out of water 3 days after oviposition and held for storage at 20°C, 80% RH and L:D=12:12 for 4–50 days. Each batch was transferred to tap water at a scheduled time and larval hatch was examined daily for 35 days. Unhatched eggs were dissected to confirm the embryonic death. The hatch rate of the experimental batches was corrected by that of the control.

MATING AND OVIPOSITIONAL BEHAVIOR. Mating and ovipositional behavior was observed in a screened cage or a glass box (20 × 20 × 30 cm). Some observations were facilitated with close-up photographs.

RESULTS

LENGTH OF THE DEVELOPOMENTAL STAGES. The duration of each developmental stage under two different food regimens is presented in Table 1. The mean duration time from oviposition to adult emergence under an ample food supply was ca. 3 weeks. The duration time for males was a little shorter than that for females. The total length of the larval stage under the insufficient food regimen was greatly increased, due mainly to retarded growth at the 4th instar. Females required 20 days more than males for development.

REPRODUCTIVE PHYSIOLOGY. Some males transferred sperm less then 24 hr after emergence (Fig. 1). Females were refractory to insemination 24 hr after emergence. Some were inseminated during 24–48 hr after emergence, and most all by 72 hr. Inseminated females appeared to be refractory to subsequent copulations.

Females could produce first clutch eggs to maturity without a blood meal (Table 2). The autogeny rate was higher, and the mean number of matured eggs greater for females fed on ample food during both larval and adult stages than for those given insufficient food as larvae or adults. When underfed during both larval and adult stages, most females did not produce mature eggs.

Rates of autogeny and numbers of mature eggs were related to wing length (Fig. 2). Females with wing lengths less than 2.1 mm never produced mature eggs. All females with wings more than 2.6 mm long had mature eggs. Between these extremes the rate of autogeny and number of mature eggs was positively correlated with wing length. The average fecundity (Y) is given by the following equations:

$$Y = RZ = 202.91X^3 - 1170.82X^2 + 2253.33X - 1446.84$$
$$R = 1.69X - 3.43$$
$$Z = 119.92X^2 - 448.66X + 421.45$$

where R is rate of autogenous females, Z is number of mature eggs for autogenous females and X is wing length.

Oviposition of the autogenous first clutch began 4 days after emergence and lasted for ca.

Table 1. Duration of developmental stages and mortality of *Tripteroides aranoides* under two nutritional regimes.

Stage		0.2/0.5* (g/day/tray) Duration (days)	0.2/0.5* (g/day/tray) Mortality (%)	0.02 (g/day/tray) Duration (days)	0.02 (g/day/tray) Mortality (%)
Egg		4.2 (4–12)**		4.2 (4–12)	
Larva***	I	2.9	1.3	2.9	2.7
	II	1.5	0.3	2.2	3.8
	III	2.1	0.3	3.2	5.0
	IV	5.4	1.4	30.9	1.1
Pupa	♂	4.9 (4–6)	1.7.	4.9 (4–6)	7.6
	♀	5.0 (4–6)		5.0 (4–6)	
Total (Larva + Pupa)	♂	16.2 (14–19)	4.9	37.6 (15–68)	18.7
	♀	17.9 (15–24)		58.1 (20–85)	

* Larvae were fed on 0.2 g before and 0.5 g after appearance of 4th instar.
** Ranges in parentheses.
*** Obtained by graphic method.

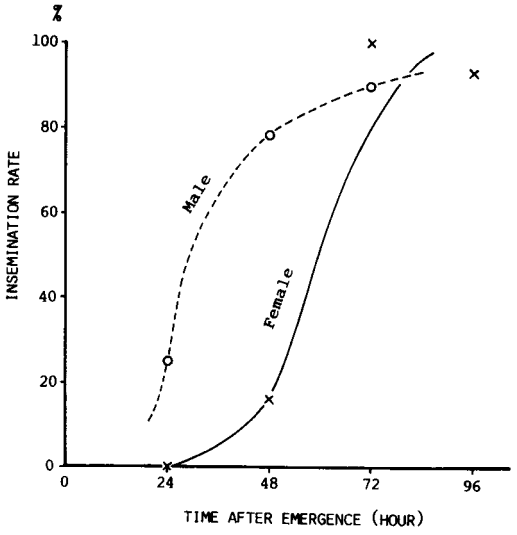

Fig. 1. Time required for insemination of *Tripteroides aranoides*. Ten to 20 females were dissected at each point.

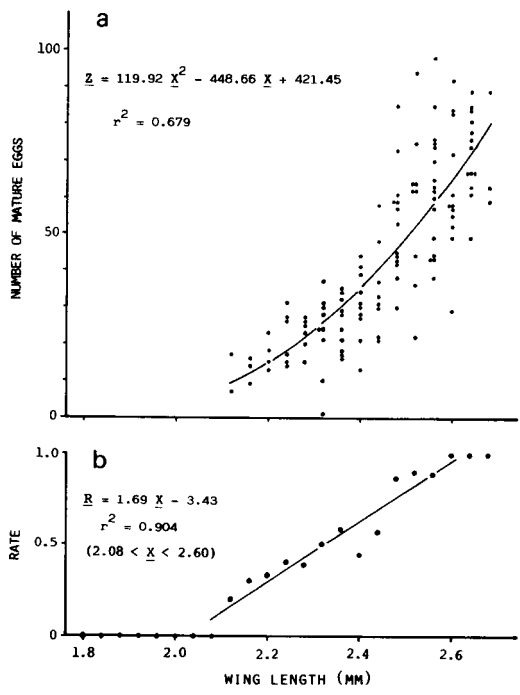

Fig. 2. Autogenous fecundity of *Tripteroides aranoides* in relation to wing length. Adults were fed on 5% sugar solution. a. Number of mature eggs (only females with mature eggs). b. Rate of females with mature eggs.

12 days (Fig. 3); that of the anautogenous second clutch began 4 days after engorgement. Females differed in their egg laying patterns: some laid the majority of the clutch in one day, while others laid small egg batches on different days.

Blood feeding activity started after the beginning of oviposition. Females provided with a bamboo oviposition cup upon emergence took the first blood meal 6 days after emergence or 2 days after the first oviposition. More than 95% of them took a blood meal within 15 days (Fig. 4a). Females not provided with an oviposition cup rarely consumed blood before oviposition (Fig. 4b). Dissection of females soon after taking a blood meal revealed many mature eggs, which indicates that even partial oviposition can release the blood feeding activity.

A blood meal was required for the maturation of the second clutch of eggs. All 25 females which consumed blood had mature eggs. The mean number of mature eggs was greater for the anautogenous clutch (108.1 eggs) than for the preceding autogenous clutch (63.7 eggs). Without a blood meal, follicular development for the second clutch stopped at IIb or earlier stages of Macan's system (1950) in 14 of 16 blood-starved females. The remaining 2 had a small number of mature eggs (1 and 17 eggs). It was uncertain whether those were eggs of the second autogenous clutch or remnants of the first.

EGG RESISTANCE TO DESICCATION. Soon after being laid, eggs could not be stored at 20°C and

Table 2. Effects of larval and adult nutrition on the expression of autogeny in *Tripteroides aranoides*.

Larval diet (g/day/tray)		Sugar solution for adults	No. females		No. mature eggs**	
1st-3rd	4th	(%)	Dissected	Autogenous (%*)	Mean*	Range
0.2	0.5	5	61	57 (93.4a)	65.7a	28–98
		0.1	51	24 (47.1b)	35.0b	7–63
0.02	0.02	5	72	33 (45.8b)	32.4b	13–59
		0.1	35	3 (8.6c)	25.3b	17–36

* Percentages and means followed by the different letters were significantly different by χ^2-test (percentages) or *t*-test (means) ($P < 0.01$).

** For autogenous females.

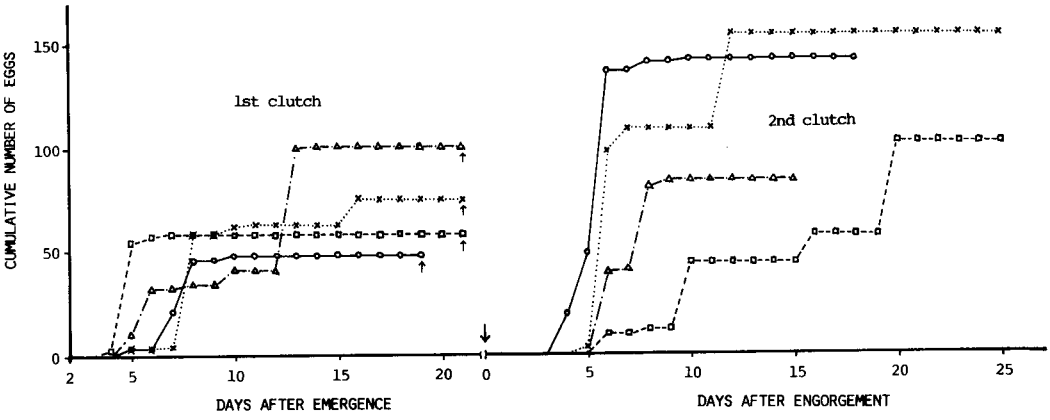

Fig. 3. Egg laying of *Tripteroides aranoides* females individually maintained. Arrows indicate blood-feeding.

80% RH as all the eggs caved-in within a day. Three days after being laid, eggs retained the original shape after drying, but the percentage of flattened eggs increased with time. The hatching rate was above 70% for the first 16 days of preservation, and decreased sharply thereafter (Fig. 5). A few eggs hatched after 35 days of drying, but none hatched after 40 days.

MATING BEHAVIOR. Mating was initiated while both sexes were in flight. A male dashed to a flying female and caught her legs. Then the male slid under the body of the flying female and faced her. The couple flew to the cage wall or to the floor and rested. As soon as connecting his terminalia to that of the female, the male released his grasp and turned his body down; their bodies were then arranged in a straight line, with heads in opposite directions (Fig. 6a). Turning of the male was sometimes finished before reaching the cage wall or floor. In such cases, both sexes beat their wings, but the couple flew in the direction of the female. Copulation continued for an average of 134.3 seconds (range 106–165, n=15) after joining terminalia. The male beat his wings throughout the copulation. When the legs of a copulating male touched the cage floor, wing beating sometimes stopped. Males often hovered above bamboo cups awaiting females coming for oviposition.

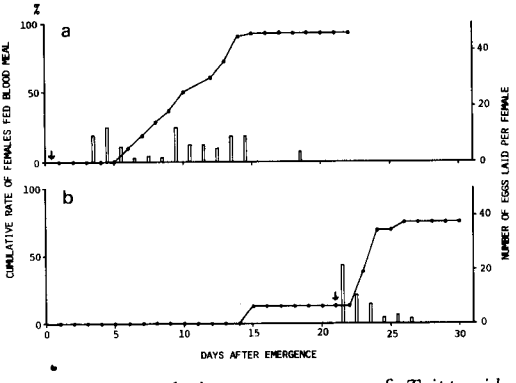

Fig. 4. Cumulative percentage of *Tripteroides aranoides* females taking a blood meal. a. Females given an oviposition cup soon after emergence. b. Females given a cup 3 weeks after emergence. Number of females observed was 43 for a, and 31 for b. Bars show number of eggs laid per female. Arrows indicate when a bamboo oviposition cup was given.

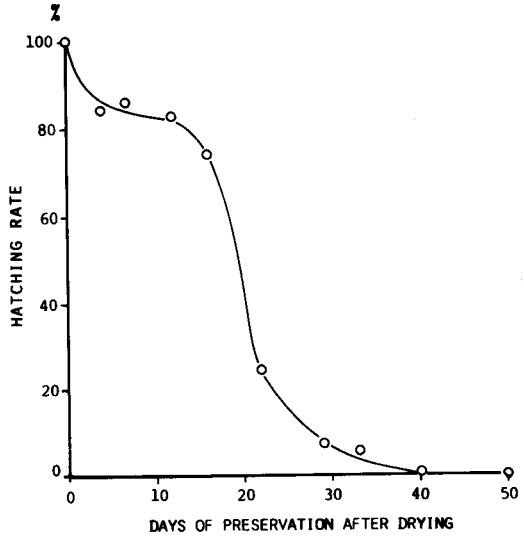

Fig. 5. Relation of hatching rate to duration of dry condition. Eggs were taken out of water 3 days after oviposition. Number of eggs observed was 152–292 per point.

OVIPOSITIONAL BEHAVIOR. Females oviposited in flight. When a closed bamboo internode (12 cm diam and 12 cm height) with a lateral aperture (11 × 4 mm) was offered, gravid females flew around the bamboo internode. A female slowly approached the aperture with her proboscis directed downwards and antennae towards the aperture (Fig. 6b-left). In front of the aperture the female hovered in a small oblique loop several times, whereupon a single white egg appeared at the tip of the abdomen. Occasionally we observed females bearing 2 or 3 eggs. Hovering still for a moment at a distance of 0.5–1 cm from the aperture, the female swung her abdomen towards the target (Fig. 6b-right). The egg was propelled 2.7–4.8 cm horizontally into the aperture. Unless the female swung her abdomen, the egg remained attached to the tip of the abdomen. Sometimes females rested at the aperture with an egg on the tip of the abdomen, but we have never observed oviposition by females at rest on the bamboo, nor did females enter into the bamboo internode through the aperture.

When an open bamboo cup was provided, gravid females hovered in oblique loops at a distance of 2–10 cm from the water surface, whereupon an egg appeared. The female propelled the egg while swinging her abdomen towards the water surface. Some females faced the inside wall of the bamboo cup at a distance of 1–2 cm, and the projected eggs attached to the moist bamboo wall.

In successive ovipositions, the female swung her abdomen once every 10–30 seconds. Since the number of the eggs laid in the bamboo cup was the same as that of the swinging motion, it is assumed that the female released an egg at each time of swinging. When a bamboo internode with an aperture was placed in the cage, the female laid an average of 4.6 eggs (range 1–31, nn = 98) in a single oviposition bout without rest or without flying to any other part of the cage from the oviposition site.

DISCUSSION

Reported lengths of immature stages in *Tripteroides* are 5–6 weeks for *Tp. aranoides, Tp. bambusa* and *Tp. nepenthis* (Edwards) collected from *Nepenthes* in Malaysia (Beaver 1979), 4–6 weeks for *Tp. bisquamatus* Lee (Assem 1959) and 20–29 days for *Tp. bambusa* (Yamada) (Miyagi 1973). These values were 1–2 weeks longer than the present observations for *Tp. aranoides* supplied ample food and reared at higher temperature. The different food conditions and temperatures may have produced different developmental times. Inadequate food prolonged the larval stage in *Tp. aranoides* as in other mosquito species (Moeur and Istock 1980, Mori 1979, Trpis 1979). Frank and Curtis (1977) found that delay in development of *Wyeomyia* larvae occurred principally in the 3rd instar under suboptimal nutrition. In *Tp. aranoides* the developmental delay occurred mainly in the 4th instar. Such instar specificity of developmental delay has not been identified in the other *Tripteroides* species.

Some *Tp. bambusa* females whose immature stages were reared at 15°C and 10 h photophase were autogenous, but at a higher temperature and longer photophase, autogeny was inhibited (Mori 1976). In contrast the rate of autogeny in

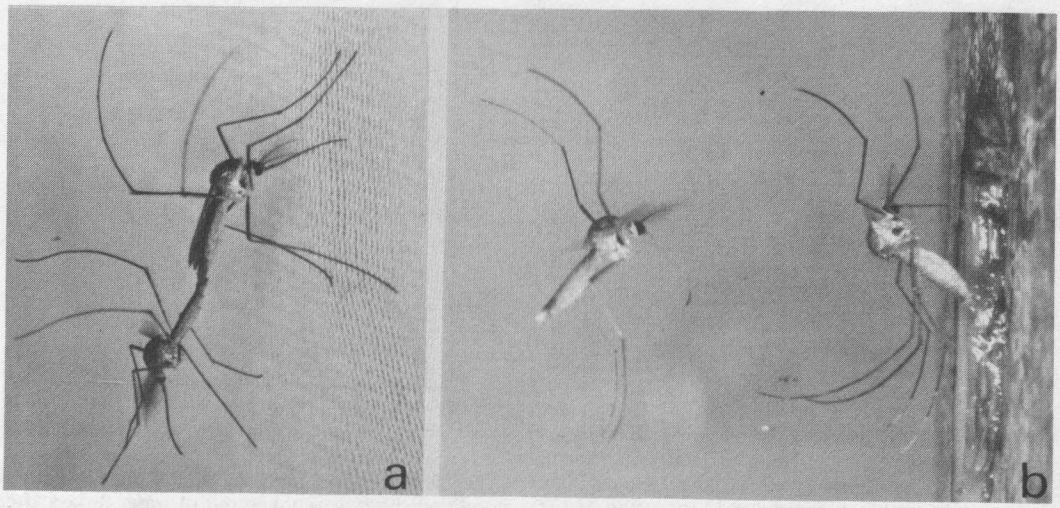

Fig. 6. Behavior of *Tripteroides aranoides*. a. Copulation. Upper mosquito is female and lower male. b. Oviposition into the aperture. Left female in approach and right in oviposition.

Tp. aranoides was very high, even under higher temperature (28°C) and longer photophase (15.5 h). This species may be autogenous for the first egg clutch under natural conditions in Thailand.

Istock et al. (1975) reported an effect of food level on egg production in *Wyeomyia smithii* Coquillett. The number of eggs produced is greater for females fed on adequate food during the larval stage than for those fed on a limited quantity of food. Insufficient food at the adult stage reduced the autogeny rate and the egg number in *Wyeomyia vanduzeei* Dyar and Knab (Nayar et al. 1979). Insufficient food during the larval and/or adult stages affects the autogeny rate and the number of eggs produced by *Tp. aranoides*. This means that the carbohydrates which *Tp. aranoides* adults take can be diverted to oogenesis, or that adults consume nutrients stored during larval life.

In the Chiang Mai area the dry season continues for ca. 6 months (November–April), during which bamboo stumps (the main breeding site for *Tp. aranoides*) seem to dry up. *Tripteroides aranoides* was found only in the rainy season in Burma (Jolly 1933) and Yunnan (Chow 1949). Mattingly (1981) therefore inferred that this species passes over the dry season in the adult or egg stage. Macdonald (1957) reported the eggs of *Tp. aranoides* resistant to desiccation for at least 10 days. In our study, eggs could not withstand desiccation over 40 days under 80% RH, which is 20% higher than the mean humidity in March and April at Chiang Mai. We assume that *Tp. aranoides* continues to breed in concealed habitats such as bored bamboos, in which sap accumulates even in the dry season, rather than pass the dry season in the egg stage.

In the mating behavior of *Tp. aranoides*, two successive steps were recognized: 1) contacting terminalia face to face, and 2) copula with heads in opposite directions. Mosquitoes with a short duration of mating, e.g., *Aedes aegypti* (Linn.) (Gwadz et al. 1971), usually complete insemination at the former step, the latter being absent. Mosquitoes with a long duration, e.g., *Aedes iriomotensis* Tanaka and Mizusawa (Miyagi and Toma 1981), require the latter step for insemination.

The ovipositional behavior of *Tp. aranoides* is similar to that of *Sabethes chloropterus* Humboldt observed by Galindo (1958). Both species oviposit in small vertical holes while flying.

Compared with the clutch size, the number of eggs laid by *Tp. aranoides* females in a single oviposition bout was fairly small. And females often lay eggs of a clutch on different days. This suggests that *Tp. aranoides* females deposit eggs in small batches at several different breeding places. Such low numbers of eggs in egg batches may decrease competition among offspring under limited food supply in small water collections such as bamboo internodes.

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