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**Behavioral ecology of the Mediterranean fruit fly: Leks,
courtship, nuptial gifts, and average offspring**

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University of Hawaii, 1993

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BEHAVIORAL ECOLOGY OF THE MEDITERRANEAN FRUIT FLY:
LEKS, COURTSHIP, NUPTIAL GIFTS, AND AVERAGE OFFSPRING

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ENTOMOLOGY

AUGUST 1993

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Kenneth Y. Kaneshiro for the continuous support and opportunity he has provided for me during my graduate education. He has been an ideal advisor and mentor. Dr. Kaneshiro's guidance has helped me to enjoy science and research and to view science in a very global perspective. I would also like to express my gratitude to my committee members for their sacrifice in time to help me accomplish this degree. Their guidance and suggestions have broadened my perspective and made me struggle to improve my skills.

In addition, I would like to thank Todd E. Shelly for his considerable input. His arrival to Hawaii to work on fruit flies was a great blessing. He patiently taught me how to write and conduct fieldwork and has been a great support to me and my family.

Lastly, I thank my family: Diann, Jeremy & Danton. Although small compared to the sacrifice they have made, I dedicate this dissertation to them. May we always have such positive challenges to share with each other in our journeys together.

ABSTRACT

Observations of the mating behavior of wild Mediterranean fruit flies, Ceratitis capitata (Wiedemann) in a semi-isolated orchard in Kula, Maui, Hawaii, revealed that matings occur on only a small proportion of available trees. Most matings occur during midday and in almost all cases 1-10 other males are present in the same tree as the mating pair. Intruders win more than two-thirds of all male-male interactions and females terminate most failed courtship attempts. Male size and territory location do not correlate with mating success and a small percentage of the males mate more than once.

Mating frequency in the laboratory is non-random. Variation in male activity level accounts for some of the differential mating success. Male copulatory success is positively correlated with the number of courtships performed, attempted copulations (mountings) and females courted. Male-male aggression, on the other hand, has a negligible effect on male mating success. Female choice also appears to influence male mating frequency. Less than 10% of courtships result in mating, and in most cases females terminate courtship by simply moving away from the male. Females that do mate generally select males

having higher copulatory scores than previously rejected males.

The non-random mating of males does not appear to be a result of female preferences for heritable differences among males in the ability to obtain copulations. Father/son correlation and sibling analysis both show no additive genetic variance for male copulatory success. However, female preferences may act to increase fecundity. Females which mate with virgin males enjoy increased longevity and fecundity. Furthermore, there is a non-linear relationship between a male's copulatory success and female fecundity. Females mating males that obtain few (<2) or many (>6) matings enjoy increased fecundity. The basis for these relationships between male mating success and female fecundity is not known but may be based on sex-peptides passed to the female during copulation. Such direct benefits may help explain the lek mating system and the evolution of female choice in this species.

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CHAPTER 1

INTRODUCTION

The Mediterranean fruit fly, Ceratitidis capitata (Wiedemann) is one of the most significant economic pests in the world. More than 200 species of plants serve as host, and this species is currently found throughout the world with the exception of North America and Japan. In addition, these flies are often located in ecologically sensitive areas making the use of insecticides undesirable. At present, the most widely used alternative technique to control these flies is sterile insect releases (Knipling 1955; Cunningham et al. 1980). The aim of the sterile insect release method is to disrupt reproduction of the target population through the effects of wild females mating with sterile males. Crucial to the success of such a program is an understanding of the mating system of the target animal. That is the goal of this dissertation.

Chapter two of this dissertation is a description of quantitative observations of the mating behavior of wild Mediterranean fruit flies in a natural environment. Although the mating system of this species had been previously described as a lek (Prokopy & Hendrichs 1979; Arita & Kaneshiro 1985; Hendrichs & Hendrichs 1990), none of these studies recorded the relative roles of male size, male

territory location, male-male interaction and male courtship ability in a completely natural environment. This paper was submitted to the Annals of the Entomological Society of America and published in 1992 (Ann. Entomol. Soc. Am. 85: 214-218).

The term lek as used throughout this dissertation is operationally defined as an aggregation of males which females visit for the sole purpose of copulation. Males do not control resources nor do they provide anything to the female except gametes. Mediterranean fruit fly leks are aggregations of males on single leaf territories. These territories are concentrated on a small area of the tree. Unfortunately, the exact parameters of the lek are not known but most likely include host status of the tree, exposure to direct sunlight and wind direction. In addition, the ability to communicate with other members of the lek via pheromones, visual signals or auditory signals will certainly delineate who participates in a given lek. As a result, the exact meaning of lek will certainly evolve as new parameters are discovered and described.

A follow up to this study was a laboratory study of mating behavior. Previous studies have shown male mating success to vary greatly and those authors have suggested that female choice is an important source of this variability (Whittier 1986; Arita & Kaneshiro 1985). However, the cause and degree of non-random mating, the role

of male-sorting and the presence of female discrimination had never been quantified.

In chapter three, the relationships between male mating success and male-male interactions, male size, male pheromone calling, male courting and attempted copulations were examined. I also estimated the degree of female choice present in this species by recording the number of courtships each female elicits as well as the number of different males she visits. In addition, whether females ultimately copulate with males of higher quality than those rejected during previous courtships was investigated. This paper is currently under review by the Journal of Insect Behavior.

Because previous studies suggested that C. capitata form leks, show strong differential mating success for the males and exhibit female choice, I examined what benefits a female receives by mating with particular males. In Chapter four, I discuss an observation that females mate most often with a small subset of non-virgin males although mating with virgin males enhances female fitness. Both the number of offspring produced per female and female longevity is greater for females that mate with virgin males. This phenomenon is not explained by temporary sperm depletion and may be the result of some nuptial gift or sex-peptide. This paper was submitted to the Annals of the Entomological

Society of America and Published in 1991 (Ann. Entomol. Soc. Am. 84: 608-611).

Chapter five follows up on the question of females benefiting by mating with particular males. I try to determine whether females gain directly through mate choice by increasing fecundity, or whether they benefit indirectly in the form of gametes that increase offspring quality or attractiveness. This study shows that the nonrandom mating observed in the C. capitata does not appear to be the result of female choice for heritable difference among males for the ability to obtain copulations. However, female choice may be based on direct benefits to the female in the form of increased fecundity. These benefits may be in addition to those benefits discussed in chapter four.

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CHAPTER 2

MATING BEHAVIOR OF MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN A NATURAL ENVIRONMENT

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This chapter was previously published in the
Annals of the Entomological Society of America 85: 214-218

ACKNOWLEDGEMENTS

We thank the Earl Fujitani family for their generous hospitality and use of their farm, and K. M. Kanegawa for technical assistance and T. E. Shelly for valuable suggestions concerning the manuscript. This work is part of a dissertation by T. S. W in partial fulfillment of the Ph.D. degree at the University of Hawaii. It was funded in part by California Department of Food and Agriculture project 88-0536 and USDA-ARS grant 58-9142-6-42 to K.Y.K.

ABSTRACT

Systematic quantitative observations of the mating behavior of wild Mediterranean fruit flies, Ceratitidis capitata (Wiedemann) were made in a semi-isolated orchard in Kula, Maui, Hawaii. Matings occurred on only a small proportion of the available trees, and most occurred on only three trees. All matings occurred on the bottom surfaces of sunlit leaves. Most matings occurred during midday and lasted nearly 3 h. In almost all cases 1-10 other males were present in the same tree as the mating pair. Intruders won more than two-thirds of all male-male interactions and females terminated most failed courtship attempts. Male size and territory location did not correlate with mating success, and a small percentage of the males mated more than once.

KEY WORDS Insecta, Ceratitidis capitata, lek, sexual selection

MALE MEDITERRANEAN FRUIT FLIES, Ceratitis capitata (Wiedemann), aggregate in leks during the late morning and early afternoon and emit sex pheromone ("call") from the undersurface of leaves to attract females (Prokopy & Hendrichs 1979). Field studies have shown that the preferred sites for lek formation are sunlit tree canopies of host trees, particularly citrus trees (Hendrichs & Hendrichs 1990). Males defend territories by aggressively lunging at and pushing intruders or through passive defense in which the resident and intruder touch heads for several minutes without any visible force (a behavior termed "facing off"; Arita & Kaneshiro [1989]). Upon the arrival of a female to the male's territory, the male orients towards her and fans his wings, providing potential visual, acoustical, and pheromonal cues (Feron 1962; Webb et al. 1983, Arita & Kaneshiro 1989). If the female is receptive to this elaborate courtship, copulation occurs on the territory.

What traits female Mediterranean fruit flies use to choose their mates are not known. These traits may be arbitrary (O'Donald 1980, Lande 1981) or may confer some advantage to the offspring (Borgia 1979, Kodrick-Brown & Brown 1984). Identification of these traits is complicated by the difficulty of separating the relative importance of male-male interactions from female choice in effecting the unequal mating success seen in classical lek species. Some

(see Halliday 1983) argue that males sort themselves through male-male aggression and effectively eliminate the 'need' or opportunity for mate choice by the females. This sorting process often involves male-male aggression for territories with a size and resident advantage (Scott 1942, Floody & Arnold 1975). As a result, it is often unclear whether the female mates with a male based on his territory location (i.e., the dominant male) or because of some inherent quality she perceives in him or perhaps a combination of both. The relative roles of male size, male territory location, male-male interaction, and male courtship ability in the mating system of the Mediterranean fruit fly are not well known. To understand these various roles, we systematically observed the mating behavior of wild Mediterranean fruit flies under natural conditions.

Materials and Methods

Our study was conducted on 6 consecutive d during March 1990 and 16 d between 13 June and 17 July, 1990. Observations were made at a 0.75 ha fruit tree orchard located at 1,150 m elevation in Kula, Maui, Hawaii. The fruit trees are surrounded on three sides by vegetable plots and on one side by a highway and forest with no apparent hosts. The fruit trees are thus isolated by at least 200-300 m from any other suitable host plants. This farm has >118 host trees including 5 lemon (Citrus limon L.), 4 peach

(Prunus persica L.), 1 nectarine (Prunus persica var. nectarina (Ait.) [Maxim]), 18 Kelsey plum (Prunus sp.), 3 red plum (Prunus sp.), 79 persimmon (Diospyros kaki L.), 5 loquat (Eriobotrya japonica (Thunb.)), 2 avocado (Persea americana Mill.), and 1 apple (Malus sylvestris L.). Only the two avocado, two of the loquat, and one red plum tree are taller than 3 m; most are pruned regularly and have a round canopy. The loquat trees were fruiting during the March observations, and the lemon, peach, nectarine, plum, and avocado trees possessed ripe fruit during July. The persimmon tree had only very small immature fruit. Each tree was individually numbered with a small aluminum tag. A trimedlure-baited trap placed by the USDA at one edge of the orchard was allowed to remain during the study period.

We have defined a lek as an entire tree where calling males have established territories rather than as a specific distance or volume, a definition used in previous studies (Prokopy & Hendrichs 1979, Sivinsky 1989, Hendrichs & Hendrichs 1990). We use this definition because 1) there was usually only one aggregation per tree, 2) the territory locations of the males seem to be determined more by the structure of the tree rather than by the flies themselves (see results), and 3) flies readily moved between leaves within the canopy of a single tree.

Censuses of all trees on the farm were conducted at 1030 and 1400 hours on each day of field work during June

and July. The observer stood near the trunk and surveyed the leaves, branches, and fruit. The observer then walked around the perimeter of the tree to observe flies not visible from beneath the tree. Ladders were used as necessary. Each tree was observed for 4 min, except the two large avocado, two large loquat, and one large red plum trees, each of which were observed for 10 min.

During each observation day in both March and July, we recorded the distribution and activity of males and females present on the two largest active lek sites between 0830 and 1500 hours. Observations were not continued past 1500 hours as no secondary mating strategy such as that reported by Prokopy & Hendrichs (1979) was observed at our study site. At these lek sites, we recorded the outcome of male-male interactions and identified the "winner" as the male that remained on the leaf after the interaction. Male-female interactions were also recorded. Courtship was scored as either accepted or rejected, and the manner of rejection was recorded. The location of all mating sites (leaves) were inconspicuously marked with a number using indelible black ink. The positions of the leaves relative to the trunk were recorded using a compass. At the time of mating, we also recorded the number of other flies present in the tree where the mating occurred.

In addition to these observations, we captured as many copulating pairs as possible using glass vials. The

duration of copulation was recorded for flies that were not captured and for flies that continued in copulo after being captured. After copulation, the flies were anesthetized in ice, and the thoraces of all captured mating flies were measured. Thoracic length was significantly correlated with dry body weight in this population ($P < 0.002$; T.S.W., unpublished data). Each fly was then individually marked on the thorax with small dots of Testor's paint and released at the capture site. Resightings of marked flies were recorded. A small sample of flies was removed from the USDA trap each week and measured to compare sizes of males that mate against this random sample of flies in this orchard. Data were analyzed statistically by analysis of variance (ANOVA) (Proc GLM; SAS Institute 1986), and means were separated using Duncan's (1955) multiple range test. Voucher specimens have been placed in the collection of the Hawaiian Evolutionary Biology Program at the University of Hawaii.

Results

The male flies preferred to perch on only a subset of all available trees. Of 118 trees, 10 trees accounted for 577/721 (80%) of all sightings, and 79 trees had zero sightings (Fig. 2.1). The type of tree did not seem important. For example, 5 persimmon trees had flies sighted on more than 10 censuses, whereas males were never sighted on 42 of the persimmon trees. The peach trees showed a

similar pattern. The trees with the most abundant sightings included two persimmon trees and one lemon tree.

Seventy-one copulating pairs were recorded during our study: 4 during March and 67 during July. All matings were on the undersides of leaves. Seventy-three percent (52/71) of the matings were recorded from just two persimmon trees and one lemon tree (these three trees also had the most abundant sightings; Fig. 2.1). These three trees did not differ in any obvious way from the other trees except they were next to each other near the middle of the orchard. Matings were recorded on only 12 trees, even though flies were sighted on 39 trees during the study period. Matings were observed on trees with ripe fruit, those with only very small immature fruit, and those with no fruit at all. Most matings occurred on the sunlit portion of the tree on the hemisphere facing the rising sun (Fig. 2.2). Several of the outliers in this figure represent flies that mated on protruding branches which, despite their position, received direct sunlight. Of the 71 matings, all but 2 occurred on different leaves. Most of the matings occurred during midday, with a peak at 1200 hours (Fig. 2.3). Mean duration of copulation was 179.3 min ($n = 19$; SD, 50.62). In nearly all cases, other flies were present within the same tree as the copulating pair (Fig. 2.4). The size of the mating males did not differ significantly from those captured in the trimedlure trap. In addition, neither group of males

differed significantly in size from mating females (Table 2.1). Three males mated more than once.

Intruders won 99/144 of the male-male interactions, residents won 39/144 of the interactions, and both left 6/144 of the time. Intruders won significantly more of the aggressive interactions (binomial; $Z = 5.58$, $P < 0.001$). Of those 144 interactions, 30% involved the "face-off" behavior. When face-off behavior occurred, the resident male won 14/33, intruders won 16/33, and both left 3/33 of the time. Thus, if the resident male displayed a passive defense, the resident and intruder were equally probable of winning the conflict (binomial; $Z = 0.24$, $P > 0.05$). The mean duration of the face-off behavior was 93 s ($n = 32$; SD, 70.70).

Twenty-five courtship rejections were observed, 24 of which were initiated by the female. Thirteen females left during the male's wing fanning, 10 left or dropped from the leaf when the male attempted to mount the female, and one courtship was disrupted by another male.

Table 2.1. Size (mm) of males and females captured in copulo, and of males removed from a trimedlure trap

	n	X \pm SD	Range

Mating σ	45	2.29 \pm 0.09 a	2.10 - 2.50
Mating φ	42	2.27 \pm 0.15 a	1.85 - 2.50
Trapped σ	140	2.30 \pm 0.14 a	1.80 - 2.70

^a Means within columns followed by the same letter are not significantly different (P = 0.05; Duncan's [1955] multiple range test).

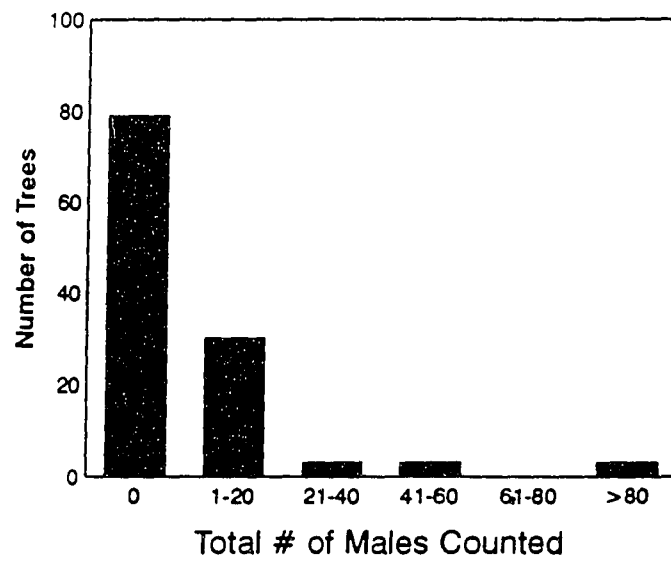


Figure 2.1 Counts of males on individual trees for all censuses in June and July.

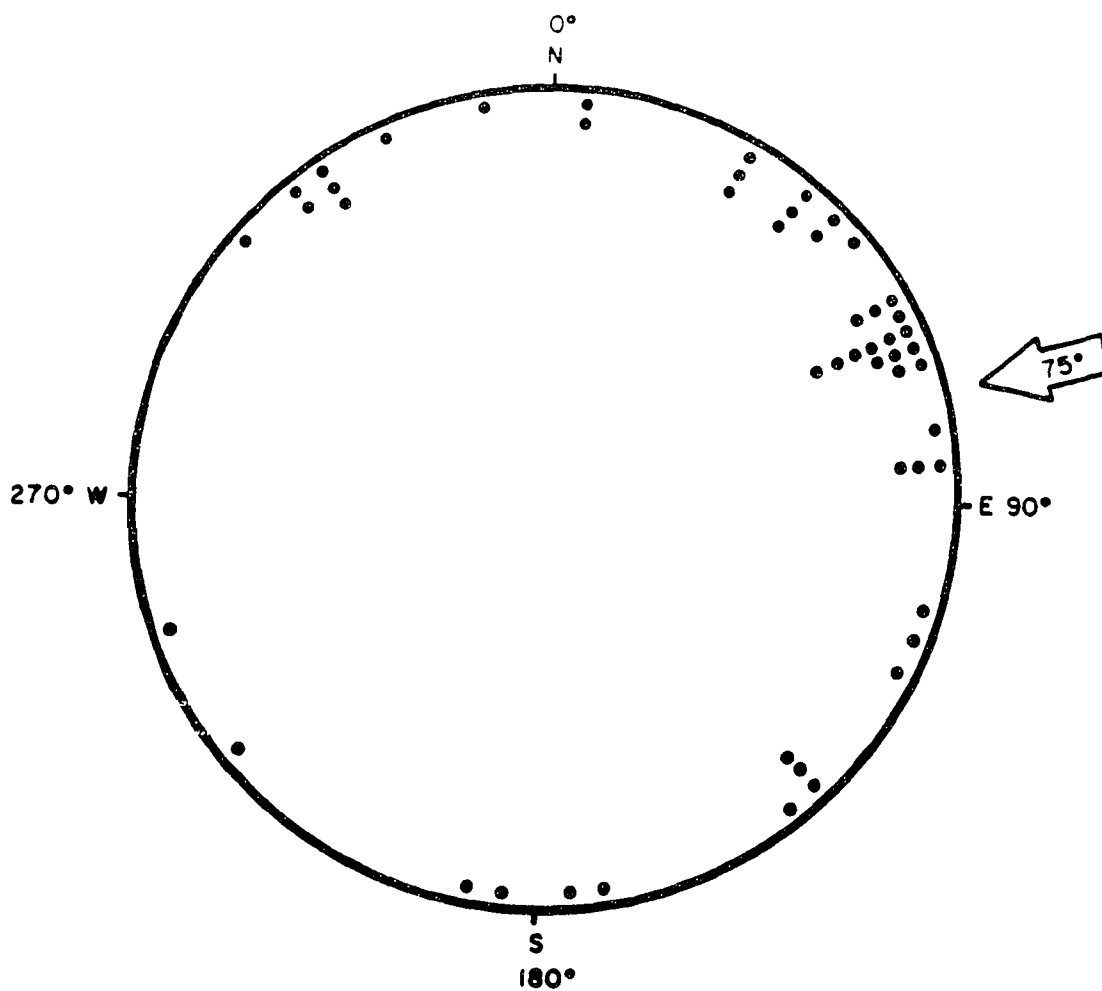


Figure 2.2. Location of matings on tree. The circle represents a tree canopy; north was determined by placing a compass at the base of the tree trunk. Arrow represents direction of morning sun.

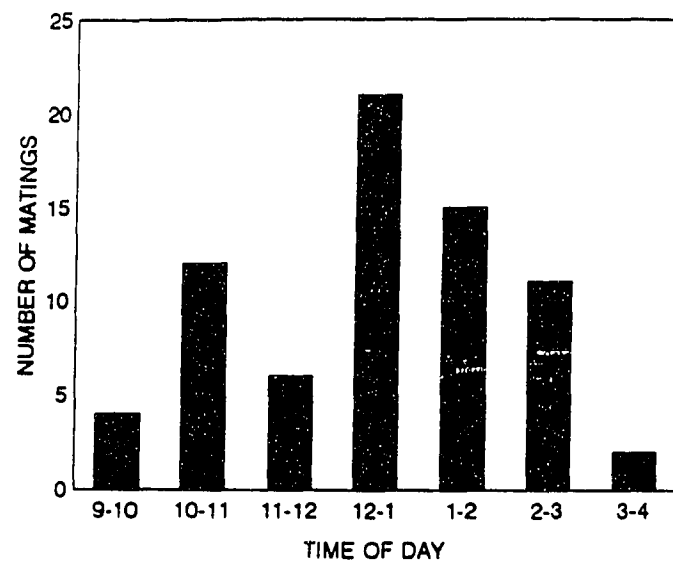


Figure 2.3. Frequency distribution of time of matings.

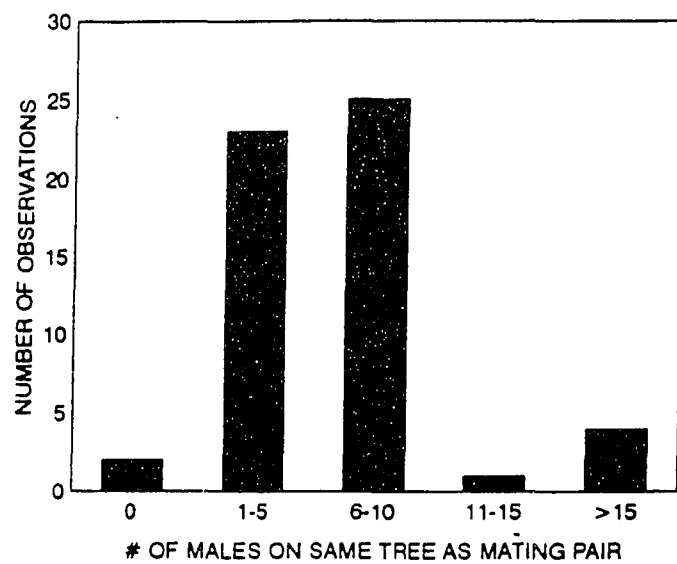


Figure 2.4. Frequency distribution of males on same tree (lek) as mating pair.

Discussion

Our results suggest that the male Mediterranean fruit fly's ability to locate specific lek sites and adequately perform courtship determines whether or not he will successfully mate. However, why males aggregate at specific lek sites is not understood. It is possible that these leks may have evolved because they enable females to make comparisons between large numbers of mating partners (Bradbury 1981). This requires the female to be able to discern differences in males and suggests that she should receive some type of benefit by choosing. The females in this species appear to receive only gametes. Such an evolution leads to the problematical "lek paradox" (Borgia 1979; Taylor & Williams 1982). The lek paradox is the observation that females which gain only gametes from their mate exhibit strong preferences, whereas in paternal species where there are clear benefits gained by mating with particular males, the female's do not exhibit as strong a preference.

There is, however, growing evidence that direct selection is important in the evolution of mating preferences, even in species that form leks (Kirkpatrick & Ryan 1991, Reynolds & Gross 1990). However, recent laboratory studies of the Mediterranean fruit fly weaken the direct selection argument. It was found that females that

mate with virgin males enjoy increased fecundity and longevity (Whittier & Kaneshiro 1992). Thus, unless there is some indirect benefit to females to mate with these preferred males it would be to the female's advantage to select a virgin (less successful) male. These indirect benefits could include one or more of these models: The sexy son hypothesis (Weatherhead & Robertson 1979), runaway selection, or the parasite hypothesis (Kirkpatrick & Ryan 1991). Hendrichs et al. (1991) feel that Mediterranean fruit fly leks have evolved in response to both direct and indirect selection, and that females, subject to high predation on fruit, may have driven the evolution of male calling and the lek mating system by having selected for ready location of males on the host foliage. In addition, they feel this may serve as an arena in which males are sorted by intrasexual selection and in which female choice is facilitated.

There was no serious predation threat at this study site, but an historical selection could account for behaviors seen in Hawaii. In addition, the lek sites did not appear to have any special resource such as fruit, bird droppings, or unusual deposits of honeydew. During our study, <10% of the potential lek sites were used. Three different species of host trees served as mating sites in this study, and at least seven other different species of host trees have been recorded as mating sites in other

studies (Prokopy & Hendrichs 1979, Hendrichs & Hendrichs 1990). However, citrus does seem to be preferred here as well as in Egypt (Hendrichs & Hendrichs 1990), as three-fourths of the citrus trees at this study site served as mating sites. Most important in determining the location of the lek site appears to be the host status of the plant, combined with environmental factors that create a favorable microhabitat. Each of our mating sites had dense foliage and close branch structure (Hendrichs & Hendrichs 1990) and direct sunlight during the morning (Arita & Kaneshiro 1985, 1989).

Most copulations occurred in trees with 2-10 other males. This number corresponds closely to the 2-12 male leks reported earlier, even though these authors defined the lek as only a small portion of the tree canopy (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989). There does, however, seem to be some optimal size of these leks. Most lek sites in this study and in some other tephritid species contain 2-10 males (Aluja et al. 1983, Burk 1983, Shelly & Kaneshiro 1991). Larger leks may be disadvantageous for all males on the lek, if increased numbers disrupt male pheromone calling and thus reduce a male's ability to attract and court females.

Female choice seems to operate in this species, as nearly all courtships that were not successful were terminated by the female. In addition, the relative

importance of male-male interactions seems to be minimal. Although it has been reported that Mediterranean fruit fly males defend their territories from other males (Arita & Kaneshiro 1989, Hendrichs & Hendrichs 1990), the term "defend" may be somewhat misleading. Resident males successfully "defended" their territory less than one-third of the time. Indeed, territory defense may not be very important, as adequate territories do not seem to be limiting, and usually the losing male merely flew to an adjacent leaf and began calling from this new territory immediately. Females did not appear to cue into "preferred" territories, as only two matings during the entire study occurred on the same leaf. As a result, it is not surprising that male size did not correlate with mating success. Similar results have been found with the Mexican fruit fly, Anastrepha ludens (Loew) (Robacker et al. 1991). This contrasts with studies of the Caribbean fruit fly, Anastrepha suspensa (Loew), in which size and mating success were correlated (Burk & Webb 1983, Sivinski 1984).

Matings occurred in a unimodal distribution throughout the day, as they do in Guatemala (Prokopy & Hendrichs 1979) and in Greece (Hendrichs et al. 1991), rather than in a bimodal distribution as seen in Egypt (Hendrichs & Hendrichs 1990). In addition, mating pairs remained at the lek site and usually upon the initial territory where copulation occurred.

Our emphasis here has been to identify cues used in mate choice. Studies now should determine the effects of the females mate choice upon the offspring. Moreover, there appears to be some variation in the behavior of the Mediterranean fruit fly populations in Egypt, Hawaii, and possibly those in Central America.

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CHAPTER 3

MALE COURTSHIP SUCCESS AND FEMALE DISCRIMINATION
IN THE MEDITERRANEAN FRUIT FLY
(DIPTERA: TEPHRITIDAE)

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ACKNOWLEDGEMENTS

We thank X. Cao, A. Dewire, and X. Gao for help in rearing the flies and tabulating the data. This research was funded in part by California Department of Food and Agriculture agreement # 90-0581 and USDA-ARS grant 59-5320-1-189 to K. Y. K.

ABSTRACT

The mating success of individually marked male Mediterranean fruit flies was monitored over six consecutive days in the laboratory. Mating frequency was non-random as both the numbers of males that failed to mate and males that mated many (>4) times were much higher than expected by chance alone. Differential mating success resulted in part from inter-male variation in activity level. Male copulatory success was positively correlated with the numbers of courtships performed, attempted copulations (mountings) and females courted. Male-male aggression, on the other hand, had a negligible effect on male mating success. Female choice also appeared to influence male mating frequency. Less than 10% of courtships resulted in mating, and in most cases females terminated courtship by simply moving away from the male. Females that did mate generally selected males having higher copulatory scores than previously rejected males.

KEY WORDS: mating behavior; courtship; lek; Mediterranean fruit fly; Ceratitidis capitata.

INTRODUCTION

Lek mating systems have been reported for many insect species (Spieth 1968; Prokopy & Hendrichs 1979; Kimsey 1980; Burke 1983). Consistent with studies of vertebrate leks (Wiley 1973; Apollonio et al. 1989), differential mating success among males - where a few individuals account for most of the matings - may be a common feature of insect leks (Arita & Kaneshiro 1985; Robacker et al. 1991). In some cases, nonrandom mating may depend directly on male performance in intrasexual aggression. Male-male interactions may result in the males sorting themselves by territory location or position in a dominance hierarchy, thereby eliminating the opportunity for females to independently choose males (Lederhouse 1982; Shelly 1987; Sivinski 1989). However, because females of lek mating species are often observed to discriminate among different males and reject most courtships (Robacker et al. 1991; Whittier et al. 1992), non-random mating may also be a result of female choice for the "best" possible male with regards to advantageous heritable differences in the offspring (Watt et al. 1986; McClain & Marsh 1989; Moore 1989).

Identifying the underlying causes of nonrandom mating is complicated by the possibility that male sorting and female choice may not have independent effects but instead

may covary in some way. Thus, males that are successful in intrasexual aggression may also be preferred by females exhibiting "active choice" (sensu Parker 1983; Poramarcom & Boake 1991). Conversely, in other species female choice is apparently based on independent cues, and male fighting ability and mating frequency are unrelated (Robacker & Hart 1985; Apollonio et al. 1989; Boake 1989). In addition, just as males vary in their ability to obtain mates, females may vary in their levels of discrimination. As a result, even poor quality males may be able to mate by chance meetings with non-discriminant females (Kaneshiro 1989). While this may not have a strong effect on the mating skew, it may act to distribute the matings to a larger group of males. Unfortunately, very little quantitative data exists concerning the effects of male aggression, male courtship, and female discrimination on mating success for lekking insects.

The Mediterranean fruit fly, Ceratitidis capitata (Wiedemann), displays a lek mating system (Prokopy & Hendrichs 1979; Arita & Kaneshiro 1985, 1989; Hendrichs & Hendrichs 1990; Whittier et al. 1992), and though data are scant, male mating success appears to vary greatly. However, the cause and degree of non-random mating, the role of male-sorting, and the presence of female discrimination have never been quantified. In this laboratory study, we first quantified the degree of non-random mating present

among Mediterranean fruit fly males. Second, we examined relationships between male mating success and male-male interactions, male size, male pheromone calling, male courting and attempted copulations. Last, we estimated the degree of female choice present in this species by recording the number of courtships each female elicits as well as the number of different males she visits. In addition, we investigated whether females ultimately copulated with males of higher quality than those rejected during previous courtships.

Mating Behavior of Ceratitis capitata

Males aggregate on specific host trees during midday and perch on leaf undersurfaces. While perching, males often display "calling" behavior in which the abdomen is curled upward, a bubble-like structure (terminal end of the rectal epithelium) is extruded, and a sex pheromone attractive to females is emitted (Feron 1962; Prokopy & Hendrichs 1979; Arita & Kaneshiro 1986; Whittier et al. 1992). Males weakly defend their individual leaf territories by lunging, pushing or chasing intruders. Possession of a particular leaf does not seem critical, since no resident advantage was detected in aggression, and in most cases the losing male simply established a new territory on a nearby leaf and begins calling with little disruption of activities (Whittier et al. 1992).

After the arrival of a female, the male initiates vigorous wing fanning, thus providing potential visual, acoustical, and pheromonal cues (Webb et al. 1983). The female, if receptive, approaches the wing-fanning male face-to-face (Arita & Kaneshiro 1989). The male continues wing-fanning for 1 - 140 seconds and then jumps on the female and attempts to copulate. Copulation occurs on the undersides of the leaf and lasts about 3 hours (Whittier et al. 1992). If unreceptive, the female decamps during the male wing-fanning or responds to the male's mount by dropping from the leaf.

Materials & Methods

Flies used in this study were from a laboratory colony started with 200 - 300 adults reared from peaches, Prunus persica L., collected in Kula, Maui, Hawaii. The colony was maintained using standard rearing procedures (Tanaka et al. 1969) for about 10 generations. The laboratory was maintained at 20-23 °C and 65%-75% relative humidity. The laboratory received both natural and artificial lighting, and an approximate 12h:12h light:dark cycle was employed. Because this species become sexually mature in 4-6 d (Arita & Kaneshiro 1982), the sexes were separated within 24 h of eclosion.

Observations were made on 6-day old virgin males and 10-day old virgin females that were first cooled in ice and

then individually marked with enamel paint on the thorax. Cooling has no adverse effect on the behavior of male (Arita 1979). Five groups of flies, each consisting of 10 males and 10 females, were placed in cubical glass containers (volume 12 l) with ample food and water. One side of the container was open and fitted with a cloth sleeve to allow access to the flies and air flow with the outside. Male density in the cages was similar to that observed in natural leks, where males typically cluster in a small portion of the canopy of host trees (Whittier et. al. 1992). However, in order to facilitate data collection on male-female interactions, we used a female density that undoubtedly exceeded natural levels. Despite their high density, females rarely interacted with one another, and therefore we assume that the nature and outcome of intersexual encounters were not markedly altered under this density regime.

The following protocol was used to observe all groups of flies. Males were placed in the cage one day prior to the start of observations and left there for the remainder of the experiment. On the following day, ten females were introduced into the cage at 0930 hrs, and an observer continuously recorded all male-male and male-female interactions until 1130 hrs. These interactions included male aggression (lunging, pushing, facing-off), male wing fanning (courtship), attempted copulation (male mounting of the female) and copulation. Courtship duration was timed

from the initiation of male wing fanning (after the female came to a stop in front of the male) until the male mounted the female, the male ceased wing fanning, or one individual moved more than 3 cm away from the other.

Following the observation period, the females which had not copulated were removed from the cage and placed into a plastic bucket (volume 5 l) with food and water until the next morning when they were re-introduced into the observation cage. Copulations were allowed to continue in the cage until completed. Females which copulated were not re-used but were replaced with new individuals (as were individuals that died during the observation period). This same procedure was followed for six consecutive days. The males were then assigned a "copulatory score" which is the total number of copulations achieved for that male during the six days of observations. After observations, we obtained measurements of male size by drying the flies in an oven at 65 °C for 18 hours and then weighing to the nearest 0.1 mg.

Because of the difficulty of recording courtship behavior and pheromone calling behavior of individual males simultaneously, we recorded pheromone calling behavior in a separate experiment. Four groups of six males each were painted and placed into cages with six females in the same manner as the experiment described above. Females were not painted. All courtships and copulations for each male were

recorded for four consecutive days. In addition, each male was checked every two minutes to determine if he was actively pheromone calling. Because copulating males were unable to call, we determined the male's pheromone calling activity as a ratio of observed calling/opportunity to call.

Results

The Data Set

All 50 males originally placed in the cages survived the entire observation period and were included in the data analysis. A total of 136 females were used in the study but analyses were restricted to females which copulated (n=84) and females which did not copulate and were observed a minimum of five days (n=23). Twenty-nine females did not mate and were not observed for five days. All interactions involving these individuals were considered, even those with flies excluded from the final analysis.

Incidence and Outcome of Courtship

In a given cage, an average of 42.7 (SD = 23.0) courtships occurred per day for a total of 1282 courtships over all cages during the entire study. Courtships involved an average of 7.6 (SD = 1.63) males per cage per day, and all males performed at least one courtship during the study. However, large variation was observed among males in both the total number of courtships performed and the number of different females courted. Over the entire observation

period, individual males performed between 1 - 75 courtships ($x = 24.2$; $SD = 19.7$) for 1 - 20 different females ($x = 9.2$; $SD = 5.4$).

Large variation in courtship participation was also evident among females. In a given cage, courtships involved an average of 8.2 ($SD = 1.8$) different females per day, and all females elicited at least one courtship. As with males, females varied greatly in both the total number of courtships elicited and number of different males visited. Over the entire observation period, individual females elicited between 1 - 67 courtships ($x = 9.2$; $SD = 11.9$) from 1 - 10 different males ($x = 3.8$; $SD = 2.5$). For a given female, courtships received were distributed fairly evenly among different males, and the total number of courtships elicited was directly related to the number of males visited ($r_s = 0.76$; $n = 107$; $P < 0.0001$).

Approximately 42% (525/1236) of the courtships ended with the female leaving during the wing-fanning stage, and 51% (630/1236) failed after the male mounted the female. Unsuccessful mounting attempts invariably resulted from the female dropping from the side or top of the cage. In some cases the male maintained his grasp of the female and attempted to force copulation on the floor of the cage. However, none of these attempts were successful. Only 7% (81/1236) of all courtships resulted in copulation. Courtship duration varied between 1 - 140 s and did not

differ significantly between those courtships that ended in copulation ($\bar{x} = 15.0$ s; $SD = 14.2$; $n = 66$) and those that did not ($\bar{x} = 18.0$ s; $SD = 16.4$; $n = 884$; $t = 1.43$; $P > 0.05$).

Male Copulatory Success

Of the 50 males observed in this study, 62% (31/50) copulated 1-5 times and 38% (19/50) did not copulate at all ($\bar{x} = 1.6$; $SD = 1.6$; Fig. 3.1). The observed distribution of copulations among males deviated significantly from random ($n = 50$; $\chi^2 = 21.9$; $df = 5$; $mean = 1.6$; $P < 0.001$; Poisson distribution), with a coefficient of dispersion of 1.7 indicating a clumped distribution of copulations. This clumping results primarily from the higher than expected numbers of males that did not copulate and males that copulated five times.

The copulatory success of males was related to their overall level of sexual activity. Male copulatory success was positively correlated with total number of courtships performed ($r_s = 0.44$; $n = 50$; $P < 0.001$), number of attempted copulations ($r_s = 0.42$; $n = 50$; $P < 0.002$), and the number of different females courted ($r_s = 0.62$; $n = 50$; $P < 0.001$). For example, on average, males which did not copulate ($n = 19$) performed 9.3 courtships, attempted 4.8 mountings, and courted 4.7 different females. By comparison, males that copulated three or more times ($n =$

17) averaged 32.9 courtships, 19.2 attempted copulations, and courted 13.0 different females (all differ at $P < 0.001$; Wilcoxon rank sum).

Data from the pheromone calling experiment also revealed large variability among males in the amount of time spent calling. Even though all males called, some males called as much as 86% of the available observation period, while others called as little as 22% of the available observation period ($x = 59$; $SD = 15$). The proportion of time a male pheromone called was correlated with the number of courtships he accomplished ($r_s = 0.45$; $n = 23$; $P < 0.05$). Interestingly, however, time spent pheromone calling did not correlate with copulatory success ($r_s = 0.23$; $n = 23$; $P > 0.05$).

In addition to the general activity of the male, variation in copulatory success appeared to reflect differences in courtship success after mounting the female. Variation in copulatory success did not appear to arise from individual differences in the "propensity" to mount females. The proportion of courtships that led to mounting (mounts/courtships) varied independently of male copulatory success (Fig. 3.2). Among males that copulated three or more times, for example, courtship resulted in mounting in 58% (327/559) of the cases compared to 57% (288/509) for males that did not copulate and males that copulated one-time ($P > 0.05$; Wilcoxon Rank Sum). In contrast, male

copulatory success was directly related to the probability of obtaining copulation once the female was mounted (Fig 3.2). For example, approximately 18% (61/327) of mountings ended in copulation among males that copulated at least three times compared to only 5% (9/196) for males that copulated only one time.

Female Discrimination and Copulation

Females varied greatly in the number of males visited prior to copulation. Females that did not copulate during their five observation days (23/107) elicited courtship 1 - 67 times ($x = 15.0$; $SD = 17.5$) from 1 - 10 different males ($x = 5.0$; $SD = 2.9$). On the other hand, females that eventually copulated were courted 1 - 47 times ($x = 7.6$; $SD = 9.2$) by 1 - 9 different males ($x = 3.4$; $SD = 2.3$). Much of this activity occurred on the day of copulation as females were courted an average of 4.3 times ($SD = 4.8$) by 2.5 different males ($SD = 1.6$) on the day of copulation.

Among females that copulated, 26% (22/84) copulated with the first male that courted them. On the other hand, nearly 50% (38/84) of the females were courted by more than five different males before copulating. Of the females that were courted by more than one male, 75% (42/56) ultimately copulated the male which had the highest "copulatory score" among all courters (Fig. 3.3). The "copulatory score" for all unsuccessful courters of a particular female ($x = 1.98$;

SD 1.1) and the "copulatory score" of the male which eventually copulated with the female ($x = 3.2$; $SD = 1.2$) were significantly different ($T = 229.5$; $n = 54$; $P < 0.001$; Wilcoxon paired-sample test). However, we recognize that there may be potential circularity in this argument because by mating with a particular female a male's score increases by one. To adjust for this, a more conservative test was performed by adding one mating to the sum of matings of previously rejected males. Males selected as mates were still found to have higher scores, on average, than rejected males. ($T = 312.5$; $n = 46$; $P < 0.05$; Wilcoxon paired-sample test). Also, among those females that mated after the first courtship, most selected males that had higher mating scores than males rejected by females that ultimately mated ($n = 22, 56$; $P < 0.005$; Wilcoxon rank sum). In addition, these males also had significantly higher scores than the first male that courted females that were courted by more than 1 male and mated ($n = 22, 56$; $P < 0.01$; Wilcoxon rank sum).

Male-Male Interactions

Male-male interactions were rare and, on average, fewer than 2.5 interactions were recorded per cage on a given day. Fighting ability was unrelated to mating success. Males that were successful in mating won 29/55 (53%) of their male-male interactions, and males which never mated won 10/20 (50%) of their interactions ($G = 0.1$; $P > 0.05$; G-test

with Yates correction factor). When only the interactions between mating and non-mating males are considered, mating males won these interactions 13/24 times ($Z = 0.2$; $P > 0.05$; Binomial test). All of these interactions were brief (1-4 seconds) and did not seem to disrupt pheromone calling for more than just a few seconds. Most of the interactions that were observed appeared to be accidental, possibly caused by the increased chance of encounters due to the confined space. No interactions occurred during courtship. Consistent with the low numbers of male-male interactions, male size in this study did not correlate with number of courtships ($r_s = 0.09$; $n = 19$; $P > 0.05$) or with the number of copulations ($r_s = 0.06$; $n = 19$; $P > 0.05$).

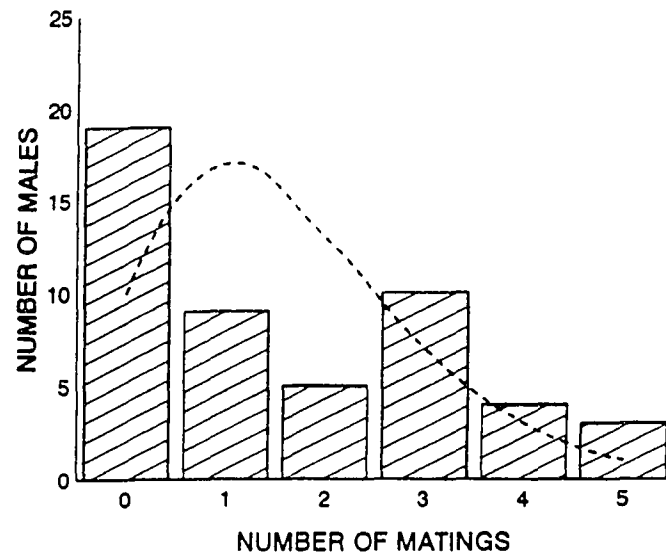


Figure 3.1. Copulation frequency distribution of males from all cages. Dashed line is the expected Poisson distribution.

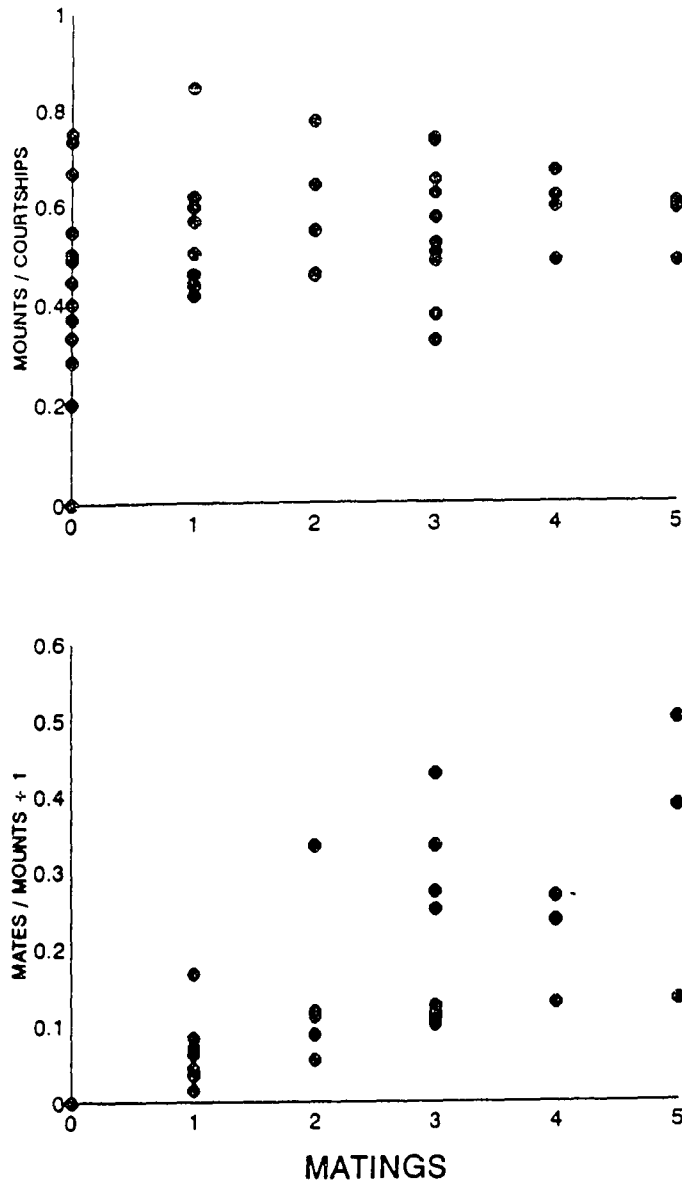


Figure 3.2. Relationship between male mating frequency and the probability that (a) courtship results in mounting and (b) mounting results in copulation. Each point represents a single male with data pooled over all observation days.

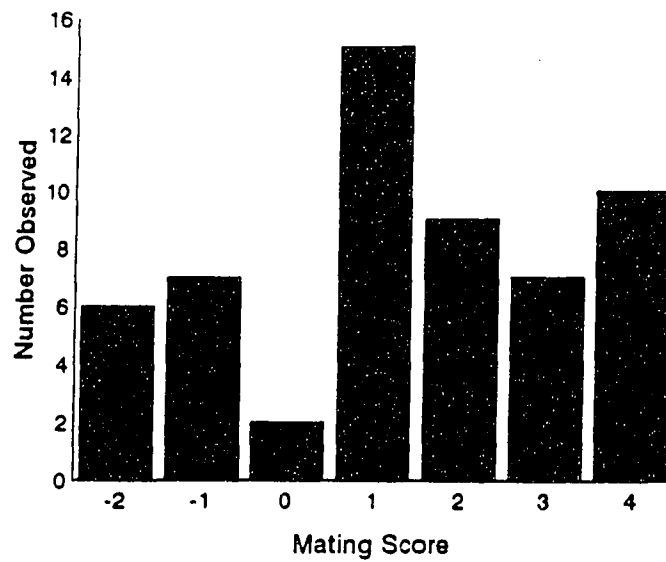


Figure 3.3. Frequency distribution of the differences between copulation scores of males selected as mate and the average copulation scores of males previously rejected by females. Positive values represent matings in which the male had a higher score than the average for all males previously rejected by a given female.

Discussion

Variation in copulatory success among males in this experiment appeared to result from both the activity of the male prior to the arrival of the female and the actions of the male in the presence of the female. While size and aggressiveness are often an important criteria in establishing dominance (Borgia 1979; Shelly 1987), size differences and success in male-male interactions did not appear to influence copulatory success in this study. This result is consistent with field observations (Whittier et al. 1992) that revealed no resident advantage in male-male interactions, no position effect on male mating success, and uniform size between mated males and males caught in traps. One male behavior that we identified as being an important determinant of courtship success was pheromone calling. Success in attracting females appeared to reflect individual differences in the time spent pheromone calling and possibly in the quality of the pheromone. While we did not examine potential differences in pheromone quality, we found that males which pheromone called for a longer period did attract more females.

Attracting a female is, of course, a critical step in achieving copulation. However, attracting a female was apparently only one of several factors necessary for successful copulation, because even though signaling effort was correlated with courtship activity, it was not

correlated with copulation frequency. This absence of a significant relationship between pheromone calling and copulatory success reflects the weak correlations between pheromone calling and number of courtships as well as the weak correlation between number of courtships and copulatory success. The weakness of these relationships suggests that male activity alone was not the sole determinant of male copulatory success.

In addition to the male's behavior, female choice appears to be an important factor in male copulatory success. Evidence for this includes the observation that the females were responsible for the termination of all unsuccessful courtships and appeared to make the ultimate decision of whether to accept or reject potential mates. In addition, mate rejection was commonly observed, and consistent with field observations, few courtships led to copulation (Whittier et al. 1992). For those courtships that did lead to mating, there appeared to be some consensus among the females as to what is a "good" mate. We observed significant nonrandom mating among the males, with a small number of males performing a large percentage of the matings.

This nonrandom distribution suggests that there may be a range of mating types among the males. In addition, as Kaneshiro (1989) has suggested, there may be a range of mating types segregating in both sexes. In this study the

females were very active in eliciting courtship, and most females elicited courtship from several males prior to copulation. The males which females eventually accepted had higher copulatory scores than males that were previously rejected. In addition, even though some females copulated with the first male they encountered, these males typically had higher copulation scores than males rejected by other females. However, there was variation in female choosiness. First, it was observed that 20% of the females never mated. It seems unlikely that these females were unable to mate, since all elicited courtship at least one time and overall averaged 15 courtships from 5 different males. In addition, even though those females that accepted copulation from the first male encountered tended to mate with males of high average score, 14% (3/22) chose males that had very low copulatory scores (1 mating) and 36% (8/22) of the females chose males with below average scores (<2 matings). Even though copulation score may not be extremely sensitive to measuring male quality, it does at least suggest that there is variation in the female's choice of the "best" mate.

While it is still not known whether differences in male ability to mate and female choice are genetic or environmental, understanding both the reasons for skewed male copulatory success as well as the basis for female discrimination may be needed to maximize the efficiency of sterile insect release programs. Recent studies by Shelly

et al. (unpublished data) showed that after releasing sterile laboratory-reared Mediterranean fruit flies into a small fruit orchard containing a wild population of Mediterranean fruit flies, sterile released males mated with wild females significantly less often than expected. While the reasons for this result are not known, a search for ways to improve the mating behavior of laboratory-reared males of Ceratitidis capitata should be of high priority to insure the success of eradication plans throughout the world.

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CHAPTER 4

MALE MATING SUCCESS AND FEMALE FITNESS IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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This chapter was previously published in the Annals
of the Entomological Society of America 84: 608-611

ACKNOWLEDGMENTS

The authors thank Alean Cook and Kyle Serikawa for laboratory assistance. This research was partially supported by a grant from the California Department of Food and Agriculture project 88-0536 and an USDA-ARS grant 58-9142-6-42 to K.Y.K.

ABSTRACT

Females of the Mediterranean fruit fly, Ceratitis capitata Wiedemann, mate most often with a small, non-virgin subset of males although mating with virgin males enhances female fitness. Both the numbers of offspring produced per female and female longevity is greater for females that mate with virgin males. This phenomenon is not explained by temporary sperm depletion, nor does it easily conform to any of the classical models of sexual selection. This apparently maladaptive mating system can be explained by Kaneshiro's model of differential selection.

KEY WORDS: Insecta, Mediterranean Fruit Fly, sexual selection, evolution

The mating system of the Mediterranean fruit fly, Ceratitis capitata Wiedemann, has been described as being characteristic of a "lek" mating system (Prokopy & Hendrichs 1979; Arita & Kaneshiro 1989). Lek behavior constitutes an aggregation of territories utilized by males for the sole purpose of mating (Wilson 1975). Mediterranean fruit fly males are polygamous (Nakagawa et al. 1971) and a small proportion of males in the population perform a majority of matings (Arita & Kaneshiro 1985). C. capitata males compete for territories (i.e., the undersurface of leaves of host trees), and it is here that mating occurs. Females that visit these territories show varying degrees of discrimination in selecting mates, as indicated by the high proportion of courtships that are elicited but ultimately rejected (T.S.W., unpublished data). In field studies of both the Mediterranean fruit fly and the melon fly, Dacus cucurbitae Coquillett, >90% of the male-female encounters result in female rejection of courtship attempts by the male (Kaneshiro et al., unpublished data). The pronounced differential mating success among males and the high number of females that reject courting males suggests sexual selection via female choice. The reason why particular males are successful whereas others fail is not clear. Females may "accept" their mates based upon qualities such as size, secondary sexual characters, "good" genes,

courtship display, or the possession of preferred territories within the lek. Confounding each of these possibilities is the unknown importance of male-male competition and how this competition influences male mating success. Unfortunately, data that could help clarify the role of each of these qualities are lacking.

Even though we do not understand why some males are more successful than others, we can investigate the effects that mate choice can have upon the female. If males are selected because of some superior fitness component that they offer to females, this should be reflected in some fitness component of the female or in the offspring. In this study, we attempt to determine what effects mate choice has upon the fitness of individual females. More specifically, we wish to determine if there is a cost or benefit to the female in mating with males that have mated more than once.

Materials and Methods

Flies used in this study were obtained from infested coffee, Coffea arabica L., collected in Kona, Hawaii; loquat, Eriobotrya japonica (Thunb.) Lindl., collected in Kula, Maui; and peach, Prunus persica L., collected in Kula, Maui. More than 300 flies were reared from the infested coffee and were maintained in culture following the method of Tanaka et al. (1969). All experiments with flies reared

from loquats and peaches were conducted using F₁ generation flies. Adult flies were fed a mixture of two parts honey: one part protein hydrolysate. The sexes were separated within 24 h of eclosion. Male and female Mediterranean fruit flies become sexually mature in 4-6 d (Arita & Kaneshiro 1982). To ensure that all flies used in this study would be sexually mature, only 12-d-old virgin males and 10-d-old virgin females were used. Mating activity was observed continuously between 0800 and 1500 hours because most natural matings occur between 0900 and 1500 hours (Arita & Kaneshiro 1989). Copulating pairs were carefully removed without agitation or disturbance of sperm intromission and all flies remained in copulo for at least 120 min. Voucher specimens of the various cultures used in this study have been preserved in alcohol or pinned and deposited in the Hawaiian Evolutionary Biology Program Reference Collection at the University of Hawaii. All analyses were performed using the GLM procedure of the Statistical Analysis System (SAS Institute 1986) and mean separations were accomplished using Duncan's multiple range test (Duncan 1955).

Mate Type and Number of Progeny. Fifty males and 50 females were placed into a screen cage (60 by 60 by 60 cm) and allowed to copulate. All males that did not mate were discarded. After copulation was completed, mated males were

returned to the cage and mated females were placed individually into 2.5-liter buckets covered with organdy that contained a water source, an adult food source, and a nectarine (Prunus persica var nectarina) as an oviposition substrate. The females were designated as females that mated with virgin males. All nectarines were of uniform size and ripeness and were used because they are an excellent host. The nectarine was replaced every 5 d for the life of the female. The infested nectarine was then placed upon a 2-cm bed of vermiculite within a 5-liter plastic bucket, which was covered with nylon organdy, and the larvae were allowed to pupate. Because natural oviposition substrates were used, no attempts to count eggs were made and only adult progeny from each fruit was counted.

Mated males were again offered virgin females during the next 15 d and each mated female was isolated, this time designated as a female that mated with a nonvirgin male. Progeny from these females were reared as described above.

The experiment was replicated 4 times. For two of the replicates, flies were taken from the coffee culture that had been in the lab about 10 generations. Flies used for the other two replicates were taken from F₁ flies reared from the loquat and peach stocks. In addition, females that died within 5 d of copulation were not included in the results.

Mate Type and Longevity of Female. This experiment was set up in the same manner as described above, except that a third group of virgin females was used. This third group of females was also individually isolated and was provided a nectarine for oviposition. However, for this experiment however, no progeny were counted from any of the three groups of females (i.e., those mated with virgin males, those mated with nonvirgin males, and unmated females), because the nectarines were discarded after allowing the females to oviposit. Only longevity was recorded for each female in this experiment. Five replications were conducted. Flies from the coffee culture were used for three of the replicates and F_1 flies obtained from loquats and peaches were used for the other two replicates.

Test for Sperm Recovery in Mated Males. Because of the possibility of temporary sperm depletion in mated males, a separate group of 50 males was placed with 75 females and allowed to copulate. All males that did not copulate were discarded. Males that mated were presented with more virgin females the following day and allowed to mate. Males that failed to mate on this second day were also discarded. All males that had mated on day one and day two were then held together for 5 d and then allowed to mate again on day seven. All mated females were isolated as in the first experiment. However, all females that mated with males on

day one were discarded. Offspring number and longevity were obtained only from those females that mated with males that had <24 h to recover from a previous mating and from those that had at least 5 d to recover from a previous mating. This experiment was replicated twice using F₁ flies from peaches.

Results and Discussion

The number of offspring produced per female differed significantly depending on whether she mated with a virgin or nonvirgin male (Table 4.1). Similar differences in Drosophila have been explained by a reduction in sperm or other components of the male ejaculate (Lefevre & Jonsson 1962). Markow et al. (1978) showed that Drosophila females that mate with nonvirgin males produce 33% less offspring than females that mate with virgin males. However, if mated males were allowed a recovery period of 24 h, the number of offspring produced by females mating with nonvirgin males did not differ from those mating with virgin males. In our study, we allowed a recovery period of 5 d to see if these differences between virgin and nonvirgin males were merely a result of temporary depletion of sperm or some component of the ejaculate. We found no significant difference for number of offspring for males that had only a 24-h recovery period versus those that had 5 d to replenish their sperm supply ($F = 0.0$; $df = 1,75$; $P = 0.96$).

In addition to significant differences in offspring produced by females that mated with virgin or nonvirgin males, there were also significant differences in longevity of the female depending on whether she had mated with a virgin or a nonvirgin male (Table 4.2). Furthermore, females that mated with virgin males lived significantly longer than females that did not mate. Although we can currently only speculate as to why this difference exists, it is possible that a "gift" may somehow be incorporated into the ejaculate in much the same way that males contribute to oocytes and to somatic tissue in Drosophila mojavensis Patterson & Crow (Markow 1984) and in several species of Lepidoptera (Boggs & Gilbert 1979). This could account for both the increased number of offspring and increased longevity of females that mate with previously unmated males. However, because females in this experiment were provided unlimited access to food, it is more likely that this gift involves components of the ejaculate other than nutritional products. Furthermore, increased fecundity is usually negatively correlated with longevity and the reproductive schedule of a population should affect the senescence pattern within it (Edney & Gill 1968), but there are exceptions (Rose & Graves 1990).

Whatever the mechanism, females apparently do not recognize virgin or nonvirgin males, or prefer nonvirgin males if they do recognize different males. Whittier (1986)

showed that Mediterranean fruit fly females preferentially mated with nonvirgin males even though virgin males were present. In view of these differences of fecundity and longevity, it would seem that females mate most often with a small, nonvirgin subset of males, although mating with virgin males apparently enhances female fitness. This apparently maladaptive mating system could be explained by a closer examination of the dynamics of the sexual selection system in this species.

Females may mate with particular males in response to a preference for a particular male trait (Fisher 1958). This possibility seems unlikely because of the selective disadvantage that appears to accompany such a preference. Females may also mate with a particular male because he possesses "good genes" and has demonstrated his "quality" by being able to mate several times already. This idea cannot be currently discounted, but we know of no mechanism by which the female can recognize male quality. However, the female may be willing to suffer reduced fecundity in order to increase the quality of her offspring, much like that of the "sexy son" proposed by Weatherhead & Robertson (1979).

Another possibility suggested by Kaneshiro (1989) is a model of differential selection between the two sexes for opposite ends of the mating distribution. It is suggested that there is a range of mating behavioral phenotypes segregating in the population. There are males that are

highly successful in mating ability and they accomplish the majority of matings in the population. However, there are also males that are not so successful in satisfying the mating requirements of most females in the population. Similarly, among females, there are some that are highly discriminant in mate choice and some that are not so discriminant. Classical sexual selection models (Fisher 1958; Lande 1981, 1982) suggest that female preference for superior male mating qualities coevolve. This coevolution results in a runaway selection for any male phenotype which gives certain males an advantage over other males in mating success. Such runaway selection for elaborate secondary sexual characters in males is then countered by natural selection in maintaining the optimum phenotype for the particular environment in which the species lives. However, the differential selection model suggests that individuals more likely to mate are the males with high mating success and females that are nondiscriminant. There is cost to females that are too discriminant, because they may never encounter males that are able to satisfy their courtship requirements. The genetic correlation between the two behavioral phenotypes in the two sexes (Kaneshiro 1989) acts as a stabilizing feature of the sexual selection process as visualized by the differential selection model. Production of the entire range of mating phenotypes in both sexes generation after generation can thus be explained by this

model, without having to invoke natural selection as the stabilizing factor.

The mating system in the Mediterranean fruit fly would appear to provide further support for the differential sexual selection model. Because of the apparent reduced fitness of females that mate with nonvirgin males (i.e., fewer offspring and shorter life span), one might expect stronger selection for female ability to detect males that had mated once before and "prefer" to mate with virgin males. However, mate preference experiments that have been conducted on this species (Arita & Kaneshiro 1985; Whittier 1986) demonstrate that $\approx 30\%$ of the males continue to be more successful in mating with more than one female (sometimes as many as 12 females), although females that mate with such non-virgin males will produce fewer offspring and will not live as long as the first female to mate with the male. We suggest that the evolutionary paradox in the mating system of the Mediterranean fruit fly ensures the differential mating between the opposite ends of the mating type distribution in the two sexes. That is, the first matings among a cohort of flies would most likely occur between males of high mating ability and the least discriminant females. Although subsequent matings still involve the same males, they involve ever more discriminant females. In the mating system of C. capitata, there are even higher costs to females that are discriminant and this actually strengthens

the differential selection process by enforcing selection for matings between males with high mating ability and nondiscriminant females.

Table 4.1. Mean number of offspring produced per female mating with a virgin or nonvirgin male

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Mate type	n	Mean	\pm SE

Virgin male	97	110.25	± 1.15
Nonvirgin male	239	73.11	± 0.65

The effect of mate type is significant for number of offspring ($F = 7.70$; $df = 1, 334$; $P < 0.01$).

Table 4.2. Mean longevity of females mating with virgin or non-virgin males and females which never mated.

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Mate type	n	Mean	\pm SE
<hr/>			
Virgin male	121	38.67	\pm .44 a
Nonvirgin male	253	32.31	\pm .28 b
Never mated	117	32.42	\pm .42 b
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Means followed by the same letter are not significantly different. The effect of mate type is significant for longevity ($F = 3.92$; $df = 2, 488$; $P < 0.05$).

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Chapter 5

INTERSEXUAL SELECTION IN THE MEDITERRANEAN FRUIT FLY: DOES FEMALE CHOICE ENHANCE FITNESS?

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ACKNOWLEDGEMENTS

We wish to thank X. Cao, X. Gao, A. Laurie-Dewire and J. Whittier for assistance on this project. This study was aided by funds from the California Department of Food and Agriculture project #90-0581 and USDA/ARS grant 58-9142-6-42 to K. Y. K.

ABSTRACT

Direct and indirect effects of mate choice on females was examined using a laboratory population of a lek mating species, the Mediterranean fruit fly. The nonrandom mating observed in this species is thought to be strongly influenced by female choice. Whether female choice acts to increase fecundity or offspring quality was assessed using two different statistical tests. Multiple regression showed that females obtain direct benefits as a result of mating with males which are successful in copulating with many females, but the relationship between male quality and female benefit is nonlinear. Females which mate with males that obtain few matings (< 2), and females which mate with males that obtain many matings (> 6) enjoy increased fecundity. Mate choice does not, however, appear to enhance offspring quality as father/son correlation and sibling analysis showed no heritable component to male copulatory success.

INTRODUCTION

Sexual selection has the potential of being very strong in lek mating species. In such mating systems there is often marked non-random mating, with females receiving only gametes and no parental care or resources from the male (Wiley 1973; Apollonio et al. 1989; Bradbury 1981; Trail 1985; Robacker et al. 1991). In some cases, non-random mating depends directly on male competitiveness in intrasexual aggression. Male-male interactions may result in the males sorting themselves by territory location or position in a dominance hierarchy, thereby eliminating the opportunity for females to independently choose males (Lederhouse 1982; Shelly 1987; Sivinski 1989). However, in many lek mating species, male-male interactions appear to be minimal and females are often observed discriminating among different males and rejecting most courtships (Andersson 1982; Gibson & Bradbury 1985; Robacker et al. 1991; Whittier et al. 1992).

While females of many resource based mating systems show preferences for males based on the quality of male resources (Emlen & Oring 1977; Gwynne 1988; Markow & Ankney 1984), the reason females prefer particular males in lek mating systems is not as clear. It has been hypothesized that female preferences for males may be based on indirect benefits in the form of gametes that increase offspring

quality or attractiveness (Borgia 1979; Hamilton & Zuk 1982; Moore 1989). The observation that lek mating females receive only gametes, yet seem to show strong preferences has been termed the "lek paradox" (Borgia 1979). This paradox is based upon the theoretical prediction that heritable variation for male quality should diminish due to selection by the females, yet such variation is necessary for females to realize a benefit by choosing males of high genetic quality.

However, some possible resolutions to this paradox have been suggested. Some studies have shown that genetic variation of male traits on which female preferences are based may not diminish over time (Hamilton & Zuk 1982; Lande 1982; Turelli 1984; Hedrick 1988). In these studies genetic variation is maintained due to cycling interactions between hosts and parasites, mutation, recombination, negative genetic correlations or fluctuating selection pressures. Furthermore, Reynolds & Gross (1990) have suggested that various direct benefits may accrue to females via natural selection acting directly on females and their offspring. It is also possible that female discrimination may be based on a more complicated package of benefits that include interactions of both direct and indirect benefits. For example, females may tolerate reduced fecundity or survival to increase the quality of their offspring (Weatherhead & Robertson 1979) or females may mate with "quality" males and

enjoy both increased fecundity and higher "quality" sons (McLain & Marsh 1990). In this study, we use a laboratory population of Mediterranean fruit flies, Ceratitis capitata (Wiedemann), a lek breeding species, to test the hypothesis that female choice acts to increase fecundity and/or offspring quality. Using quantitative genetics we estimate additive genetic variance for male copulatory success and use multiple regression to test for possible correlates of male copulatory success and female fitness.

Biology of Ceratitis capitata

The Mediterranean fruit fly infests more than 200 different species of fruits and vegetables (Christenson & Foote 1960). The egg stage last about 2 days, the larval period 12 days, and the pupal stage about 14 days (Christenson & Foote 1960). The adults become sexually mature in 4 - 6 days (Arita & Kaneshiro 1982).

Mediterranean fruit flies display a lek mating system (Prokopy & Hendrichs 1979; Arita & Kaneshiro 1989; Hendrichs & Hendrichs 1990; Whittier et al. 1992) as males aggregate and "call" on host trees. When calling, males curl the abdomen upward and extrude a bubble-like structure (terminal end of the rectal epithelium) and emit a sex pheromone attractive to females (Feron 1962; Prokopy & Hendrichs 1979; Arita & Kaneshiro 1986). After the arrival of a female, the male initiates vigorous wing fanning thus providing

potential visual, acoustical, and pheromonal cues (Webb et al. 1983). The female, if receptive, approaches the wing-fanning male face-to-face. The male continues wing fanning for 1- 140 seconds and then jumps on the female and attempts to copulate. Copulation occurs on the undersides of the leaf and will continue for about 3 hours (Whittier et al. 1992). If unreceptive, the female decamps during the male wing-fanning or responds to mounting by dropping from the leaf. Most courtships appear to end in failure with only about 7% of all courtships resulting in copulation (Whittier et al. 1992; Whittier et al. in review). In spite of this low success rate, males have been observed to mate more than one time in nature (Whittier et al. 1992) and in the laboratory (Nakagawa et al. 1971; Whittier et al. In Review).

Materials and Methods

Male Copulatory Success

All flies used in this study were from a laboratory colony started with 200 - 300 adults reared from peaches, Prunus persica L., collected in Kula, Maui, Hawaii, and maintained using standard rearing procedures for about 15 generations (Tanaka et al. 1969). The laboratory was maintained at 20-23°C and 65% - 75% relative humidity. The laboratory received both natural and artificial lighting,

with approximately 12h:12h light:dark cycle. The sexes were separated within 24 h of eclosion.

After eclosion, individual flies were cooled in ice and then uniquely marked with enamel paint on the thorax. Cooling has no adverse effects on behavior. (Arita 1979). After painting, fifteen sets of flies, each consisting of 16 7-d old virgin males (240 males total), were placed in cubical screen cages (32 cm x 32 cm x 32cm) with ample food and water. Male density was similar to that observed in natural leks where males typically cluster in a small portion of the canopy of host trees (Whittier et al. 1992). All flies within a set were taken from the same larval batch (created with eggs from > 100 females) to minimize environmental differences. The following day, and each day for 10 consecutive days, five 10-11 day old virgin females were placed into the cage and allowed to mate.

The flies were observed every 15 minutes between 1030 - 1300h, for 10 consecutive days, recording the times at which copulations occurred, the identities of the males, and copulation durations. Observations in nature show that most copulations occur between 0900 - 1500h and that flies copulate an average of 179 minutes (Whittier et al. 1992). Copulating flies were isolated by coaxing them to walk inside the vials, a procedure that did not disrupt copulation. Flies were allowed to complete copulation in these vials. Upon completion of copulation, the mated

female was discarded, and the male was returned to the cage. All unmated females were removed from the cage at the end of each observation day and discarded. Males that died during the ten days of observations were replaced with a stock male of the same age and same larval batch to maintain constant density within the cage. No data were used for either the male that died or his replacement. Copulatory success (= copulation score) of a male was measured as the number of copulations achieved during the 10 days of observation.

After 10 days of observation, all males were individually placed with three 10-day old virgin females in 5 l clear plastic tubs with ample food and water and a cotton sleeve over the opening to allow access to the flies and air flow with the outside. Males were isolated to eliminate the effects of male competition, limit any possible effects of female choice and increase each male's chances of copulation. These flies were observed every 15 minutes between 0800 - 1500h. Upon observing a copulating pair, the two unmated females were removed and copulation was allowed to proceed without interruption. If no copulations occurred in a particular tub after four days, all three females were replaced.

Mated females were individually placed in a 250 ml plastic cup that was covered with nylon organdy and provided food, water and an artificial oviposition substrate (a perforated plastic vial containing a water moistened sponge

and a small amount of peach baby food). To avoid environmental differences within the lab, the female's location on the shelves was randomized every 2 days. Every 2 days for 3 weeks the eggs were removed from the plastic eggling vial with a small paintbrush and placed on top of 40 grams of standard rearing media. The cup of media was then placed on a 3 cm bed of vermiculite inside a 2.5 l plastic bucket with a nylon organdy cover. Adults that later eclosed were removed and separated by sex within 24 hours.

Of the 240 males placed into cages, 125 (52%) produced offspring that were able to be scored. Ninety-nine males (41%) died during scoring or while isolated in the 5 l tubs. Sixteen males (7%) mated but did not produce offspring.

Heritability of Male Copulatory Success

Narrow sense heritabilities of male mating success were estimated from offspring-parent regression for 125 fathers that produced offspring (Falconer 1989). The average score of the first three male offspring to be scored and that were sired from the male's first mating after being isolated was used. In addition, the heritability of male copulatory success was measured on 80 of these fathers by analysis of half-sib and full-sib families (Falconer 1989). Two offspring from each of the male's first two matings were included in the half-sib and full-sib analysis. One son from the first larval batch and one son from the second

larval batch of each female was randomly selected and scored in the same manner as their father. Each son (8 days old) was placed into a cubical 10 l glass cage with 15 other 8 day old males. To minimize environmental contributions to within family correlations, sons of the same father or mother were not placed into the same cage and all sons scored for each mother were reared from different larval batches. All females used in scoring the sons were from the original stock used to score the fathers. Since some males were measured on two matings, the sources of variation in copulatory success were between males, between females within families, and within families.

Heritability of size was also calculated by offspring/parent regression and by half-sib and full-sib analysis. Size for all fathers, mothers, and sons was determined by drying the flies at 65°C for 18 hours and then weighing to the nearest 0.1 mg.

Male Copulatory Success and Female Fitness

A different set of males was scored exactly as described above. However, each mated female was isolated into a 5 l organdy covered plastic bucket with food, water, and a peach as an oviposition source. Peaches were of uniform size and ripeness and were changed at least every 5 days for the life of the female. Peaches that were removed from the female were placed on a 3 cm bed of vermiculite

inside a 2.5 l plastic bucket, covered with nylon organdy. Larvae were allowed to pupate in this bucket, and all adult flies that eclosed were counted. However, to avoid problems of independence only the first female that a particular male mated was included in the analysis. All fathers from the original cages were killed by freezing after the 10 days of scoring, and all isolated females were allowed to die natural deaths. All flies were then dried and weighed as described above.

Multiple regression using the number of progeny produced by an individual female as the dependent variable and male copulatory success as the explanatory variable of interest was then performed. A backward selection was accomplished using the backward selection option of Proc Reg in SAS (SAS 1988). Variables were removed if their type II sums of squares had an F statistic with a $P > 0.05$. Other explanatory variables, which may confound the effects of male mating success, were also included in the model. These include female size, female longevity, copulation duration, male size, and block. These variables may be confounding, because large females or longer lived females may produce more offspring (Sivinski & Dodson 1992; Whittier & Shelly in press), copulation duration may affect the amount of sperm transferred (Farias et al. 1972), and male size may affect the amount of sperm or accessory fluids received by the female (Leopold et al. 1971; but see Sivinski and Smittle

1987). In addition to these explanatory variables, all possible interaction terms as well as quadratic terms for the explanatory variables were included.

Two replicates consisting of 38 and 48 pairs of flies were conducted and have been treated as blocks. Because of the two blocks, we also included block as a possible confounding variable and a block x male copulatory success variable to test for differences in mating success between blocks. The F value for the block x male copulatory success variable was less than 0.21, so we have used the pooled error mean square. Data were centered by subtracting the mean of the variable from each observation. This was done to avoid any possible collinearity problems between variables and between linear and quadratic terms included in the model. Any males or females with missing data were discarded.

After model selection, the resulting model included 5 variables. Diagnostic procedures were then conducted. Colinearity was not present, and there were no significant jackknife residuals, leverage or influence. Visual observations of residuals and normal quantile-quantile plots showed no serious deviations from normality.

Results

Male copulatory success was found to be non-random for the fathers ($n = 236$; $\chi^2 = 36.8$; mean = 1.7; $P < 0.001$;

Poisson; Fig. 5.1), confirming previous observations (Whittier et al. 1993). Much of the deviation from randomness is a result of larger than expected numbers of nonmating males and males that mated four or more times. For males, remating provides a large fitness benefit. Regression of the number of offspring sired against number of copulations was significant and revealed that 103.4 additional offspring were sired per mating (model = Number of offspring = $-9.9 + \text{number of matings} (103.4)$; $df = 1, 45$; $r^2 = 0.39$; $P < 0.001$).

Even though non-random mating was observed for both fathers and sons, no heritable genetic component to male copulatory success was found. Father/son regression of the average copulation score of three sons on father copulation score showed no significant additive genetic variation for copulatory success (Fig. 5.2). In addition, sibling analysis showed no significant sire effect on male offspring copulatory success (Table 5.1). There was, however, a marginally significant dam effect on son copulation success. This larger between-dam component suggests non-additive genetic variance or variance due to common environment. Thus, only the sire component can be used to estimate heritability.

Females did not appear to discriminate among males on the basis of heritable variation in size. Father/son regression of father size and average size of the first

three sons was not significant ($df = 1, 81$; $F = 0.25$; $P > 0.05$) (Fig 5.2) and sib analysis showed no significant additive genetic contribution for size (Table 5.2).

In addition to the possibility that females may choose males on the basis of heritable differences in copulatory success, we also assessed whether the female gains any direct benefit via increased fecundity from the male. Based on multiple regression, five variables showed significant contribution to explaining the number of progeny produced by an individual female. These variables included the number of copulations the male obtained, number of copulations a male obtained (squared), female longevity, copulation duration (squared) and the differences between blocks (Table 5.3). Both replicates were consistent in their selection of variables included in the final model and were consistent with the final model of the pooled data. Even though copulation duration (squared) is included in the final model, the effect on female progeny number is negligible. That is, for every 12 minute difference in copulation time, fecundity only increases by one. The only two variables that have a substantial effect on the number of offspring are female longevity and male copulatory success.

If all variables except male copulatory success are held constant, the number of offspring produced does not follow a linear pattern (Fig 5.3). Females that mate with low scoring males (<2) and females which mate with males

that scored very high (>6) enjoy enhanced fitness. On the other hand, females which mate with males with some intermediate score appear to gain no fitness advantage or even appear to suffer some fitness disadvantage.

Table 5.1. Analysis of variance of parent and offspring copulatory success.

Variance					Variance		Percent
Source	Error	df	MS	F	P	component	of Total
=====							
Sire	MS(Dam(Sire))	79	2.35	0.84	0.7	-0.10	0.00
Dam	MS(Error)	80	2.79	1.29	0.08	0.31	12.88
Error		160	2.16			2.15	87.12

Table 5.2. Analysis of variance of parent and offspring size.

Variance				Variance		Percent	
Source	Error	df	MS	F	P	component	of Total
=====							
Sire	MS(Dam(Sire))	73	19.31	0.89	0.7	-0.54	0.00
Dam	MS(Error)	74	18.28	1.06	0.4	0.59	3.01
Error		148	20.45			19.26	96.99

Table 5.3. Results of multiple regression using backward selection of number of progeny per female regressed against male copulatory success and other possible confounding variables. Model sum of squares = 340045.23; df = 80; r^2 = 0.41.

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	P
=====					
Intercept	182.95	23.85	249951	58.8	0.0001
Mate Score	-22.37	7.43	38439	9.0	0.0035
Female Longevity	1.81	0.40	85678	20.2	0.0001
Mate Score ²	4.88	2.17	21538	5.1	0.0271
Copulation Time ²	0.007	0.00	41341	9.7	0.0025
Block	-71.07	14.38	103817	24.4	0.0001

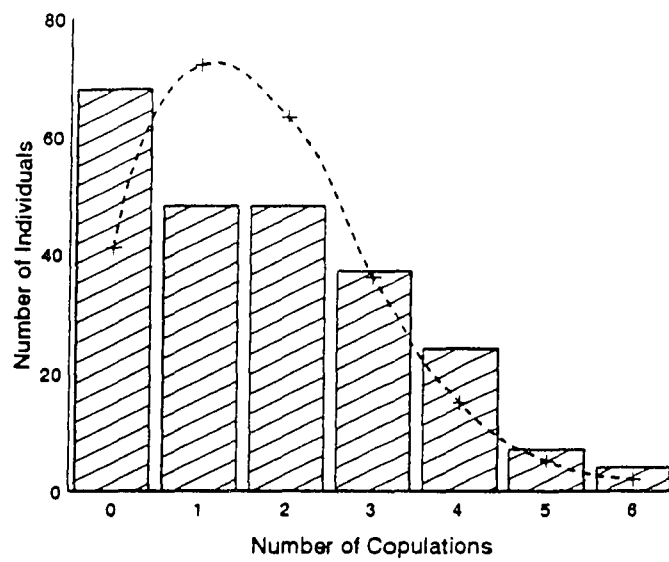


Figure 5.1. Copulation frequency distribution of fathers. Dashed line is expected frequencies of Poisson distribution.

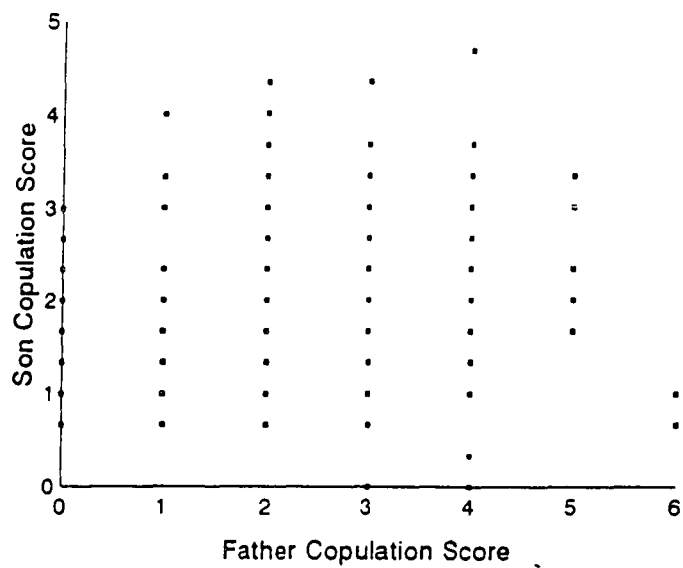


Figure 5.2. Regression of son copulation score on father copulation score (df = 1, 124; $F = 0.04$; $P > 0.05$; $r^2 = 0.0003$).

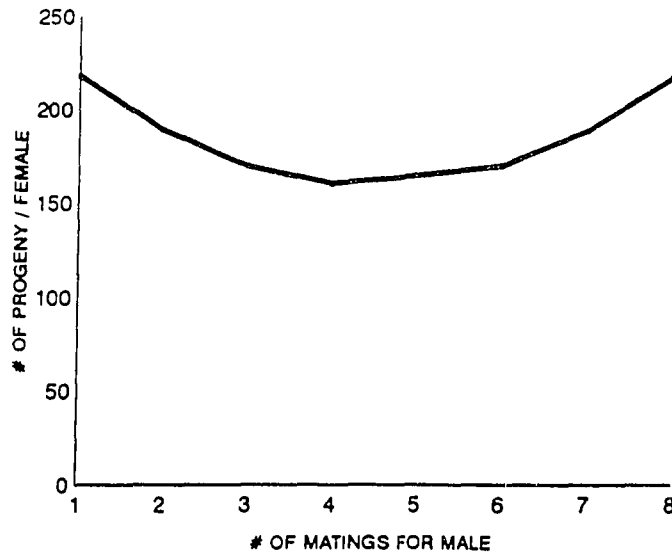


Figure 5.3. Regression of number of progeny per female on number of copulations obtained by the male with the variables copulation duration and female longevity held constant. Regression equation derived from multiple regression: Number of offspring = $-22.37 (\text{male mating score} - \text{mean of male mating score}) + 4.88 (\text{male mating score} - \text{mean of male mating score})^2$. In this study mean male mating score was 2.24 matings.

Discussion

Nonrandom mating observed in the Mediterranean fruit fly males does not appear to be the result of female choice for heritable differences among males for the ability to obtain copulations. Both father/son correlation and sib analysis show no relationship between father copulatory success and son copulatory success. This lack of relationship is consistent with theoretical predictions that male traits preferred by females might have low or zero heritabilities (Fisher 1958; Maynard Smith 1978). It is also possible that the tests used in this study are not sensitive enough or have inadequate sample size to confirm heritability (Klein et al. 1973). However, this is unlikely to be the principal factor as there were no trends toward significant heritability in this study.

Even though there were no apparent indirect benefits to the female by choosing successful mates, female choice may be based on direct benefits to the female in the form of increased fecundity. This result is a little more difficult to assess as the benefits are not linearly correlated with male copulatory success. Females mating with males that copulate one or two times and males that copulate 6 or more times enjoy enhanced fecundity. The increased fecundity of the one and two time mated females may be explained by a nuptial

gift that may be passed to the female during copulation. Whittier and Kaneshiro (1991) showed that females that mated virgin males enjoyed both increased longevity and fecundity. This "gift" was shown to occur only with virgin males and did not regenerate even after five days. However, studies by Shelly and Whittier (1993) indicated that, despite apparent fitness costs, females selected mates independently of their status as a virgin or non-virgin. One possible explanation of the increased fecundity of females which mate very successful males is selection for males that maximize fertilization (Markow et al. 1978). However, there is no evidence that females in this species can recognize such males.

Perhaps the difficulty in identifying significant benefits to the female due to female choice is not in the statistical tests used above but in the assumption that males actually mate nonrandomly. Kearns et al. (1990) points out that experimental design, choice of statistical test and interpretation of results can result in dramatic differences between different studies in the mating distributions of males. However, male Mediterranean fruit flies seem to show a consistent mating distribution. Three previous studies of the Mediterranean fruit fly have used different experimental designs and have shown differential mating success. In each of these studies the large number of nonmating males and few highly successful males were the

basis for their conclusions of nonrandom mating. Arita & Kaneshiro (1985) used males that were 7 days old and tested groups of three males with one virgin female. Matings were allowed until three matings were recorded per group of males. Whittier (1986) placed groups of 50 newly eclosed males into a cage with females at a 1:1 ratio and allowed mating. Mated females were replaced with virgin females maintaining the 1:1 ratio for the entire life of the male. This same experiment was repeated with a ratio of 5 males: 1 female. Whittier et al. (1993) placed groups of 10 six day old males and 10 six day old females into a cages and recorded mating behavior for six days. Mated females were replaced. In spite of the differences in experimental design in each of these studies, the mating distributions are very similar.

Arita & Kaneshiro (1985) recorded 29% of the males not mating and 20% of the males performing >50% of the matings. Whittier (1986) recorded 25% of the males not mating and 20% of the males performing >50% of the matings when the ratio of males to females was 1:1. When the ratio of males to females was 5:1, 22% of the males did not mate and 20% of the males performed more than 50% of the matings. Whittier et al. (In Review) recorded 38% of the males not mating and again showed that 20% of the males performed >50% of the matings. In this current study, the distribution of the matings for the fathers are consistent with previous studies

with 28% of the males not mating and 22% performing >50% of the matings. It appears that the distribution of matings among the males is somewhat independent of experimental design for this species. However, data for field studies are lacking and may show a different distribution.

Furthermore, if females mate randomly, male copulatory success should strongly correlate with increased male activity. Whittier et al. (In Review) showed that male pheromone calling (signaling) was correlated with number of courtships performed but was not correlated with copulatory success. However, the number of courtships performed was positively correlated with copulatory success even though this correlation was fairly weak ($r_s = 0.44$). In the same study, though, female choice also appeared to play an important role in male copulatory success. Less than 10% of the courtships resulted in mating, and in most cases females terminated courtship by simply moving away from the male. Nearly 25% of the females did not copulate during the study even though some were courted 67 times. Of the females that did copulate, nearly 50% were courted by five different males. Of these females that were courted by more than one male, females generally selected males having higher copulatory scores than previously rejected males.

The Mediterranean fruit fly lek remains paradoxical. It does appear that females exercise choice, yet the basis for this choice has not been elucidated. Documented

benefits of mating with particular males may include direct benefits such as increased longevity and fecundity if a virgin male was mated or increased fecundity for mating with low or high scoring males. However, females select mates independently of whether they are virgin or non-virgin. (Shelly & Whittier 1993). Intuitively, good genes would seem to be the most simple explanation for the observed nonrandom mating but the data and many theoretical predictions do not support this hypothesis. If the entire mating system is based only upon environmental differences, then it is difficult to explain the formation of leks in this species and the amount of female choice observed. Females gain nothing by being choosy and could possibly incur large costs by delaying mating or by not even mating at all.

Several questions remain to be answered. First, field studies need to confirm that non-random mating due to female choice does occur. Second, it would be helpful to identify the character or suite of characters the females use in choosing mates. Third, the non-linear relationship between mate choice and the obtaining of benefits needs to be further evaluated. Does this non-linear relationship pose problems for linear analysis of heritability? Certainly, the potential for complex interactions and antagonistic relationships exist and could make conventional ANOVA methods inappropriate.

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CHAPTER 6

CONCLUSIONS

The studies included in this dissertation confirm that Mediterranean fruit fly males aggregate and form leks. Within these leks, male-male interactions seem to play a minor role in determining mating success. On the other hand, it appears that female choice plays a significant role in determining male mating success. Female discrimination leads to large variability in male mating success with a few males doing most of the matings. However, even though females seem to have no problem distinguishing good males, I cannot show what cues the females use in selecting mates. Females do not seem to mate with males based on their success in male - male interactions nor could I show that females mate particular males in order to enhance the mating success of their sons. However, I was able to show that females benefit directly by mating with particular males. Females that mate with virgin males, males that ultimately obtain few matings (<2) or males that ultimately obtain many matings (>6) enjoy enhanced fecundity. However, it appears that females select males independently of whether males are virgin or not.

Because the distribution of mating in nature is so important to sterile insect release programs, future research should concentrate on efforts to identify the importance of leks. The current definition of lek is used operationally and does not provide an opportunity to statistically test for the existence of leks. To define a lek in a testable manner, it will become crucial to understand the communication between males and females. The lek should ultimately be defined as the area within which participating individuals interact. Currently, my one tree definition does not account for variation in tree size. Furthermore, the area that these flies effectively communicate with each other may overlap parts of several trees. An understanding of the parameters of a lek will allow us to determine the importance of the lek to the mating system of this species and provide us an opportunity to quantify the proportion of matings, if any, that take place outside of leks.

In addition to understanding the parameters of the lek, the cues females use to select mates should be investigated. Ideally, this work should be done in the field. However, care must be used in this type of study. This dissertation has shown that the mating system of this species is complex and relies on the interactions of several different behaviors. Whether there is a single cue, a whole suite of cues, or no cues at all needs to be determined. Intense

study for any particular behavior may not prove fruitful in identifying why some males are more successful at mating. Males can possibly compensate for some weaknesses by being strong in other areas.

Ultimately, the understanding of the mating system of this species would allow us to select for better maters that could be incorporated into mass-reared strains used in SIT programs. Perhaps additive genetic variance can be found by trying more painless methods of study such as selection experiments. It would also be worthwhile to use more non-traditional approaches to selecting for mating ability such as that suggested by Kaneshiro in which the traits of male mating success and female discrimination are treated as antagonistic to each other. Other studies should focus on non-genetic ways to improve male mating success. Larvae diet, adult diet, exposure to trimedlure or other para-pheromones may be a good place to start. The potential benefit of being able to manipulate male mating ability is immense. Super-males could be released in sterile insect release programs increasing the effectiveness of these important programs and reducing the cost.

Lastly, these flies provide a unique opportunity to bridge the gap between applied sciences and basic biology. Leks continue to be one of the least resolved issues in evolutionary biology. By understanding the mating system of these flies, scientists can potentially reduce the huge

economic losses caused by this fly throughout the world as well as provide more information concerning the lek paradox.