LABORATORY BIOLOGY OF THE RICE MIDGE, *CHIRONOMUS TEPPERI* SKUSE (DIPTERA: NEMATOCERA): MATING BEHAVIOUR, PRODUCTIVITY AND ATTEMPTS AT HYBRIDIZATION

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Abstract

Single pair matings of *Chironomus tepperi* were carried out with the female active or anaesthetized. Males mated readily in both situations, with little evidence of courtship behaviour. Mated females resist further matings, passively at first but later adopting the "avoidance posture". Females deposited eggs in gelatinous masses: unfed females laid only one or two egg masses for an average of 351 eggs while fed females laid up to six egg masses of progressively reduced size for an average of 531 eggs. *Ch. tepperi* males were also mated to anaesthetized females of other species. Although successful copulation occurred, no fertile egg masses were obtained.

Introduction

Previous studies have indicated that the formation of large swarms is probably a prerequisite to mating in most Chironomidae (see Gibson 1945, Downes 1969, Oliver 1971 for reviews) and that successful laboratory colonization seems to be restricted to those species in which such large swarms are not essential. It has been noted by Scharf (1969) that the widespread Australian species, *Chironomus tepperi*, can be readily bred in the laboratory for at least 13 generations. This species is of considerable interest since it rapidly colonizes freshly inundated areas (Edward 1964), sometimes in such density that it causes severe damage to newly sown rice crops (Hely 1958, Jones 1968).

The ability to establish laboratory stocks offers potential for genetical, behavioural and biochemical studies. Information on the actual sequence of events associated with the mating process and estimates of the productivity of females are essential to provide baseline data both on the laboratory behaviour of the insect and on its potential use in hybridization studies.

Materials and methods

The *Ch. tepperi* strains used in this study came from: Somerset Dam in Queensland; Arrawarra, Deniliquin, West Wyalong in New South Wales; Carrum, Harrow, Jamieson, Werribee, Woohlpooer in Victoria; Kimba in South Australia and Kalgoorlie in Western Australia. Strains were established either from wild caught larvae or from egg masses deposited by wild caught females. As far as possible the individuals used were adults from the first generation after the strain was established in the laboratory.

Other species used in hybridization experiments were: *Chironomus magnivalva* Kieffer from Sarina, Queensland and Nadi, Fiji; *Ch. oppositus* Walker from North Kew, Wartook Reservoir (Grampians) in Victoria and Warrens Gorge (Flinders Ranges) in South Australia; *Ch. cloacalis* Atchley and Martin from Fernvale, Queensland and Werribee, Victoria; *Ch. alternans* group sp.a[1] from Fernvale, Queensland; *Ch. alternans* group sp.b[2] from Eildon, Victoria and Warrens Gorge, South Australia; *Ch. nepeanensis* Skuse from Somerset Dam, Queensland; *Kiefferulus intertinctus* (Skuse) from Somerset Dam, Queensland; *Kiefferulus* sp.[3] from Werribee, Victoria; and *Nilodorum biroi* (Kieffer) from Somerset Dam, Queensland.

1. Mating experiments

These experiments were of two types: (a) those in which both sexes were active, and (b) those in which the male was active but the female was anaesthetized.

(a) Both sexes active: One male and one female were introduced into a $15 \times 2\frac{1}{2}$ cm vial with a cotton wadding or foam plastic stopper. To determine "mating speed" as in *Drosophila* mating experiments (see for example Kaul and Parsons 1965), the stopper was pushed halfway down the vial (to restrict the space available) and the vial was placed upright on the bench. The length of time the flies remained in copulation (the "Duration of Copulation" of *Drosophila* experiments) was also determined from these experiments as well as from others in which the vial was shaken to bring flies into contact before placing the vial on the bench. Matings of single pairs in vials will be referred to as "pair matings".

(b) Male active but female anaesthetized: In these experiments the female was lightly anaesthetized with anaesthetic ether before being introduced into the shell vial with a male. The vial was then shaken to bring the male and female into contact as quickly as possible so that copulation could occur before the

[1] In other publications this species has been referred to by the *nomen nudum Ch. februarius* (Martin 1967) under which name it will ultimately be described

[2] This species will ultimately be described as *Ch. pseudoppositus* (see Martin 1966).

[3] This species will ultimately be described as *Kiefferulus cornishi*.

female revived. After initial experiments with *Ch. tepperi* females, this technique was subsequently used with females of other species in attempts to obtain interspecific crosses. The females often appear not to recover fully from the effects of the ether. Fowler's (1972) experiments with mosquitoes suggest that an alternative anaesthetic, such as nitrogen, may be more suitable.

2. Oviposition

Mated females were isolated in a vial containing about 2 cm of distilled water. A toothpick angled across the vial was provided for the female to rest on while laying. In experiments set up after November 1972, food in the form of a dilute treacle solution was provided on a strip of paper tissue, since Goff (1972) reported that fed *Chironomus* females survived longer and laid more fertile egg masses.

Results

1. Mating experiments

(a) Both sexes active: *Chironomus tepperi* appears to have little recognizable courtship behaviour prior to mating. No activity similar to the "boxing-like" behaviour reported in *Glyptotendipes paripes* Edwards by Nielsen (1962) was observed. Sometimes the male would show obvious signs of preparing to mate prior to approaching the female, by rotating the hypopygium and opening and closing the claspers. More often he would attempt to copulate with the female as soon as they came into contact, the coupling process occurring so rapidly that it could not be readily followed by eye. Mating speed ranged from one second to 560 s for 17 matings. In experiments with *Drosophila* the mean value is generally quoted (e.g. Kaul and Parsons 1965), 136 s in the present experiment, but the median value of 70 s is probably more meaningful in view of the spread of the data.

The process of mating was similar to the "substrate copulations" of *Allochironomus crassiforceps* Kieffer (Syrjämäki 1964), with the male mounting in the "face-to-back" position and then generally switching to the "end-to end" position. Also, on occasions, the male would mount the female in the "face-to-face" position, particularly if the two midges had been shaken down together at the bottom of the vial.

A mean duration of copulation of 44 s was obtained in 45 matings (range: 18 to 147 s).

Males were able to mate many times. Quite often the male would attempt to mate again within 30 s of the initial copulation. Several males were mated to more than one female and, when sufficient virgin females were available, attempts were made to find out how many times a male could mate, and whether all such matings were fertile. The results were variable as was reported for *Ch. riparius* Meigen (Downe 1973). However, on two occasions males were mated ten times over a period of three days, using a different female for each mating. In one case the third female laid an infertile egg mass, probably due to an unsuccessful mating (see p. 8). For the other nine matings the fertility of the egg masses was over 90% until the tenth, when it dropped to about 50%. This male provided enough sperm to fertilize 4339 eggs out of the total 5025 eggs laid by the ten females. The other case provided incomplete data but the result was different in that the egg masses laid by at least the last two females were infertile.

Females, on the other hand, will apparently mate only once. If a male attempts to copulate with a previously mated female within a few hours of her mating, she will press herself close against the surface on which she is resting, but otherwise appears completely passive. The male, however, is quite unable to couple with her. This is apparently not due to any physical change in the female terminalia because, in experiments with etherized females and in one case where the female was accidentally squashed during the experiment, the males coupled readily; so that the female must be taking some action to prevent further matings.

Some hours after mating, the female ceases passively to resist the male and instead actively tries to escape his attentions by running away and tucking the abdomen well in under her as in the "avoidance posture" (Beerman 1955, Hein and Schmulbach 1971). This behaviour was also noted in some females more than 24 hours old who had not mated. This is a partly subjective observation since females which would not mate were often not recorded. However, in the recorded data there were four out of 14 females more than 24 hours old which would not accept the male, compared with only three of 45 females less than 24 hours old. It may therefore be a reaction partly due to age rather than specifically due to previous mating. Mating can apparently occur at any time of the day as in *Ch. pallidivattatus sensu* Edwards (Hein and Schmulbach 1971).

(b) Male active but female anaesthetized: In these experiments with *Ch. tepperi* the behaviour of the males was similar to those in which the female was active, except that the initial coupling was almost always in the "face-to-face" position with the female lying on her back. After coupling, the male generally switched to the "end-to-end" position by either turning the female upright or by flipping on to his back so that both were lying ventral side upermost. The mean duration of copulation in 23 trials was 43 s (range: 24 to 130 s). This figure is almost identical to the result in the experiments using active females.

2. Hybridization studies

The success of matings using an anaesthetized female opened the possibility of using *Ch. tepperi* males for hybridization studies. With the exception of *Ch. magnivalva*, which also "pair mates" and where matings in both directions were obtained, no successful copulations could be obtained with active females because the female always showed the "avoidance posture". A series of hybridizations, using anaesthetized females of species which appeared cytologically closely related to *Ch. tepperi*, as well as others quite distantly related and often quite different in colour pattern, was attempted. A total of seventeen copulations was obtained with *Ch. magnivalva* (2 matings), *Ch. oppositus* (3), *Ch. alternans* gp.sp.b. (2), *Ch. nepeanensis* (2), *Ch. cloacalis* (7) and *Nilodorum biroi* (1). The mean duration of copulation was 44 s, which is almost identical to the value obtained for conspecific matings. This suggests that the copulations were successful in terms of the male transferring sperm, and one *Ch. oppositus* female dissected following mating did contain sperm. However, no fertile egg masses were obtained, the majority of females laying no egg mass at all.

The main barrier to successful copulation did not appear to be phylogenetic relationship, as evidenced by intergeneric matings, but size disparity. The smaller *Ch. tepperi* males had most difficulty trying to mate with females of a larger species such as *Ch. nepeanensis*. The male appeared to orient himself over the female thorax and, if he could not reach the female terminalia from this position, attempted to copulate with any part of her body with which his terminalia came in contact—the edge of the wings, the hind legs or, on turning around, with her head and neck. Only rarely did he move away from his position over the thorax to follow the abdomen down to the terminalia. Similar behaviour was noted in males attempting to copulate with mated females during their passive stage and also in males attempting to copulate for the first time, as noted in *A. crassiforceps* (Syrjämäki 1964).

3. Productivity Estimates

Productivity was estimated using the number of eggs produced, the fertility estimated by the percentage of eggs showing some evidence of development, and the hatchability.

Results from all mating experiments can be treated together as all females were set up in identical conditions once the anaesthetized females had recovered. However, anaesthetized females were more likely to drown, and so only those cases in which females actually laid were used in subsequent calculations.

In early experiments, prior to November 1972, the females were not provided with any source of food and generally laid only one egg mass. Some did lay a second, and one a third egg mass. From November 1972, when the females were provided with food, their survival was much better and they regularly laid three or more egg masses. Several laid five egg masses and three laid six. The usual time interval between successive egg masses was about two days. The average number of eggs laid by 27 fed females was 531, with about 85% fertility, compared with 351, with about 87% fertility, by 40 unfed females. This difference in fertility is not significant. About six percent of mated females laid an infertile egg mass and these have been included in the calculation of fertility and hatchability. A maximum estimation of fertility of about

TABLE
SIZE AND FERTILITY OF SUCCESSIVE EGG MASSES OF *CH. TEPPERI*

Egg mass	Size range	Average size	Average fertility (%)	Average hatchability (%)
First	120-679	344.0 (63)	86.2	76.5
Second	68-266	131.8 (27)	85.7	74.7
Third	20-150	75.9 (15)	71.5	65.8
Fourth	11-56	29.1 (9)	49.8	49.8
Fifth	7-31	16.8 (6)	54.6	54.6
Sixth	1-7	4.3 (3)	0	0

Figures in brackets indicate the number of egg masses on which the averages are based.

93% can be obtained by excluding these cases from the calculation on the basis that they were due to failure to transfer sperm. There are however other possible causes for such infertility, including sterility of one of the parents.

Successive egg masses showed a progressive reduction in size (Table). First egg masses ranged from 120 to 679 eggs and the size of the egg mass appears to be correlated with the size of the female (correlation coefficient of egg number to wing length = 0.89). Average size was 344 eggs and the average fertility was 86%. However, some of the eggs which began developing did not hatch and the average hatchability was only 77%. This was particularly evident in the material from Kalgoorlie, W.A., in which some egg masses showed about 99% fertility but only between one and 15% hatchability.

Second egg masses were about one third of the size of the first egg masses and averaged 132 eggs. The average percentage of fertile eggs was 86 and an average of 75% hatched. The third egg masses were about one sixth of the size of the first and averaged about 76 eggs of which, on average, 72% developed and 66% hatched. The fourth egg masses averaged about 29 eggs with an average of 50% developing and hatching. The fifth egg masses had an average of 17 eggs and in this small sample about 55% developed and hatched. This apparent increase in fertility from fourth to fifth egg masses can be ascribed to sampling error. Fourth and fifth egg masses showed a great variability in fertility ranging from complete infertility to complete fertility (in fourth egg masses) or 75% fertility (in fifth egg masses).

The three sixth egg masses contain one, five and seven eggs respectively. None of these showed any development. It would therefore seem that a single female is capable of laying over 1000 eggs (largest number recorded 1164) with an overall hatchability of about 90%.

There was no evidence of any genetical incompatibility in crosses involving geographically widely separated populations. The results are a little difficult to interpret because the material from Kalgoorlie, W.A. used in many of these crosses, had a broad range of fertility even in intrapopulation crosses. However, some crosses between individuals from Kalgoorlie and from Woohlpooer, Vic. did produce egg masses with a fertility of over 95%. Other crosses involving Harrow, Vic. and Arrawarra, N.S.W. or Werribee, Vic. and Somerset Dam, Qld. were virtually 100% fertile. Unfortunately no crosses could be made between Western Australian and Queensland material because the appropriate strains were not available in the laboratory at the same time.

Some observations were made on virgin females. When given the opportunity, many will lay an egg mass, some may lay two and one laid three. However, the time between layings is longer and much more variable than with mated females. The first egg mass may not be laid for 12 days although the average is about three days after emergence with about three days between successive egg masses.

Discussion

Relatively little is known about the mating behaviour of *Ch. tepperi* in the field. It is not certain that males swarm prior to mating, or if they mate with any female on contact, or whether both methods are utilised as in *Chironomus (Camptochironomus) tentans* F. (Sadler 1935), *Glyptotendipes paripes* (Nielsen 1962) and *Allochironomus crassiforceps* (Syrjämäki 1964). Syrjämäki (*op. cit.*) suggests that very high population densities lead to contact matings with no complex courtship pattern, similar to the matings of *Ch. tepperi* under laboratory conditions. However, it does not appear that such high densities of *Ch. tepperi* regularly occur in the wild. For a colonizing species such as *Ch. tepperi* (Martin 1974 and unpublished data) it would seem of advantage to be able to dispense with the necessity to swarm prior to mating. Oliver (1971) suggests that chironomids are specifically adapted to long flights on the wind after emergence. Normally this is to a resting site beside the larval habitat (Nielson 1962) but a colonizing species could use this wind blown flight for dispersal provided high densities in a swarm were not subsequently required for mating. No field observations on mating or dispersal have been recorded for *Ch. tepperi*. It is also unknown how many times a male mates in the field, but these laboratory experiments indicate that he is capable of mating many times and fertilizing as many as 4000 eggs.

Since males of species which lack courtship behaviour are liable to attempt copulation with dead females or females of other species (Syrjämäki 1964), we attempted to obtain interspecific and intergeneric matings using anaesthetized females. No fertile egg masses were obtained, but sperm transfer was demonstrated. The technique thus seems to have promise and suggests that post-mating genetic sterility barriers exist between *Ch. tepperi* and those species used in the experiments.

The females mate only once under laboratory conditions, therefore it is almost certain that this will be the situation under natural conditions. Similar results have been reported for *Ch. tentans* (Beermann 1955), *Ch. pallidivittatus* (Hein and Schmulback 1971), *Ch. riparius* (Downe 1973) and in *Clunio aquilonius* Tokunaga and *C. tsushimensis* Tokunaga (Hashimoto 1969). *A. crassiforceps* shows a rather different behaviour since the females will copulate repeatedly (Palmen 1962, Syrjämäki 1964). In another Australian and Pacific species, *Ch. magnivalva*, some females will mate twice (in three of fourteen tested) sometimes within a few minutes of the first mating (Martin, unpublished data). Data from a much greater range of species is necessary before making any general conclusion as to which type of behaviour is the most common.

The situation with respect to egg laying as reported here is somewhat different from previous reports for *Chironomus* species, possibly due to the provision of food to the females. The earlier reports have indicated that females were capable of laying three egg masses of progressively smaller size (Wensler and Rempel 1962) although none have reported cases of females laying more than two (Sadler 1935, Fischer 1969, Oliver 1971). The present experiments confirm that the successive egg masses are smaller but do not agree with the inference by Wensler and Rempel (1962) that a female may only oviposit three times. The additional egg masses may be due to asynchrony in the development of oocytes in the three series of follicles, or to subsequent oviposition of eggs trapped in the oviduct. Providing the females with food has led to an increase in the estimates of their productivity. Thus, using the earlier techniques with unfed females, we obtained an average of 351 eggs, but with feeding this has increased to an average of 531 with some females laying over 1000 eggs. It would seem likely that females feed in the wild, but the number of egg masses they lay is unknown. Some wild caught females have laid what appears to be a second egg mass shortly after capture, but not a later egg mass.

Ch. tepperi obviously has considerable potential for use in genetical and other studies. Individuals will "pair mate" and a single mating is capable of producing large numbers of offspring. Thus genetically defined stocks could be produced and maintained although periodical outbreeding might be needed as individuals appear to become smaller and weaker after some generations of inbreeding (Martin and Porter, unpublished data). Males of *Ch. tepperi* could be used in hybridization experiments with a broad range of other species from the genus *Chironomus* or other genera. The main restriction, at least with related genera, would appear to be extreme differences in size between the male and the female. Whether viable offspring may be obtained will depend on the nature and extent of a post-mating genetic sterility barrier which appears to be present in the crosses performed so far.

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