Multiple mating by females of two *Bactrocera* species (Diptera: Tephritidae: Dacinae)

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Abstract

Multiple mating was investigated in two tephritid species when females were under minimal male pressure because they were each confined with a single male in cages $20 \times 20 \times 20$ cm and observed daily until they died. Laboratory-reared females of *Bactrocera cucumis* (French) lived up to 274 days and refractory periods averaged 59–63 days. However, the distribution of matings among *B. cucumis* females was not significantly different to that expected by chance. Wild females of *Bactrocera cacuminata* (Hering) reared from field-collected fruits of *Solanum mauritianum* Scopoli lived for up to 134 days and mated up to three times with refractory periods between matings averaging 27–39 days. The distribution of the number of matings among females of *B. cacuminata* was non-random because of the high proportion of non-maters (50%); but, when only females mating more than once were considered, there was no significant departure from random expectation.

Keywords: refractory period, Bactrocera cacuminata, Bactrocera cucumis, SIT

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Introduction

Multiple mating, especially polyandry, is common in tephritids and refractory periods for females have been reported in the range of one day to four weeks, whereas males are generally found to be able to mate daily for several days or maintain the ability to mate for several weeks (Tychsen & Fletcher, 1971; Fletcher & Giannakakis, 1973; Fay & Meats, 1983; Prokopy & Roitberg, 1984; Robacker *et al.*, 1985; Kuba & Itô, 1993; Landolt, 1994; Meats & Fay, 2000; Kraaijeveld *et al.*, 2005; Radhakrishnan & Taylor, 2007, 2008).

*Author for correspondence Fax: +61 7 3735 3697 E-mail: d.drew@griffith.edu.au The fitness consequences of multiple mating have been extensively reviewed (Ridley, 1988; Birkhead & Møller, 1998; Arnqvist & Nilsson, 2000). For tephritid fruit flies, it is possible that repeated mating can shorten the lifespan of females, but this may be offset by increased fecundity (Chapman *et al.*, 1998).

Much research on multiple mating of tephritid females has focussed on the means by which male ejaculate inhibits remating during the refractory period (Fletcher & Giannakakis 1973; Kuba & Itô, 1993; Mossinson & Yuval, 2003; Kraaijeveld & Chapman, 2004; Harmer *et al.*, 2006; Radhakrishnan & Taylor, 2007, 2008). Other factors that affect the length of the refractory period and remating frequency include density, biased sex ratio and long-term adaptation to crowded culture conditions (Robinson *et al.*, 2002; Vera *et al.*, 2002; Kraaijeveld *et al.*, 2005). Notwithstanding the fact that many tephritids in the field can form mating aggregations (Tychsen, 1977; Sivinski, 1984; Arita & Kaneshiro, 1989; Prokopy *et al.*, 1996), the high densities in some laboratory cultures can be as artificial as those used in the technique of 'forced mating' that is used to produce unusual events, such as interspecific hybrids (Takai *et al.*, 1984; Taylor & Craig, 1985; Ye *et al.*, 1992; Pike & Meats, 2002). Thus, different laboratory protocols can yield different results on multiple mating, even for the same species. It would be desirable to use conditions of density and sex ratio similar to those found in the field; and, indeed, multiple mating can be detected in the field by finding DNA microsatellite evidence for polyandry (Tripet *et al.*, 2003; Kraaijeveld *et al.*, 2005; Song *et al.*, 2007). However, this does not give precise evidence of the numbers of matings per female and cannot provide any on refractory period.

This paper describes research that used conditions that reduced any potential for forced mating to a minimum because flies were caged in male-female pairs in $20 \times 20 \times 20$ cm cages, and each pair was observed daily during the normal dusk mating period until the female died. Thus, polyandry was not possible unless a male that died was replaced. Two species studied were *Bactrocera cucumis* (French) and *Bactrocera cacuminata* (Hering). The former is a serious pest of commercial cucurbits in Queensland; whereas the latter is widespread along the entire Australian eastern seaboard; and, although it is not a pest, it has some potential to be one because it can be cultured in a variety of commercial fruits (Fitt, 1986; Drew, 1989).

Materials and methods

Study insects

B. cucumis were obtained from a laboratory colony approximately ten generations old and held at the ICMPFF laboratories, Griffith University, Brisbane. Laboratory colonies were maintained accord to the methods of Swaine *et al.* (1978), with about 6–8 weeks elapsing between generations. *B. cacuminata* were reared from infested fruits of its major host plant, *Solanum mauritianum* Scopoli, that were collected from the field at Mt Gravatt, Brisbane.

All flies were reared, and the experiments conducted, under constant environmental conditions of 25°C (\pm 1°C), 60–70% relative humidity and a photoperiod of 9h:15h, light:dark cycle with a natural dusk from a window occurring at the start of the dark phase. Under these conditions, fly mating activity occurred under dusk illumination between 1730 to 1930 hours. Flies for the experiments were allowed to emerge from puparia within 30 × 30 × 30 cm stock cages, enclosed with a fine synthetic mesh, immediately separated into sexes in separate cages and then provided with a diet of sugar, yeast protein (MP Biomedicals LLC enzymatic yeast hydrolysate) and water.

Mating study

For *B. cucumis*, mating observations were conducted on 62 pairs of virgin flies combined at three days after emergence from puparia. Each pair was confined in a cage $(20 \times 20 \times 30 \text{ cm})$ and provided with the same diet as in the stock cages of emerged males and females noted above. Over time, males that died were replaced with males from the same cohort that had also been held with a single female under the same environmental conditions. Females that died

were not replaced. Each pair of flies was monitored daily during the dusk phase and records made of mating pairs. These observations were conducted using a dull red torch light in order not to disturb mating pairs. Observations continued for 274 days. It was not possible to systematically record courtship activity because of the number of pairs and the poor light.

For *B. cacuminata*, two sets of observations were conducted along similar lines to those above. In the first case, 22-day-old virgin males and females were combined in 35 single pairs in separate cages. Observations continued until the last female died at 134 days. In the second case, all procedures were identical except that 45 pairs of virgin flies were set up when the flies were seven days old.

Statistical analyses

Statistical analyses established the significance (*P*) and predictive power (percentage of variance explained, 100 r^2) of various correlations. These correlations were: (i) between the number of matings and age of death; (ii) the delay between the first and second mating, and the second and third mating; (iii) the delay between the first and second, and the third and fourth mating; (iv) the delay between the second and third, and the third and fourth mating; and (v) the delay between the age of first mating and each of the aforementioned delays. Significance was defined in relation to a critical *P* of $\alpha = 0.05$ that was subjected to Dunn-Sidâk's adjustment to α' , where $\alpha' = 1 - (1 - \alpha)^{1/n}$ and *n* = the number of comparisons in the analysed group (Sokal & Rohlf, 1995).

Comparison was also made between the distribution of number of matings per female and that expected by chance from the binomial distribution. When a fly can mate no more than once per fortnight, the binomial probability of mating r times in n fortnights is:

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

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

The number of flies expected to mate $0, 1 \dots n$ times was found by multiplying the pertinent value of W_r by the number of flies starting a trial.

Results

Survival and mating in B. cucumis

Sixty of the 62 pairs of *B. cucumis* that were caged together at three days old mated at least once and, of these 60, 53 pairs (88%) mated twice, 31 pairs (52%) mated three times, 11 pairs (18%) mated four times and 2 pairs (3%) mated five times. The mean refractory period between matings was remarkably constant but the standard deviations were wide; the means (\pm SE) in days were, respectively, 59 ± 40.4 , 60.6 ± 29.4 and 63.0 ± 17.0 . This was associated with a wide asynchrony in mating times with a great deal of variation between pairs in terms of the number of matings achieved by a given age (fig. 1).

B. cucumis females lived up to 274 days (mean 171 ± 7.5 SE). There was a significant relationship between the number of matings and the time of female death (*P* = 0.005; *n* = 58), indicating that the longer-lived flies had mated more often. The correlation explained 13% of the variance.



Fig. 1. Percentage of female *B. cucumis* (white symbols) surviving and (black symbols) accumulated percentages of first, second and subsequent matings.

Relation of periods between successive matings in B. cucumis

The times (refractory periods) between the first and second matings in *B. cucumis* and the times between the second and third matings were significantly correlated if treated as a single comparison but not in terms of the Dunn-Sidâk adjusted level of significance (α') for multiple comparisons, (P=0.04, $\alpha'=0.017$; n=31). The correlation accounted for 14% of the variance. The times between the first and second, and the third and fourth matings were not correlated (P=0.64; n=11, accounting for 2.4% of variance) and neither were the times between the second and third, and the third and fourth mating (P=0.36; n=31, accounting for 9.4% of the variance).

There was no correlation between age of first mating in *B. cucumis* and the time between the first and second mating or the third and fourth mating (P = 0.46; n = 53 and 0.56; n = 31, respectively).

Survival and mating in B. cacuminata

For *B. cacuminata*, 23 of the 35 pairs caged together at 22 days old mated once and, of these 23, 17 pairs (74%) mated twice and 5 pairs (22%) mated three times. Similarly, 27 of the 45 pairs caged together at seven days old mated once and of these 27, 15 pairs (56%) mated twice and 4 pairs (15%) mated three times. The mean refractory periods were lower than the values found for *B. cucumis*, being about 27–39 days with SEs ranging from 6.3–14.6.

B. cacuminata females caged together at 22 days old lived for a maximum of 134 days (mean 92 days \pm 4.5 SE) and those caged together at seven days lived a maximum of 130 days (mean 86 days \pm 5.2 SE). There was no significant difference between these two means (t = 0.84, df = 78, *P*(twotailed) = 0.4). The correlation between lifespan and the number of matings in *B. cacuminata* that mated was significant (P = < 0.001, n = 50), indicating that the longer-lived flies tended to mate more often, and this relationship accounted for 40% of the variance.

The correlations between the time of first mating in *B. cacuminata* and the time between the first and second matings, and second and third were not significant (P = 0.95; n = 32 and P = 0.65; n = 9, respectively). Likewise, the



Fig. 2. The distribution of frequency of matings per female in (a) *B. cucumis* and (b) *B. cacuminata* compared to the binomial expectation (\square , observed; \square , binominal expectation).

correlation between the time between the first and second matings, and the second and third was not significant (P=0.66; n=9).

Mating, chance and the expectation from the binomial model

For *B. cucumis*, the relationship between the distribution of matings per female and the binomial expectation is shown in fig. 2a. There were 62 *B. cucumis* females in the study, and a total of 159 matings were recorded in the 20 fortnights of observation; thus, the binomial probability of observing a mating by a given female in a given fortnight was 159/20/62 = 0.1282. The distribution of matings per female expected over the whole period according to this probability and the number of periods is compared to the observed frequencies in fig. 2. The two distributions do not deviate significantly ($\chi^2 = 9.21$, P = 0.101, df = 5).

For *B. cacuminata*, the relationship between the distribution of matings per female and the binomial expectation is shown in fig. 2b. The binomial probability for this species (following the procedure outlined above) was calculated as 91/8/100 = 0.11375. In this case, the two distributions are significantly different ($\chi^2 = 21.1$, P < 0.001, df = 3). Because the result could have been influenced by the large proportion of non-maters, a comparison was made using only the numbers of *B. cacuminata* females that mated more than once. In this case, 18 females mated once only (thus, mated zero times more than once), 23 mated once more than once and 9, and zero mated twice and three times more that once, respectively. The binomial probability (deducting a fortnight for the first mating period) was calculated as 41/7/50 = 0.1171. The binomial expectations using this probability did not differ significantly from the observed values ($\chi^2 = 3.56$, P = 0.313, df = 3).

Discussion

Multiple mating and survival

A high percentage of females of both *B. cacuminata* and B. cucumis mated at least twice and in all experiments. The refractory period for B. cacuminata exceeded that recorded for B. tryoni by Fletcher & Giannakakis (1973) while that for B. cucumis was twice as long. The demonstrated multiple mating in monogamous pairs would suggest a potential for polyandry in the field. This has been borne out for one of our study species by Song et al. (2007) who found, through molecular examination of the progeny of wild B. cacuminata females, that they mated with at least two males. The higher number of matings per female in B. cucumis was associated with their long life spans. The maximum was 274 days compared to 134 days for B. cacuminata in this study and 140 days recorded in laboratory conditions for B. tryoni by both Drew et al. (1983) and Meats (2006). The difference in the degree of multiple mating between B. cucumis and B. cacuminata is most likely due to the greater opportunity for mating in the former afforded by its longer lifespan. Within species, female insects generally gain fitness benefits from mating frequently (generally polyandrously) but this may not be manifested in increased longevity (Arnqvist & Nilsson, 2000; Ivy & Sakaluk, 2005; Gershman, 2007; Seslija et al., 2008).

The role of chance in individual pairings

The very weak or non-significant relationships between the intervals between matings in individual *B. cucumis* mating pairs are consistent with the distribution of matings among pairs being not significantly different to that expected by chance. However, the distribution of the number of matings among females in *B. cacuminata* was non-random, despite non-significant relationships between the intervals between matings. In this case, the cause appears to be the unexpectedly high proportion of non-maters (50%) because, when the distribution of mating frequencies of females mating more than once are considered, there is no significant departure from random expectation.

The importance of null models (such as the Poisson) to the analysis of mating data has been recognised by several authors because what appears to be governed by sexual selection or other biotic influences may also be explained as the outcome of random processes (Sutherland, 1985; Bradbury & Andersson,1987; Hartley & Shepherd, 1995; Friedl & Klump, 2005; Snyder & Gowaty, 2007). Meats & Fay (2000) established that matings per male in *B. tryoni* were consistent with a random model, whereas Focardi & Tinelli (1996) found that a model of behaviour in leks governed by random processes not only produced results usually consistent with random expectation but occasionally produced results that were not.

The SIT and female refractory periods

The SIT, or the control of a pest by the mass release of conspecific sterile males, relies on the ability of the sterile males to mate with wild females and cause sterility of any eggs and, failing that, to impose as long a refractory period as possible. However, remating frequency may be increased by crowding, biased sex ratio and long-term adaptation to crowded laboratory culture (Robinson et al., 2002; Vera et al., 2002; Kraaijeveld et al., 2005). Thus, adaptation to mass rearing can decrease the refractory period of females, although whether this is solely due to a deficiency in sterile males is not certain. For example, Radhakrishnan & Taylor (2007) using *B. tryoni* from an SIT factory found about 15% of females remated within one day rather than the period of four weeks reported by Fletcher & Giannakakis (1973). Kraaijeveld et al. (2005) considered that, under such circumstances, polyandry could be a drawback in genetic control programmes. However, it would make no difference to the ratio of sterile males to wild males required as long as the ejaculate of released sterile males was as effective in imposing a refractory period as wild males (Curtis, 1985). An effective sterilizing dose of radiation not only causes dominant lethal mutations in spermatids and spermatozoa (fatal to any fertilized egg) but also prevents the initiation of further spermatogenesis. Thus, depending upon the timing of irradiation, a virgin sterile male will either have no sperm or a limited exhaustible supply (Ashburner, 1989). For two Bactrocera species examined to date, it is not necessary for sperm to be present in the ejaculate in order for mating to cause a refractory period (Kuba & Itô, 1993; Harmer et al., 2006; Radhakrishnan & Taylor, 2007, 2008). If the ejaculate of the sterile males does not cause such a long female refractory period as that of wild males, this would have to be rectified by releasing sterile males at a higher rate.

When small production areas of about 1 km across have to be treated with the SIT (e.g. see Barnes, 2007), polyandrous multiple mating of wild females could be advantageous, as it would result in the remating of any that invaded from neighbouring untreated sites, and such females would stand a very high probability of mating with sterile males (Helsinki *et al.*, 2008).

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