CREATING AND MAINTAINING CULTURES
OF CHIRONOMUS TENTANS
(DIPTERA: CHIRONOMIDAE)¹

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ABSTRACT: A modified procedure for continuous culture of Chironomus tentans Fabricius, which requires equipment generally available in biological laboratories, is presented. The substrate on which the larvae are reared consists of acetone-treated and boiled paper towels. Liquified vegetable diet is used for more uniform distribution of food in the culture.

Methods exist in the literature for rearing and maintaining cultures of several genera and species of Chironomidae (Biever 1965, Yount 1966; Credland 1973; Downe and Caspary 1973; Gallepp 1979; also see reviews by Fittkau et al. 1976; Merritt et al. 1978) including Chironomus tentans (Sadler 1935; Hall et al. 1970). Major difficulties in methodology have been both biological (usually low survivorship) and physical. Even the best methods require construction of special tanks and cages and then may take a considerable period of trial and error through a lack of specific detail in published methods. It is not unusual that a year or more may elapse before some methods produce enough individuals for experimental needs.

Chironomus tentans, a hardy species, has proven ideal in ecological and physiological studies, as a toxicological test organism in the laboratory, and may be used as a food source for other aquatic organisms. In designing the methods used, we have relied on basic principles, hints from the literature and three years of our own trial and error. Equipment needed is minimal and generally available in most types of biological laboratories. The methods should be applicable to any of the tube-dwelling, filter feeding or grazing Chironomidae (Leathers 1923).

The quantities given below will create one “continuous” culture in a standard 38 l (10 gal.) aquarium. We do not recommend larger aquariums as they prove to be much less productive per unit area. Aquariums as small as 4 l (1 gal. glass jars) can be used effectively. One culture should yield up to 20 larvae per day. This is equivalent to 180 mg of 3rd instar or 300 mg of 4th larval instar.

Substrate: C. tentans prefers a soft, flocculent substrate (Sadler 1935) which can be artificially duplicated by ground and shredded paper toweling. To achieve suitable texture and to remove impurities, the paper is soaked in acetone and then boiled. If the chironomid larvae are to be used in tests with

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toxic organic compounds, any residual acetone left in the toweling will affect the results even if present only in trace amounts. In this case all the acetone must be removed by keeping the paper in boiling water for at least 48 hours with four or five complete changes of water. It may be desirable to process large batches of paper at one time which then can be kept frozen until needed (R. Mazzone, pers. comm.).

Soak 12 sections (approx. 50 gms) of Scott®, Nibroc® or an equivalent type of brown paper hand towel (26x10 cm folded two-ply) in enough acetone to keep them wet in a closed glass container for at least 30 minutes. Squeeze out the acetone and replace it with a fresh amount for a second and third 30 minute period. If a Soxhlet acetone extractor is available, the acetone may be reused. Rinse the towels in distilled water or carbon-filtered water four or five times until the strong odor of acetone is removed. Reboil the paper in distilled or carbon filtered water for 1 hour or until most of the color is removed — brown towels will remain a light tan. Finally, cut or tear the towels into smaller pieces and shred to a coarse pulp using a blender.

Aquarium assembly: A simple aquarium and adult capture system is given in Fig. 1b. The aquarium is of a standard 38 l (10 gal.) size measuring approximately 26x41x21 cm. The bag (1-2 mm coarse mesh cloth) will effectively contain emerging adults even when loosely fitted to the aquarium. Access to the inside of the bag is through two overlapping flaps that may be closed and fastened by a few pins. Strings attached to the four corners are tied to any fixed structure above the aquarium to hold the bag in place.

Starting cultures: In a 38 l aquarium, place 10 l of carbon-filtered or conditioned tap-water (water exposed to the atmosphere and aerated for 3-4 hours.) Add the shredded towel, 1 ml of prepared food (see below), and mix thoroughly. Allow 1 hour of settling time which should produce a substrate layer 2.5-4.0 cm thick. Carefully add enough additional water to create a 3 cm clear layer over the substrate. If any substrate is resuspended during one of the steps, allow time for it to resettle. The air supply to the tank should be at a rate that does not resuspend the substrate. This may be done by suspending an airstone at a level just below the surface of the water. (Fig. 1b). Two or three egg masses obtained by the method below may now be placed very gently on the surface of the substrate.

Food and feeding: Several types of food have been used in maintaining larval Chironomidae with varying degrees of success (Biever 1965). We have chosen the following composition because it can be liquified and thus more uniformly distributed in the culture. Food is prepared by blending 20 gm “Tetra® Conditioning Food, Vegetable Diet for Tropical Fish” with 200 ml distilled or carbon-filtered water. Prepared food should be kept
Fig. 1. Equipment employed for forced matings and egg collection of *Chironomus tentans* (A), and oblique view of established culture aquarium with adult capture bag (B), measurements for aquarium and bag are in centimeters.
refrigerated. Shake the mixture well and add about 1 ml at the start of each culture and after every change of water. The amount of food added depends on the density and age of the larvae. If too much food has been added, the water will appear cloudy the next day. If the water remains cloudy, it should be replaced.

Maintaining cultures: Because nutrients and byproducts build up quickly, at least part of the water should be changed every 4-7 days. Surface water is siphoned off down to a level just above the substrate. Freshly prepared water plus 1 ml of food is added slowly until the original depth is reached.

Continuing and starting new cultures: At 21 C, egg masses hatch 2-3 days after deposition. 1st instars appear in 3-4 days, 2nd instars in 6-8 days, 3rd instars are present after 12-14 days, 4th instars appear around the third week, and adults begin to emerge after 4-5 weeks. The generation of larvae will be continuous to some degree if left undisturbed because a small percentage of the adults will mate and some egg masses will be deposited in the culture. To maintain healthy cultures, a more forced type of mating is recommended. Adults are aspirated into a dry 250 ml Erlenmeyer flask (Fig. 1a) which is then loosely stoppered with cotton (Fig. 1a). Three or four pairs of males and females should produce enough eggs to begin a new culture. Adults are left to mate in the dry flask for several hours, then a volume of 50 ml of conditioned water is gradually added. The flask is set at a slight angle so that most of the water is at one side. Eggs are deposited before dawn, so the age of the mass can be determined. Eggs may be used to restock old cultures, start new ones, or used in experiments that require this life stage. A new egg mass should be added to ongoing cultures every 2-3 days for maximum harvest and emergence rates.

If maintained as above, a culture should be productive for about 6 months. After that time the old culture should be discarded.

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LITERATURE CITED


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12 March, 1982

The Commission hereby gives six months’ notice of the possible use of its plenary powers in the following cases, published in the Bulletin of Zoological Nomenclature, volume 39, part 1, on 11 March, 1982, and would welcome comments and advice on them from interested zoologists. Correspondence should be addressed to the Secretary at the above address, if possible within six months of the date of publication of this notice.

Case No.

2067  *Thrips rufa* Haliday, 1836 (Insecta, Thysanoptera, Thripidae): proposed ruling that this is a nomenclaturally valid name for the type species of *Aptinothrips* Haliday, 1836.

2169  *Phrynus* Lamarck, 1801 (Arachnida, Amblypygi): proposed conservation.

2305  *Agrotis redimicula* Morrison, 1875 (Insecta, Lepidoptera): proposed conservation from 1874.

2346  *Buprestis nana* Paykull, 1799, *non* Gmelin, 1790 (Insecta, Coleoptera): proposed conservation.

2351  *Papilio fatima* Fabricius, 1793 (Insecta, Lepidoptera): request for conservation under the plenary powers.

2355  *Attus otiosus* Hentz, 1846 (Araneae, Salticidae): proposed conservation under the plenary powers.

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