

Unfaithful Mediterranean Fruit Fly *Ceratitis capitata* Females: Impact on the SIT?

M. BONIZZONI¹, L. M. GOMULSKI¹, S. BERTIN¹,
F. SCOLARI¹, C. R. GUGLIELMINO², B. YUVAL³,
G. GASPERI¹ and A. R. MALACRIDA¹

¹*Dipartimento di Biologia Animale, Università di Pavia, Piazza Botta 9,
27100 Pavia, Italy*

²*Dipartimento di Genetica & Microbiologia, Università di Pavia, via
Ferrata 1, 27100 Pavia, Italy*

³*Department of Entomology, Hebrew University of Jerusalem, PO Box
12, Rehovot, Israel*

ABSTRACT An understanding of the levels of remating and paternity skew in the field can be important for polyphagous pest species with a high colonization potential such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). The use of polymorphic simple sequence repeats on flies from two Mediterranean populations in combination with various statistical methods showed not only that Mediterranean fruit fly females remate in the wild, but most importantly, that the level of sperm precedence could influence the effect of remating itself since one male, presumably the last, tends to sire most of the progeny. Levels of remating and paternity skew may have important implications for the evolution of the species in terms of maintenance of genetic variability. Moreover, these features of mating behaviour may locally affect the efficiency of the sterile insect technique (SIT), which is a commonly applied control strategy against the Mediterranean fruit fly.

KEY WORDS multiple mating, sperm use, *Ceratitis capitata*, microsatellites, field populations

1. Introduction

Understanding the frequency of remating and the factors that may influence it, together with an understanding of the extent and mechanisms that regulate sperm use are particularly important for pest species such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) for which the sterile insect technique (SIT) is widely used as part of area-wide integrated pest management (AW-IPM) programmes (Krafsur 1998). The efficiency of the SIT depends, amongst others, on the sexual competitiveness of sterile males, in terms of mating competitiveness, sperm transfer, accessory gland secretion transfer, and sperm competition. Females mated with sterile males should ideally show the same remating behav-

our as those mated with wild males (Cayol et al. 1999). If females mate more than once they can remate with either a fertile or a sterile male, however, the release of a huge excess of sterile males in Mediterranean fruit fly SIT programmes suggests that any female that does remate is likely to mate with a sterile male.

The Mediterranean fruit fly mating system is based on arboreal leks, where females encounter several courting males and freely select a mate (Yuval and Hendrichs 2000). Less commonly, an alternative “fruit-guarding” tactic is employed, whereby males occupy oviposition hosts and copulate previously mated females that alight on them to oviposit (Prokopy and Hendrichs 1979). It is hypothesized that female monogamy could be

favoured over polyandry due to the highly male-skewed operational sex ratio at lek sites (Warburg and Yuval 1997), and the high copulation cost for females (Hendrichs and Hendrichs 1998). However, female remating has been reported in the laboratory (Saul and McCombs 1993, Whittier and Shelly 1993, Blay and Yuval 1997, Miyatake et al. 1999), in field cages (McInnis et al. 2002, Vera et al. 2003, Shelly et al. 2004), and in a wild population from the Greek island of Chios (Bonizzoni et al. 2002, Kraaijeveld et al. 2005). Depending upon the experimental conditions and the strain of flies studied, remating frequency ranges from 0.03 to 0.76. It has been proposed that renewal of female sexual receptivity is influenced by the efficiency of the previous mating, in particular by the quantity of the sperm transferred and the quality of male accessory gland secretions (Mossinson and Yuval 2003). Extensive studies have been done in *Drosophila*, where three seminal fluid peptides (the sex-peptide, ovulin and DUP99B) have been shown to play a major role in eliciting both short-term and long-term postmating responses (Liu and Kubli, 2003). Laboratory experiments using morphological mutants in the Mediterranean fruit fly have shown that, following remating, between 58 and 71% of the progeny was fathered by the male of the second mating, giving a second male sperm precedence (P2) of between 0.58 and 0.71 (Saul and McCombs 1993).

Little is known about whether Mediterranean fruit flies from different origins vary in remating frequency due to geography, climatic conditions, population density, and seasonal fluctuations. Studies conducted to date have focused on sexual compatibility among Mediterranean fruit flies from different localities, on sexual compatibility of wild flies versus sterile males (Cayol et al. 2002), and on the relative competitiveness of sterile versus wild males (McInnis et al. 2002, Vera et al. 2002, Kraaijeveld and Chapman 2004). Assessing the extent of Mediterranean fruit fly remating in the field is extremely challenging since, as in other field-based studies of multiple mating and sperm use, there is no

experimental control over the number of times that a female mates, the interval between matings or the genetic identity of multiple fathers contributing to a brood.

However, the availability of high resolution molecular markers, such as microsatellites (simple sequence repeats) (Bonizzoni et al. 2000, Baliraine et al. 2003), coupled with efficient statistical methods such as GERUD (Jones 2001) or SCARE (Jones and Clark 2003), enables the assignment of paternity in an open field situation with considerable confidence to be made even from a relatively low number of insects sampled (Imhof et al. 1998, Bungaard et al. 2004, Bretman and Tregenza 2005, Schlötterer et al. 2005). The use of the most informative loci on the basis of their polymorphic information content – a parameter dependent on the number of the alleles at each locus and its heterozygosity (Hearne et al. 1992) – allows the inference of paternity by comparing the genotypes of wild-caught Mediterranean fruit fly mothers and their progeny produced in the laboratory.

2. Populations Studied

Two geographic populations from the Mediterranean basin, one on the Island of Chios in Greece and the other from Rehovot in the central coastal plain of Israel, were chosen to determine whether the frequency of remating varies between geographically different populations. These two sampling areas have been extensively used for Mediterranean fruit fly behavioural and demographic studies (Rivnay 1950, Katsoyannos et al. 1998, Israely et al. 2005). While both Chios and Rehovot populations follow a seasonal Mediterranean pattern, the slightly different climatic conditions influence the seasonal fluctuations of their population density (Katsoyannos et al. 1998, Israely et al. 2005). On the Island of Chios, Mediterranean fruit fly adults can be captured from June to mid January, with peak densities being reached between the beginning of August and the end of November. The population remains below a detectable level for the rest of the year, over-

wintering as larvae mainly in sweet oranges and mandarins (Katsoyannos et al. 1998).

In the Rehovot area, the seasonal fluctuation in population density is bimodal with the first adults appearing in April-May. Due to favourable weather conditions and host availability, the spring population rapidly increases until July when it goes through a strong bottleneck due to both high temperatures and a relative scarcity of hosts. A second, but minor population increase is observed in September, with a peak in October. In winter (January-March), the number of flies rapidly decreases to an almost undetectable level (Israely et al. 2005).

Mediterranean fruit fly females of unknown reproductive status were captured in BioLure[®] baited traps from the two areas. The sampling procedures in Chios were described by Bonizzoni et al. (2002). In Rehovot, traps were placed in September 2001 and in April-May 2002. The number of flies trapped was generally low (less than 50 flies). Twenty females in 2001 and 30 in 2002 were removed from the traps, transported to the laboratory and allowed to oviposit individually in appropriate fruits. In 2001 each female oviposited in one fruit whereas in 2002 the fruits were removed from the oviposition cages every two days and replaced by a fresh fruit. Females were allowed to oviposit until they died. Each female and all her adult offspring were killed and shipped to Italy in 100% ethanol.

To determine the level of microsatellite polymorphism of the two populations, two additional samples of 36 and 42 flies were collected in Chios in 2000 and in Rehovot in 2002.

3. Observed Remating Frequencies

Genotyping of the two random samples from Chios and Rehovot identified the most informative simple sequence repeat loci for paternity analysis, based on the number of alleles, their frequency and their polymorphic information content (Hearne et al. 1992). In Chios, these loci were *Ccmic* 8, 3, 17 and 13;

and in Rehovot they were *Ccmic* 2, 6, 15, 17, 25, 3, 32, 13 and 23.

Based on data from these most informative simple sequence repeats, combined exclusion powers (i.e. the probability of excluding a randomly chosen candidate parent from parentage of an arbitrary offspring, given the genotype of the mother and all her progeny), of 0.725 and 0.938 were derived for Chios and Rehovot, respectively. These same loci provided correct paternity assignments of 80.0% and 95.8% respectively in Chios and Rehovot (CERVUS simulation (Marshall et al. 1998)). The lower value of the predicted success rate for Chios could be due to its lower simple sequence repeats variability and its higher level of allele sharing ($aS = 0.046$ and 0.0002 , in Chios and Rehovot; respectively (Palmer et al. 2002)), which could mask remating.

Seventy two percent of caught wild females (26 out of 36) in Chios produced progeny giving a total of 681 individuals. Paternity analysis performed on the progeny of the 18 mothers with at least 12 offspring (mean progeny size: 31.4) (GERUD (Jones 2001)) revealed a remating frequency of 5.5% (one out of 18 females).

In Rehovot, 15% of the females collected in September 2001 (three out of 20), and 30% from April-May 2002 (nine out of 30), produced progeny giving a total of 422 individuals. Paternity analysis performed on the progeny of the eight females with at least 12 offspring (mean progeny size: 36.5) revealed a remating frequency of 50% (four out of eight females; two from each of the 2001 and 2002 collections).

4. Observed and Expected Remating Frequencies

The probability of detecting two fathers was calculated from the population simple sequence repeats allele frequency data (GERUD (Jones 2001)) under different conditions of progeny size (10, 34 or 50) and paternity skew, assuming second male sperm precedence (50, 60 or 70%). The results indicated that the number of progeny influenced remating detection more than the

level of paternity skew (i.e. in Chios for a paternity skew $P_1:P_2$ of 3:7, the correct detection of the second father varied from 52.3 to 62.3%, with 10 and 34 offspring, respectively, while with a $P_1:P_2$ of 4:6, the range was from 54.1 to 62.3%. The same tendency was found in Rehovot (data not shown). The probability of detecting remating was higher in Rehovot than in Chios (Bonizzoni et al. 2006). Based on these simulations, 1.6 rematings should have been detected in Chios whereas only one was observed. In other words, among the 18 females analysed, 9% were expected to remate, but only 5.5% were observed. Consequently, the mean number of fertile matings per female (M) is estimated as 1.09 (18+1.6)/18, while the observed value was 1.06 (18+1)/18. In Rehovot, over the eight tested females, 4.3 cases of remating should have been detected compared with the four observed; M is estimated as 1.54 (4.35+8/8) versus the observed 1.50 (4+8/8).

G tests of independence were applied to test whether there were differences in the observed remating frequency between Chios and Rehovot. The test was significant at $P < 0.025$ ($G = 5.88$, d.f. = 1).

5. Breeding Behaviour

The SCARE program, which uses a Bayesian approach (Jones and Clark 2003) was used to estimate M and the proportion of offspring from the last-mating male (β). In Chios, the 95% confidence interval range for M and β were respectively 1.15-2.01 (mean 1.48) and 0.80-0.95 (mean of 0.89). In Rehovot, using the four unlinked loci with the highest polymorphic information content (*Cmic3*, 6, 15, 17), the 95% confidence interval ranges for M and β were: 1.61-4.09 (mean of 2.59) and 0.64-0.82 (mean of 0.73).

6. Is Remating Frequency Affected by the Genetic Background and/or Population Density?

Multiple matings ascertained both in Chios and Rehovot suggest that Mediterranean fruit

fly females can copulate more than once in the field. Both the GERUD and the SCARE-derived estimates of M were lower in Chios than in Rehovot. The lower number of females analysed in Rehovot, associated with the higher rate of remating detected independently in the collections from two different years with respect to Chios, renders the observed difference in remating frequency even more reliable. It has been hypothesized that different strains of Mediterranean fruit fly differ in male stimuli and in female responsiveness (Jang et al. 1998), and that the tendency to remate may be adaptive and heritable (Saul and McCombs 1993). In laboratory experiments, Whittier and Shelly (1993) showed that remating was adaptive since multiple-mated females had a significantly higher reproductive output than once-mated females. Chios and Rehovot have well-established fly populations sharing a relatively recent common evolutionary history (Malacrida et al. 1998). Consequently, they show a very low level of genetic differentiation and a high gene flow estimate (Bonizzoni et al. 2004). However, the possibility cannot be excluded that the distinct genetic background of the two populations affected the extent of the remating estimates.

Although following a similar Mediterranean seasonal pattern, the fluctuations in Chios and Rehovot populations are influenced by slightly different climatic conditions (Katsoyannos et al. 1998, Israely et al. 2005). On the island of Chios, fly sampling was done in July 2000 when the population was still expanding but had already reached a moderate size (Katsoyannos et al. 1998). The flies from Israel were collected in September 2001 and in April-May 2002, i.e. at the beginning of the autumn and spring expansions respectively. Since the Mediterranean fruit fly has a high rate of population increase (Liedo and Carey 1996), it is reasonable to suppose that at the time of the collections the Rehovot population could have been biased towards younger flies. In Mediterranean fruit fly females, the receptivity to a second mating was shown to be negatively correlated with female age

(Chapman et al. 1998, Kraaijeveld and Chapman 2004). Moreover, mated males seem to be more efficient at inhibiting remating than virgin males (Vera et al. 2002). Therefore, a physiological parameter, such as the lower average age of the Rehovot flies may account for their higher frequency of remating.

According to sexual selection theory, females of a lekking polyphagous species such as *C. capitata*, should evolve highly discriminative mate choice based on male quality (Kaneshiro 1989). Kaneshiro (2000) suggested that under conditions of small population size, choosy females that cannot encounter males satisfying their courtship requirements, might undergo a physiological change thus increasing the chance of mating with less "successful" males and as a result will tend to remate. That such a scenario occurred in Rehovot during the time of sampling cannot be excluded.

7. Sperm Use

The SCARE simulations (Bungaard et al. 2004) on the two natural populations strongly indicate that Mediterranean fruit fly populations exhibit high sperm skew (mean $\beta = 0.89$ and 0.73 for Chios and Rehovot, respectively). Since results using morphological mutants have shown that the second male sired more progeny than the first (Saul and McCombs 1993), it is reasonable to assume in the present studies that it was also the second male that contributed in a higher proportion to the paternity of the offspring, as was assumed in the SCARE simulations (Jones and Clark 2003). In previous laboratory crosses, the proportion of offspring sired by the first and second male did not change over the females' life span or with different intervals between matings (Katiyar and Ramirez 1970), suggesting a complex system of sperm storage and use. Marchini et al. (2001) and Twig and Yuval (2005) analysed the functions of the two types of sperm storage organs (i.e. the two spermathecae and the fertilization chamber) in relation to insemination, fertilization and their

control. Spermathecae function as long-term storage organs, while the fertilization chamber acts as a staging point for fertilizing sperm. Twig and Yuval (2005) concluded that the use of both sperm storage organs allows females to manipulate ejaculates by diverting and segregating them adaptively. Obviously, the storage of sperm from more than one male provides opportunities for sperm competition and/or sperm choice. Which mechanism is responsible for the high level of second male sperm precedence that was both observed and simulated is still an open question requiring further study. From the SCARE simulation, and at a very preliminary level also from the progeny analyses, the extent of paternity skew appears greater in Chios than in Rehovot where a higher frequency of remating was observed. This finding introduces the interesting question of understanding the evolutionary relationship (if any) among sperm allocation, remating frequency and sperm displacement in the Mediterranean fruit fly.

8. Conclusions

In the context of Mediterranean fruit fly control programmes based on the SIT, the low mating competitiveness of sterile males increases the probability of a female remating (Kraaijeveld and Chapman, 2004). The findings reported in this study indicate that in wild populations of the Mediterranean fruit fly (1) remating is occurring, and its level may vary, and that (2) there is preference for sperm from the second mating male. In relation to both the use of the SIT and in the analysis of the evolution of this species, these studies show the importance of acquiring knowledge on the mechanisms of sperm use and competition in Mediterranean fruit fly and couple them to studies on male mating competitiveness.

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