J.Sci.Soc.Thailand, 20(1994) 125-133

LABORATORY STUDIES ON THE LIFE CYCLE AND BREEDING OF THE MIDGES *CHIRONOMUS PLUMATISETIGERUS* (DIPTERA : CHIRONOMIDAE)

CHALIOW KUVANGKADILOK

Department of Biology, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand.

(Received June 30, 1994)

ABSTRACT

The adults of Chironomus plumatisetigerus *can be bred in the laboratory for at least 9 generations, by using mating cages 37x37x37 cm³, 30.5x30.5x30.5 cm³, 20.5x20.5x20.5 cm³ and 10.5x10.5x10.5 cm³. The adults will mate readily in a cage 10.5x10.5x10.5 cm³ while only some successful matings between individuals have been obtained in the larger cages. In addition, the number of males in the mating cages seemed to affect the success of crosses. For the parental crosses and the F1 self-crosses, at least 25 males were required, while for the self-crosses of F2 and the following generations only 10 males were needed. Laboratory egg masses were collected and checked for fertility and hatchability. The fertility and egg hatchability of the egg masses of F1 and the other generations were rather high (>93%), therefore the laboratory conditions did not seem to affect the breeding mechanisms of* Ch. plumatisetigerus.

The life cycle of Ch. plumatisetigerus *has four stages,as found in other dipterous insects, of which the longest is the larval stage. The head capsule structures of the female larvae are larger than those of the male, so it seems that the male and female larvae have different growth rates.*

INTRODUCTION

The larvae *Chironomus* (Chironomidae) are known as "blood worms" because they are red in color due to the presence of hemoglobin in the blood[1]. They occur in all geographical regions but are more abundant in warmer regions. The chironomid larvae live in the mud or decayed leaves on the bottom of standing or running water of deep or shallow streams. Most of them build larval cases, open at both ends, on or within the substrate they live. The case consists of particles from the substrate and is lined and held together with silk-like threads secreted by the salivary glands[2]. The larvae are chiefly herbivorous and feed on algae, higher aquatic plants and organic detritus. From an economic standpoint, the chironomid larvae are an important item of food for many freshwater fish and other aquatic animals.

The chironomid adults are mosquito-like in appearance but more delicate and with shorter proboscis. The adults do not feed on humans or animals. Moreover, they are not carriers of any diseases.

Many chironomid midges are found in suburban areas of Bangkok. *Ch. plumatisetigerus* is one of the most common species in shallow, permanent or semipermanent waters. It has 4 pairs of chromosomes (2n=8) with 3 metacentric pairs and one short acrocentric pair. Additionally, it is polymorphic for six paracentric inversions on five chromosome arms[3].

Little is known about the life cycle and laboratory breeding of *Ch. plumatisetigerus* in Thailand. This paper therefore reports its life cycle and breeding in the laboratory in order to establish the laboratory stocks for the behavioral, biochemical and molecular genetical studies of this species in the future.

MATERIALS AND METHODS

Larval specimens in this study were collected from a shallow ditch behind the campus of the Faculty of Science, Mahidol University, Bangkok, by using a dip net. Third and fourth instars were gently picked from the sediment, mud and decayed leaves by small forceps, then placed in a plastic container with a 250 cm^2 bottom area and 50 cm high filled to 20 cm with water from the sampled ditch.

In the laboratory the larvae were reared in a controlled temperature room (25°c) with a 12 hr light/12 hr dark cycle. The containers were aerated continuously. Ground mouse pellets (F.E.Zuellig Co., Bangkok) were provided as food at the rate of 0.4 g/week. Two or three Kleenex industrial wipers, washed in absolute ethanol, were shredded and added to provide a substrate on which the larvae could build their cases and feed.

Salivary gland polytene chromosome preparations of the fourth instar larvae were prepared and the banding patterns were checked for species confirmation[3]

1. Mating experiments

Laboratory matings were carried out in cages 37x37x37 cm^3, 30.5x30.5x30.5 cm^3, 20.5x20.5x20.5 cm^3 and 10.5x10.5x10.5 cm^3 in the controlled temperature room as described previously. In each experiment only 10 females were used, but the number of males was varied from 1 to 5, 10, 15, 20,25 and 30. A small plastic container with some water was placed in the cage, in which females could lay their egg masses. Cotton wool stoppers with diluted syrup for adult feeding was also put in the mating cage. Egg masses were collected,checked and scored for fertility and hatchability using a stereomicroscope.

2. Life cycle studies

Each fertilized egg mass was set up individually in a plastic container, containing 1.5 l of distilled water mixed with 1x Martins solution[4]. The basic Martins medium was obtained by adding 1ml/l of 1% $NaHCO_3$, 5% NaCl, 1% $CaCl_2$, 0.2% KH_2PO_4, 1% $MgSO_4$ and 1.8% $MgCl_2$. The medium was supplemented with 0.2 ml/l of either 0.1% $FeCl_3$ or $FeSO_4$. Three washed tissues were added and the ground mouse pellets were provided at the rate of 0.2 g initially and 0.4 g/week. Air was also supplied to the containers to increase the survival of larvae.

Fourth instar larvae were sexed using a stereomicroscope[5]. The growth and development of the head capsules in each larval stage of 20 male and female larvae were measured using an eyepiece micrometer with a binocular dissection microscope (Olympus BS-2).

RESULTS AND DISCUSSION

1. Laboratory self-crosses

The parental crosses and the F1 self-crosses were not successful when the number of males per cage was less than 25. The fertile egg masses were commonly obtained in the cages with at least 25 males. However, successful crosses within the F2 and the following generations occurred in the cages with at least 10 males. Successful mating could be performed for at least 9 generations. There was slight variation in fertility and hatchability of the eggs between generations. All generations showed a high fertility and hatchability of about 93-99% (Table 1). Therefore,the laboratory conditions did not seem to affect the breeding mechanisms of *Ch. plumatisetigerus*. However, the number of males in mating cages seems to affect the success of crosses as found in some *Chironomus* species, such as *Ch. oppositus* which needed at least 20 males for successful crosses[6]. In addition to the number of males, cage size also seems to affect the crosses, since fertile egg masses were more commonly obtained from the smallest cage (10.5x10.5x10.5 cm^3) than from the larger cages.

Why do the fertile crosses of *Ch. plumatisetigerus* need so many males? One possible explanation is that swarming behavior may exist in *Ch. plumatisetigerus*. Most *Chironomus* species form swarms which are mainly composed of males. It is believed that the swarm functions to bring the sexes together to mate[7,8]. In *Ch. riparius*, swarming is an essential precursor to mating and oviposition[9]. Females and males of this species swarm separately, the females swarming below the males, and mating occurs below or at the side of the swarm[10]. Very few species in the genus are known to mate without a swarm although *Ch. tentans* and *Ch. pallidivittarus* do so[11].

Few reports of mating behavior under laboratory conditions exist for chironomids. A few *Chironomus* species can be bred in the lab by single pair mating without forming a swarm, for example *Ch. tepperi*[12], although it is not certain whether the males of this species swarm prior to mating in nature. On the other hand, most species need to form a mating swarm, such as *Ch. piger* and *Ch. thummi*[10].

A knowledge of swarming and mating behavior is essential to a full understanding of the situation in *Ch. plumatisetigerus*. This aspect should be investigated in the future.

2. Life cycle

The complete life cycle of the male *Ch. plumatisetigerus* is about 25 days, while the female has a 27 day life cycle. *Ch. plumatisetigerus* has four life stages, egg, larva, pupa and adult.

Egg. The eggs are laid in a gelatinous mass which is horse-shoe shaped (Figure 1a) and attached to a solid object by a gelatinous stalk. The eggs are spherical and transparent, so the development of eggs can be studied microscopically. All eggs hatch within four days.

TABLE 1. Fertility and hatchability of laboratory egg masses of *Chironomus plumatisetigerus*

Generation	Number of eggs	Average number of eggs	Average fertility (%)	Average hatchability (%)
F1	87-693	474.33 (27)	99.36	98.95
F2	100-593	330.96 (30)	97.36	97.02
F3	119-762	399.40 (40)	95.35	94.67
F4	79-666	339.17 (122)	97.72	96.82
F5	55-724	295.69 (58)	95.13	94.38
F6	75-586	293.37 (54)	97.15	95.51
F7	68-428	238.40 (35)	94.85	93.79
F8	75-450	241.50 (34)	95.50	94.45
F9	158-491	310.50 (14)	96.94	96.46

( ) = number of egg masses

TABLE 2. Growth and development of the head capsules of male and female larvae of *Chironomus plumatisetigerus*

stage	Larval period (days)	Head capsule (average in microns)			
		width		length	
		male	female	male	female
First instar	2	89.3	90.2	110.3	111.0
Second instar	2	168.0	171.2	199.5	206.3
Third instar	2	294.0	352.0	388.5	460.2
Fourth instar (male)	7-8	441.0	504.0	598.5	682.5
(female)	9-10				

Larva. The larva has four stages, first, second, third and fourth instar.

First instar larva. The larvae are in this stage for 2-3 days. Newly hatched larvae leave the egg mass and swim around in the water. They are photopositive, colorless and lack blood gills. They build larval cases using a mixture of tissues and food debris. The larval cases are attached to the wall and bottom of containers. The average width and length of the head capsules are about 89 and 110 microns for males, and 90 and 111 microns for females, respectively (Table 2).

Second instar larva. This stage lasts about 2 days. The bodies are red and the reproductive organs are not developed. The head capsule of males is approximately 168 microns in width and 199 microns in length while the width and length of the female head capsule are about 171 and 206 microns.

Third instar larva. This stage also takes 2 days.The male and female larvae grow rapidly as can be seen from the head capsule structures. However, males and females show differences in size of head capsules (Table 2).

Fourth instar larva. This stage is the longest stage. The females spend 9-10 days in this stage while males spend only 7-8 days. The sizes of males and females are clearly different, the female larvae being larger than males.

The fourth instar larva is elongated, cylindrical and slender (Figure 1b). It consists of 2 parts, the head and the twelve segmented body (Figure 2a). The larval head is rather small and protected on its upper or dorsal surface by three plates, one median and two lateral (Figure 2b). The median plate (clypeus) is used to determine the degree of sclerotization of the head capsules of various *Chironomus* species[13]. The dorsal surface of the *Ch. plumatisetigerus* head shows a slightly dark triangular fronto-clypeus. The clypeus carries the labrum, which hangs like a flap in front of the mouth and can be bent backwards. The lateral plates have two pairs of rudimentary eyes, antennae and jaws. The labial plate consists of 15 teeth with a pointed central tooth and a distinct first lateral tooth (Figure 3a) The mandibles are strong with 4 pointed teeth (Figure 3b). The antennae are small and consist of five segments (Figure 3c).

The reproductive organs of the larva are located on the ventral surface of the twelfth segment. The respiratory organs consist of blood gills, ventral blood gills and anal blood gills. Two pairs of ventral blood gills are on the lateroventral surface of the eleventh abdominal segment. The two pairs of anal blood gills are located at the tail-end (Figure 2b). There is one pair of well developed lateral projections between the eleventh and twelfth segments. Some *Chironomus* species show different lengths of lateral projections, but some species, such as *Ch. pseudo-oppositus*, have no lateral projections[13]. The larva of *Ch. plumatisetigerus* has two pairs of prothoracic appendages. The first pair, on the first thoracic segment, is short, united at the base and armed with numerous hooks used in grasping food,in creeping and in holding on to the borrow. The second pair of appendages, on the last abdominal segment,are anal feet,long stiff locomotive appendages with a few stout curved spines.

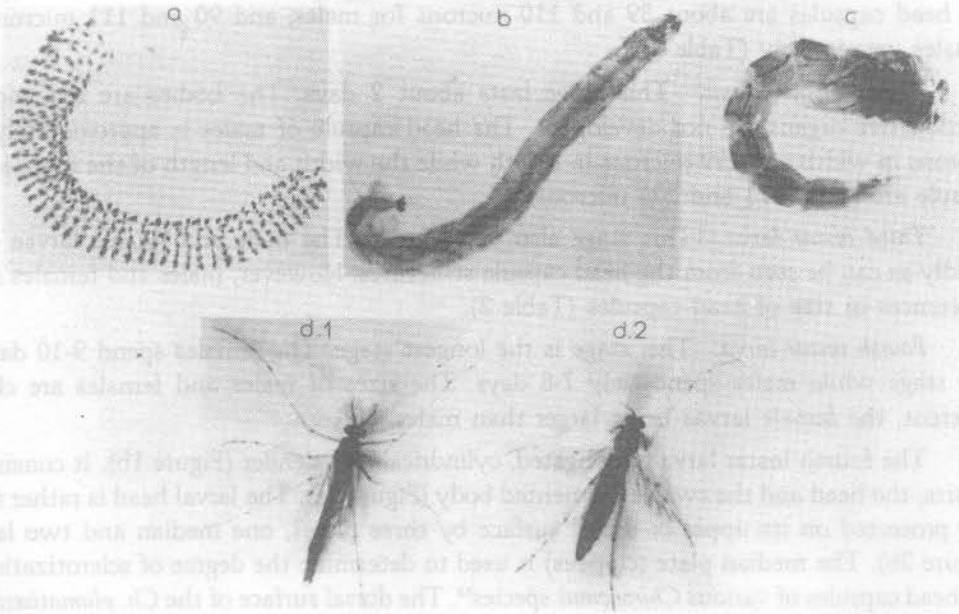

Fig. 1. 1a. Egg mass of *Chironomus plumatisetigerus.*
1b. Larva.
1c. Pupa.
1d.1 Adult,male. 1d.2 Adult,female

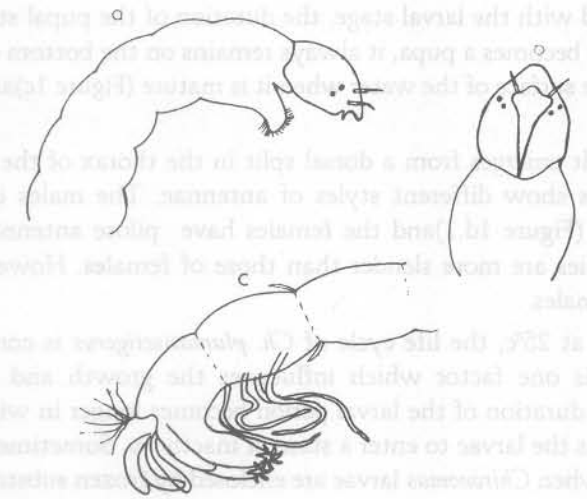

Fig. 2. Larva of *Chironomus plumatisetigerus*.
2a. Side view of a larva showing head and the first five segments.
2b. Dorsal view of the head showing the slightly dark "v" anteriorly.
2c. Side view of a larva showing the last three segments.

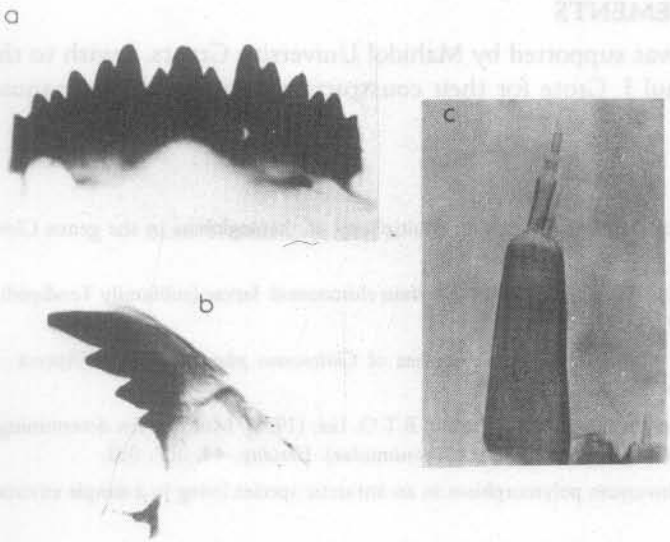

Fig. 3. 3a. Labial plate of a larva of *Chironomus plumatisetigerus* showing the rather pointed central tooth and the distinct first lateral teeth.
3b. Mandible of a larva showing the pointed teeth and the complete fourth teeth.
3c. Antenna of a larva showing five segments.

Pupa. Compared with the larval stage, the duration of the pupal stage is very brief, only 1 day. When it first becomes a pupa, it always remains on the bottom of the container. However, it moves to the surface of the water when it is mature (Figure 1c)and adult eclosion occurs.

Adult. The adult emerges from a dorsal split in the thorax of the pupal skin. The adult males and females show different styles of antennae. The males usually have the antennae very plumose (Figure 1d.1)and the females have pilose antennae (Figure 1d.2). Moreover, the male bodies are more slender than those of females. However, the females have longer lives than males.

In the laboratory at 25°c, the life cycle of *Ch. plumatisetigerus* is completed in 25 to 27 days. Temperature is one factor which influences the growth and development of *Chironomus* larvae[14]. The duration of the larval period becomes longer in winter because the lower temperature causes the larvae to enter a state of inactivity. Sometimes all growth and development will cease when *Chironomus* larvae are enclosed by frozen substrate[15]. In addition to temperature, sex also affects the growth and development of the larvae. This study showed the larger head capsule of the female fourth instar larvae. Similarly, mature females of *Ch. plumosus* were heavier than males[16]. In *Ch. nuditarsus*, the male and female larvae had different growth rates[17]. Additionally, it was found that low oxygen concentration in water reduced growth in *Ch. anthracinus*[18]. Therefore , the duration of growth and development of *Chironomus* species varies under the influence of a number of factors, especially temperature and sex.

ACKNOWLEDGEMENTS

This work was supported by Mahidol University Grants. I wish to thank Prof. Visut Baimai and Dr. Paul J. Grote for their constructive criticism of the manuscript.

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บทคัดย่อ

แมลงหนอนแดงชนิด *Chironomus plumatisetigerus* สามารถผสมและเพาะเลี้ยงในห้องทดลองได้ติดต่อกันจนถึงลูกรุ่นที่ 9 โดยทำการทดลองผสมภายในกรงขนาดต่างๆ กันดังนี้ คือ ขนาด 37x37x37 ลูกบาศก์เซนติเมตร 30.5x30.5x30.5 ลูกบาศก์เซนติเมตร 20.5x20.5x20.5 ลูกบาศก์เซนติเมตร และ 10.5x10.5x10.5 ลูกบาศก์เซนติเมตร จากการทดลองพบว่า แมลงหนอนแดงจะผสมและวางไข่อย่างสม่ำเสมอในกรงขนาดเล็กสุด (10.5x10.5x10.5 ลูกบาศก์เซนติเมตร) ได้ดีกว่ากรงขนาดใหญ่ นอกจากนี้ จำนวนตัวผู้ในกรงยังมีผลต่อความสำเร็จของการผสมพันธุ์ด้วย ในการผสมรุ่นพ่อแม่ (P) และลูกรุ่นที่ 1 (F1) ด้วยกันเองจะต้องใช้ตัวผู้อย่างน้อยที่สุด 25 ตัว แต่ในการผสมลูกรุ่นที่ 2 (F2) และรุ่นถัดไปใช้ตัวผู้เพียง 10 ตัวเท่านั้น เมื่อศึกษาเปอร์เซนต์ของไข่ที่ถูกผสมและออกเป็นตัวของแต่ละรุ่น พบว่ามีเปอร์เซนต์ค่อนข้างสูง (> 93%) และไม่แตกต่างกันมาก จึงดูเสมือนว่าสภาวะของห้องทดลองไม่มีผลต่อกลไกการผสมพันธุ์และเพาะพันธุ์ของแมลงหนอนแดง (*Ch. plumatisetigerus*)

วงจรชีวิตของแมลงหนอนแดงมี 4 ระยะ เช่นเดียวกับแมลงอื่น ๆ ในออร์เดอร์ดิบเทอร์รา (Order Diptera) ระยะตัวอ่อนเป็นระยะที่ยาวนานที่สุด จากการศึกษาการเจริญเติบโตของตัวอ่อนโดยการวัดขนาดของปลอกหุ้มหัว (head capsule) พบว่าตัวเมียมีขนาดใหญ่กว่าตัวผู้ ซึ่งเป็นสิ่งชี้ให้เห็นว่าตัวเมียและตัวผู้มีอัตราการเจริญเติบโตต่างกัน