

# DISTRIBUTION OF MATING FREQUENCY AMONG MALES OF THE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT), IN RELATION TO TEMPERATURE, ACCLIMATION AND CHANCE

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## Summary

Male *Bactrocera tryoni* tend to mate on several occasions when given the opportunity. The distribution of mating frequency among individuals at normal culture temperatures appears to be determined mainly by chance thus it is unlikely that male mating success could be improved for SIT by selection. However, at near-threshold temperatures there may be some variation in mating frequency due to variation in innate mating propensity and hence scope for selection for such conditions. There is a general lowering of mating frequency at lower temperatures. The very low mean frequency that is observed at 18°C, which is just above mating threshold, is not due to most flies being below threshold but is due to most flies mating, each with a low frequency. Prior temperature acclimation had no significant effect on the results.

## INTRODUCTION

If males of a species can mate several times, the frequency of mating can be a fitness character that would be subject to natural selection—or to artificial selection in the case of insects that are mass reared for the sterile insect technique of population control (SIT). However, because of selection, fitness characters tend to have little variance within a population and much observed variation can be due to chance, especially if the character can only be measured by frequency such as the probability of mating (Falconer 1981). Reasons for variation in mating success have been sought in terms of variation in size, symmetry, other anatomical features and behavioural traits but random variation can be a large, and sometimes apparently the only, contributing cause (Sutherland 1985; Bradbury and Andersson 1987; Ueno 1994; Delisle 1995; Parsons 1997; Liimatainen *et al.* 1997; Jaastad 1998).

Thus although it would be desirable to start and maintain a strain of insects for SIT using males that mated most often, it would be of little use if the males so selected happened to mate most often merely by chance. It is to be expected that laboratory strains of insects can be inadvertently selected for a number of characters including mating propensity (Harris *et al.* 1986). However, this selection would occur at the normal laboratory temperature which would normally be optimum. Selection for mating propensity at lower temperatures, for lowering of temperature threshold and for ability to acclimate in respect of these would not have occurred in such cultures. Thus it is possible that more variation could be seen in mating frequency at lower temperatures, especially near to the threshold and among insects previously acclimated to cold. If this were so it would be possible to select strains for SIT in colder weather as in the strategy for

prophylactic releases proposed by Fay and Meats (1987).

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) mates at dusk and there is usually only enough time in any one dusk period to mate once, although males can mate in many dusk periods over a lifetime (Fay and Meats 1983). Fay and Meats (1983) have shown that mating frequency at a given dusk varies with temperature but that cold acclimation had no significant effect. However, there has been no data available on the distribution among males of the frequency of mating at any temperature. In particular, it is not known whether the mating that occurs at temperatures near to the lower threshold is due to only a few males being 'above threshold' or to most males being above threshold but each mating at a low frequency.

This paper investigates the probability of mating during a given dusk at a range of temperatures from 16°C (virtual threshold) to optimum (25°C). It also reports on frequencies of multiple mating in males at 18°C, 20°C and 25°C in order to establish whether the distribution is non random (hence selectable) and whether a tendency for non randomness increases at lower temperatures. In particular, these data are analysed to determine whether the reduction in mating frequency at lower temperatures is due to only a small (selectable) proportion of males being 'above threshold' or whether all males are affected equally so that they all retain the ability to mate at a low temperature but do so less often.

Experiments were done with both warm-acclimated and cold-acclimated flies because variation in the ability to cold-acclimate would also be a further cause of a non-random distribution of mating and therefore a further basis for selection.

Observations were made over several weeks, so that it was also necessary to distinguish the effects of mortality from other causes of variation in mating over such an extended period.

## MATERIALS AND METHODS

### *Culture and testing procedures*

Flies were obtained from a laboratory culture (Bateman 1967) which was approximately 30 generations old. They were reared either entirely at 25°C (warm-acclimated) or were cold-acclimated during development according to the procedure of Meats and Fay (1976) and kept in a similar temperature regime (25°C for 8 h per day, 12°C for 16 h per day) except when being tested for mating frequency. In both rearing regimes the photoperiod was 16 h with the last hour being at dusk levels (10 lux); in the 'cold acclimation' regime dusk occurred during the final hour of the period at 25°C.

For testing, warm-acclimated and cold-acclimated males were caged separately with warm-acclimated virgin females for 3 h per week over a 6 or 7 week period. At each test temperature (18°C, 20°C or 25°C) both types were kept in the same environment cabinet in which lighting was reduced to dusk levels (10 lux) for the third hour; this occurred at the same time as dusk was experienced under the 'cold' or 'warm' rearing regimes.

Each set of observations at each temperature started with a cohort of 50 males of each acclimation type. In order to disperse acclimation types within a cabinet, each cohort was caged as 2 sub-cohorts of initially 25 males each; the cage dimensions were 350×350×450 mm. The number of females introduced to a cage for each test was always equal to the number of males surviving in that cage. Mating pairs were removed during the 1 hour dusk period and any unmated females removed after it. Any male mating in a particular week was marked with paint on a specific spot on the thorax and returned to its original cage.

A separate trial at 16°C was run to establish the mean mating frequency of the two acclimation types at that temperature.

### *Probability of mating and the binomial expectation*

The usual method of calculating a binomial expectation would involve estimating the probability of mating ( $p$ ) on any occasion ( $i$ ) from the average number of matings per male observed over the total number of occasions. The assumption would be that each individual has the same probability of mating and that this would be the same on any occasion. This assumption is obviously violated when the observation period (as here at six to seven weeks) is

long enough for mortality to occur. Mortality in the experiments described here, commenced at two to four weeks (depending on treatment) and accumulated to 10–18% over the remaining period. The value of  $p$  was therefore calculated so that it was based on the sum of males remaining alive at each occasion, viz:  $p = T / \sum m_i$  where  $T$  is the total number of matings over  $n$  occasions,  $m_i$  is the number of males alive on a given occasion ( $i$ ) and the summation made for values of  $i = 1$  to  $i = n$  inclusive. The assumption is henceforth that each male has the same probability of mating as any other male on any occasion as long as it is still alive. This assumption allows the calculation of the expected distribution of mating frequency for any number of occasions and any schedule of mortality.

### *Probability of mating a given number of times*

When (as here) a fly can mate no more than once per occasion, the binomial probability of mating  $r$  times in  $n$  occasions is

$$W_{r(i)} = n! / [r(i)!(n - r(i))!] \cdot p^{r(i)} q^{n - r(i)}$$

where  $r(i)$  is a number from zero to  $n$  inclusive,  $q$  is  $1 - p$ ,  $n!$  is  $n$  factorial, and  $\sum W_{r(i)} = 1$ .

If no mortality had occurred then the number of flies in any treatment expected to mate 0, 1... $n$  times could have been found by multiplying the pertinent value of  $W_r$  by the number of flies starting the trial. However, as mentioned earlier, up to 18% of flies did not survive for  $n$  occasions. Thus for each treatment we calculated a set of  $W_r$  values for each survival category, that is a set of  $W_r$  values for those surviving for  $n$  occasions, another set for those surviving for  $n-1$  occasions and so on. Each  $W_r$  value was then multiplied by the number of flies observed in the pertinent category to obtain the number of flies expected to mate in each case. Hence for any trial we obtained the number of flies that survived for  $n$  occasions that were expected to mate 0, 1... $n$  times, the number of flies that survived for  $n-1$  occasions that were expected to mate 0, 1...( $n-1$ ) times and so on.

The total number of flies in any trial expected to mate on a given number of occasions was then the sum of the values expected from each survival category for that number of occasions in that trial.

### *Mean mating frequency and temperature*

Figure 1 shows that mean mating frequency ( $p$ , or matings per surviving male per occasion) rises from virtually zero at 16°C to around 0.83 at 25°C. The rise is rapid at first with each increment of temperature producing proportionately less of an increase in frequency. There was no significant difference between the two acclimation types so that the relationship for both fits the equation—

$$p = 1 - \text{antilog}(a - bc)$$

where  $a = -0.01323$ ;  $b = 0.087$ ;  $c = ^\circ\text{C}$  above  $16^\circ\text{C}$ . In other words, the log proportion not mating is proportional to temperature.

#### Distribution of mating frequency

Figures 2 and 3 pertain to warm- and cold-acclimated flies respectively. In each case at each temperature there was a significant variation in the number of times that males mated, with the modal frequency shifting upwards with temperature (i.e. 1-3 at  $18^\circ\text{C}$ , 5 at  $20^\circ\text{C}$  and 6 at  $25^\circ\text{C}$ ). The frequencies expected from the binomial model are also shown.

The data for either acclimation type at  $18^\circ\text{C}$  or  $20^\circ\text{C}$  were sufficiently different from the binomial predictions to fail 'goodness of fit' chi squared tests at the 5% level. The data for  $25^\circ\text{C}$ , however, conformed to expectations in each case. The percentage of variance explained by the binomial model for warm-acclimated flies at  $18^\circ\text{C}$ ,  $20^\circ\text{C}$  and  $25^\circ\text{C}$  was 69.9%, 67.4% and 97.8% respectively. The equivalent values for the cold acclimated flies were 67.7%, 90.1% and 96.1%.

## DISCUSSION

#### Deviations from expected distribution at $18^\circ\text{C}$ and $20^\circ\text{C}$

Deviations from binomial expectations were significant at  $18^\circ\text{C}$  and  $20^\circ\text{C}$ . This suggests that the assumptions of the binomial model were not met. Since the method of calculating the expected values accounts for the effect of mortality, the reason must lie elsewhere. The most obvious possibility is that the assumption that every surviving fly in any one trial has the same mating propensity is wrong, at least at  $18^\circ\text{C}$  and  $20^\circ\text{C}$ . A slight variation in mating propensity in any batch of flies would lead to a distribution of mating frequencies that is more represented in the tails than would be expected from a binomial model; this is what can be observed thus there may be scope for the selection of flies that mate more often in such conditions. Nevertheless, the large

spread of 'expected' values generated by the binomial model itself and the fact that the latter accounts for 67% or more of the variance in the observed data shows that most variation is due to chance alone and not due to variation in mating propensity.

#### Proportion of males mating in near-threshold conditions

The low frequency of mating at  $18^\circ\text{C}$  is not due to a large proportion of flies failing to mate since most mated at least once. Thus the possibility (considered in the introduction) that low mating frequency in near-threshold conditions was due to only a few flies being physiologically 'above threshold' is not the case.

#### Effect of cold acclimation

Figure 1 shows that there is no significant effect of acclimation on  $p$  at any one temperature. This is in accord with the findings of Fay and Meats (1983). Figures 2 and 3 indicate that acclimation is of no significance to matings per male. It is however difficult to compare Figures 2 and 3 directly since Figure 2 involves 7 dusks and Figure 3 involves only 6. The slightly greater numbers of males failing to mate at  $18^\circ\text{C}$  when cold acclimated may be due to this fact.

#### Lack of deviation from expected distribution at $25^\circ\text{C}$

Binomial expectations were fulfilled in both trials at  $25^\circ\text{C}$ . Most flies mated on nearly every occasion, the proportion of males in the three highest frequency classes was 80%. Flies in the lower frequency classes were those that died early, but nevertheless most mated on most available occasions as long as they lived. It thus appears that  $25^\circ\text{C}$  is a virtually optimal temperature for mating and it follows that the effect of any variation in mating propensity would be hard to detect since any proposed 'above average' flies could not improve their performance above the maximum possible frequency and any proposed 'below average' flies would still be performing at close to that frequency because of the favourable temperature.

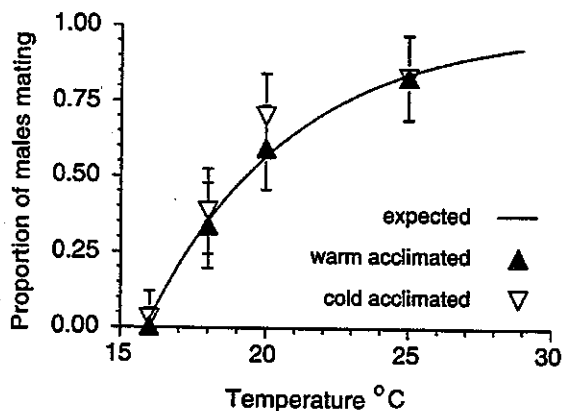


Figure 1. Proportion ( $p$ ) of mature *B. tryoni* mating during a given 1 hr dusk period in relation to temperature. 95% confidence limits shown;  $n=50$ . The log proportion not mating is proportional to temperature hence the curve indicates the mean expectation when  $p = 1 - \text{antilog}(a - bc)$

where  $a = -0.01323$ ;  $b = 0.087$ ;  $c = ^\circ\text{C}$  above  $16^\circ\text{C}$ .

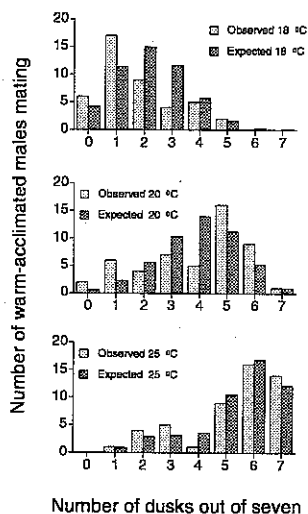


Figure 2. Warm-acclimated males: distribution matings per male over 7 dusk periods:  $n = 50$ ; expected values adjusted for mortality as explained in text.

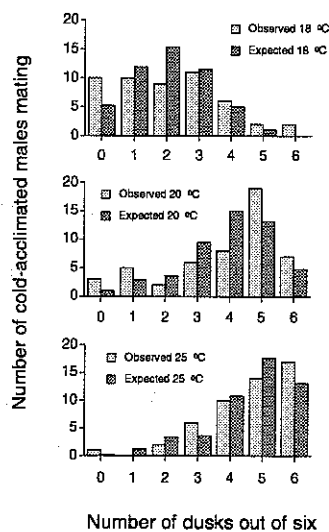


Figure 3. Cold-acclimated males: distribution of matings per male over 6 dusk periods:  $n = 50$ ; expected values adjusted for mortality as explained in text.

### Consequences of laboratory selection

It is possible that long-term laboratory selection (to  $F_{30}$ ) of the stocks used here had maximised mating propensity and hence reduced its variance. If such an effect had been produced then one would expect that it would be most pronounced at the normal laboratory temperature of 25°C. Harris *et al.* (1986) found that laboratory rearing for over 20 generations had maximized mating speed in the medfly, *Ceratitis capitata*; further selection could not improve it although selection for slow speed was successful.

### Appropriate null model for variation

The experiments described here are an illustration of the fact that a wide variation in the frequency of a discrete variable is not necessarily evidence of innate or heritable variability that could be the basis of selection. An appropriate null model (such as the binomial) for such circumstances is required to detect variability greater than that expected by chance alone. There are now several examples of experiments on mating success in insects that have revealed variation to be not significantly different from that expected from a random model (Sutherland 1985; Ueno 1994; Parsons 1997; Jaastad 1998).

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