Effect of Sexual Experience on the Mating Success of Males of the Mediterranean Fruit Fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

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ABSTRACT. Laboratory experiments tested whether females of Mediterranean fruit fly, *Ceratitis capitata*, discriminate between virgin and non-virgin males as mates. Despite a possible reduction in fecundity, results indicated that females mated with non-virgin males as frequently as virgin males in choice tests. Discussion of this finding is provided in context of a lek mating system characteristic of *C. capitata*.

INTRODUCTION

In *Drosophila*, the mating experience of males may affect the number of progeny produced by females. Females of *Drosophila melanogaster* Meigen mated to virgin males or non-virgin males allowed a 24-hour recovery period produced more offspring than females mated to twice-mated males allowed no or only a very short recovery time (Markow et al. 1978). Similarly, in *D. silvestris* Perkins, virgin males or non-virgin males allowed a 72-hour recovery period transferred more sperm to females than males that had mated previously within the past 48 hours (Schwartz 1991). Though mating with a recently mated male is disadvantageous to *Drosophila* females, the ability to detect, and thus discriminate against, non-virgin males appears to vary among females of different species. Females of *D. melanogaster* showed a strong preference for virgin males (Markow et al. 1978), while females of *D. silvestris* did not (Schwartz 1991). Female preference for virgin males has also been demonstrated in the Caribbean fruit fly, *Anastrepha suspensa* (Loew), though the negative consequences of selecting a recently mated male have not been elucidated (Sivinski 1984).

In a recent study on the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann), Whittier & Kaneshiro (1991) found that females that mated with virgin males produced more offspring than females that mated with non-virgin males. Interestingly, a decline in productivity was evident even when the non-virgin males were allowed a 5-day recovery period after the first mating. Although the exact mechanism remains unknown, Whittier & Kaneshiro (1991) suggested that these effects probably do not reflect sperm depletion but a reduction in some other component of the ejaculate.

Here, we present the results of laboratory experiments that test whether *C. capitata* females discriminate between virgin and non-virgin males as mates. As shown below, females failed to exhibit discrimination in our mating trials. We discuss this finding in context of the lek mating system characteristic of medflies.

MATERIALS AND METHODS

All flies used were from a laboratory stock started in August 1990, with approximately 200–300 adults reared from peaches, *Prunus persica* L., collected in Kula, Maui, Hawai'i. Our study was conducted in March–April 1992. Consequently, the experimental flies were approximately 20 generations removed from the wild. The stock was maintained using standard rearing procedures (Tanaka et. al. 1969). Flies used in the present study were separated by sex within 48 hours of eclosion before individuals had reached sexual maturity (Arita & Kaneshiro 1982).

In the main set of mating trials, virgin females were placed singly in closed containers with 1 virgin and 1 non-virgin male. All females used were 10–14 days old. Virgin males were taken directly from "male only" holding buckets, and unlike later trials (see below), their sexual activity was not monitored prior to use. To obtain non-virgin males, we introduced several hundred virgin individuals of each sex into a large screen cage (1.2 x 0.6 x 0.6 m) between 0800–0900 h. The cage was monitored continuously for about 3 h, and mating pairs were removed by "coaxing" them to walk into glass vials, a procedure that only rarely disrupted copulations. Pairs were then left undisturbed to complete mating in the vials. Only males that remained in copula for a minimum of 90 min (the approximate time required for complete sperm transfer, Farias et. al. 1972) were used as non-virgins in the mating trials. Non-virgin males were held either 1 or 2 days prior to their use in mating trials. When used in the mating trials, virgin and non-virgin males were 11–16 days old, and in all cases males paired together in the same bottle were the same age.

Mating trials were conducted on 12 different days using the following procedure. On the afternoon preceding a particular trial, we marked each male with a dot of enamel paint on its thorax, different colors being used for virgin and non-virgin males, respectively. To allow marking, males were cooled for several minutes in an ice chest. This procedure had no obvious negative effect on the flies, and males resumed normal activities within minutes of marking. Pairs of marked virgin and non-virgin males were then placed in each of 40 to 65 transparent plastic bottles (4 l volume) and left overnight. Bottles were placed on their side, and a cloth sleeve was fastened over the mouth. A small amount of food (a honey and yeast mixture) and a water container were placed inside each bottle. Between 0800–0900 h of the next day, we added 1 virgin female to each of the bottles and checked for matings at 0.5-hour intervals until 1500 h, a period covering the time of peak mating activity (Whittier et. al. 1992). Flies were then removed, and bottles were cleaned on the inside with a moist sponge. During the study, room temperature and relative humidity were maintained between 20–23 °C and 65–75%, respectively. The room received both natural and artificial lighting, and an approximate 12 h light:12 h dark cycle was employed.

Following these trials, we performed 2 additional sets of experiments that incorporated slight modifications of the original design. In the first, we screened the virgin males to insure that they were, in fact, sexually active. Our rationale was that a female preference for virgin males, if existing, might be concealed if a certain proportion of virgin males failed to display sexual behavior under laboratory conditions. Non-virgin males, on the other hand, by virtue of their mated status, already "proved" their willingness to copulate under artificial conditions. Thus, one day prior to the mating trials we observed a group of approximately 80 virgin males in a screen mesh cage (60 cm cube) and collected only those males that exhibited pheromone calling for inclusion in the mating trials (again using the plastic bottles). In the second modification, we also screened males but used screen mesh cages (30 cm cubes) instead of plastic bottles as mating chambers. By allowing free movement of air, the screen cages presumably reduced intermixing of male pheromonal signals and possible female "confusion" regarding the identity and location of specific signalers. In both of these additional experiments, virgin males were placed with non-virgins that had mated 1 day prior to the mating trial.

Data were analyzed using a G-test with Yates correction ($P < 0.05$; Zar 1974).

RESULTS

Female choice of mates was made independently of male mating experience in all experiments. In the main experiment, matings involving virgin males and males that had mated 1 day earlier were observed with equal frequency (47% vs 53%; $n = 69$; $P > 0.05$). Similarly, virgins and males that had mated 2 days earlier had similar mating success

(46% vs 54%; $n = 83$; $P > 0.05$). In the trials using screened males in bottles, virgin and non-virgin males accounted for 46% and 54% of the matings, respectively ($n = 58$; $P > 0.05$). In the trials using screened males in screen mesh cages, there was again no difference in the mating frequency of virgin (52%) and non-virgin (48%) males ($n = 50$; $P > 0.05$).

DISCUSSION

Medflies exhibit a lek mating system (Prokopy & Hendrichs 1979; Arita & Kaneshiro 1985, 1989; Hendrichs & Hendrichs 1990; Whittier et al. 1992). Males form aggregations (termed leks) in the canopies of host trees and rest singly on the under surfaces of leaves. Sexually active males adopt a "calling" posture by curling the abdomen upward and emitting a pheromone attractive to females (Feron 1962). Following the arrival of a female to the leaf, the male fans his wings and makes oscillatory head movements. The male then mounts the female, and (if she is receptive) copulation ensues. In sum, females appear to be the choosy sex, and mate selection appears to operate freely (i.e., without male coercion) on the basis of certain (and as yet unknown) male or territory characteristics.

Given these observations, female medflies are expected to select mates so as to maximize their reproductive success. It is therefore unclear why females apparently fail to discriminate against non-virgin males as mating partners. In fact, in a recent study (Whittier et al. 1994) groups of 10 males were presented with 10 new females daily over 6 consecutive days and observed that certain males accounted for a disproportionately large number of copulations while many males did not mate at all. Thus, though virgin males were available, females appeared to consistently prefer particular non-virgins. It is possible that by multiple matings, females could "counteract" the negative effect of mating with a non-virgin male. However, most females appear to mate just once in their lifetime: only 33% of the females observed in a laboratory study mated more than once even when provided with constant access to males and oviposition substrate (Whittier & Shelly 1993).

Perhaps the most likely explanation for this finding is that the reduction in progeny number associated with mating with non-virgin males is outweighed by an increase in the "vigor" or genetic quality of the offspring. For example, if mating with a specific non-virgin male results in the production of highly attractive sons, the female may incur reduced productivity to produce "sexy sons" that will have enhanced mating success (Weatherhead & Robertson 1979). The heritability of male mating success is currently under investigation in our laboratory (Whittier, unpubl. data).

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