1-1-1988

# Polygamous mating system of a tephritid fruit fly, Rhagoletis pomonella Walsh.

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# POLYGAMOUS MATING SYSTEM OF A TEPHRITID FRUIT FLY, *RHAGOLETIS POMONELLA* WALSH

A Dissertation Presented

by

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Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 1988

Entomology

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ACKNOWLEDGMENTS

I would like to dedicate my dissertation to the late Betty D. Erickson because she would have been so proud.

I give very special thanks to Preston and John for providing the most wonderful distractions and for being patient and loving through it all. Furthermore, I could not have gotten to this point without the loving support and encouragement of my family, James, Dorothy, and Kathryn Opp. To them, I am forever indebted.

Many people have made my tenure in Amherst a pleasant and fulfilling experience, including my fellow maggoteers: Sue B., Sylvia, Patti, Cindy, Martin, Tom, Jorge, Dan, and Scott. I thank them for their friendship, assistance, and advice, and for letting me bend their ears every so often. I owe a special thanks to Joe, for being such a good friend. I'd also like to thank the members of my adopted labs, Toxicology and the Apiary, for letting me get in the way, break a little glassware, and chat incessantly. Also, to John Edman, John Clark, and John Stoffolano, many thanks for allowing me to borrow equipment and space.

I have benefitted greatly from the expert assistance of many, many people, including Nga, Jay, Simon, Esme', Doug, Heidi, Blossom, and Mary. I wish them well in their future endeavors, and I hope they return to visit some day. Stuart Berlocher and Bruce McPheron, of the University of Illinois, graciously allowed me to use their lab space and to pick their brains regarding electrophoretic techniques. John T. Bonsignore miraculously developed computer programs which saved me from pure panic.

Finally, my work would not have been possible without the support, advice, encouragement, and friendship I received from Ron Prokopy. Although I was doubtful initially, I now believe that Ron was a fly in a former life. His insights and instincts with these flies are truly amazing. Thank you, Ron, for everything.

Chapters 2 and 3 are included with permission from Florida Entomologist and Annals of the Entomological Society of America, respectively.

ABSTRACT

POLYGAMOUS MATING SYSTEM OF A TEPHRITID FRUIT FLY,

RHAGOLETIS POMONELLA WALSH

SEPTEMBER 1988

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The purpose of this study was to investigate behavioral and ecological factors influencing the mating system of the apple maggot fly, Rhagoletis pomonella Walsh (Diptera: Tephritidae).

In nature, wild apple maggot flies (AMF) were individually marked and released on a host apple tree. Fly dispersal was influenced by the onset of reproductive maturity. Although most pre-reproductive individuals dispersed away from the host tree, once reproductively mature, males remained on the tree for more consecutive days than females.

In the laboratory, female AMF were mated different numbers of times to assess effects of mating on lifetime fecundity and fertility. Multiply-mated females demonstrated increased fecundity and fertility compared to virgin or singly-mated females. At least part of the effect was behaviorally induced because sham-mated females exhibited fecundity and fertility similar to multiply-mated females.

Based on a field cage study of marked male and female AMF on a host hawthorn tree, the mating system of the fly was characterized as dual polygamous. Observations of equal male and female variance in

mating success and of non-random mating patterns in each sex, together with indications that females benefit from multiple matings, formed the basis for this new term.

Using starch gel electrophoresis of whole insects to compare parent and offspring allozyme profiles, high degrees of second male sperm precedence were found when females mated with two males. Thus, male AMF benefitted from mating with non-virgin females by fathering a high proportion of offspring.

In a field cage, multiple matings increased the propensity of female AMF to forage for oviposition sites (host fruit), and to lay eggs compared to virgin or singly-mated females. The hypothesis of behavioral effects of multiple matings was reinforced because sham-mated females were as likely as multiply-mated females to forage and lay eggs. In the presence of males on a host tree, multiply-mated females were less inclined to lay eggs than singly-mated females, although females of each mating status increased their foraging rate (rate of fruit finding) in the presence of males. The "hazard" of male encounter might have been perceived differently by females of different mating status.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

Sexual selection is a term first proposed by Darwin (1871) in his book "The Descent of Man and Selection in Relation to Sex." Darwin (1871) saw sexual selection as being distinct from natural selection. Although both natural and sexual selection result from the differential ability of individuals to leave offspring, sexual selection alone involves the differential ability of individuals to acquire mates. Natural selection, on the other hand, may operate on a variety of traits, other than mate acquisition, which may ultimately influence reproductive success (Darwin, 1859).

Darwin (1871) proposed that sexual selection would result in conflicts between and within the sexes primarily due to the ability of males to fertilize more than one female. Furthermore, he divided sexual selection into two primary components: 1) intrasexual selection, in which individuals of one sex (usually male) compete with each other for access to individuals of the opposite sex, and 2) intersexual selection, in which individuals of one sex (usually female) exercise choice in the selection of mates.

Perhaps surprisingly, basic notions of sexual selection have undergone relatively few major changes since Darwin. One of the most noteworthy theoretical advancements has concerned ideas of parental investment in offspring (Trivers, 1972). According to one theory, the sex whose average parental investment is greater will become a limiting resource for the oppostie sex (Trivers, 1972). Because males tend to produce more numerous, small, motile gametes and invest less in

offspring than females, females tend to be a limiting resource for males, resulting in competition among males for access to females.

Polygyny (multiple male mating) is often considered to be the most common animal mating system (Thornhill and Alcock, 1983) due to the ability of males to fertilize many females and because males typically exhibit less parental investment than females. Polyandry (multiple female mating), on the other hand, is considered to be rare in animals, although several scenarios have been proposed in which polyandry may benefit a female (Thornhill and Alcock, 1983). Polygamous mating systems, in which both males and females multiply mate, are rarely discussed in the literature, either in empirical or theoretical terms. The lack of discussion of polygamous mating systems may not be an accurate reflection of the frequency of this type of mating system in nature.

Previous studies have suggested that the mating system of the apple maggot fly is polygamous (Neilson and McAllan, 1965; Prokopy and Bush, 1972; Prokopy and Bush, 1973c, Prokopy et al., 1972; Smith and Prokopy, 1980). Nevertheless, many practical and theoretical questions concerning multiple mating in this fly have remained. The behavioral-ecological studies presented in this dissertation follow the guidelines of Opp and Prokopy (1986) by beginning with general questions addressed by observational studies in nature, followed by more specific questions addressed by experimental manipulation under controlled conditions.

The first research chapter, Chapter 2, concerns an observational study of wild apple maggot flies in nature. The purpose of the Chapter 2 study was to observe wild flies from the time of first emergence through reproductive maturity (i.e. mating and oviposition) to determine

seasonal changes in dispersal and other behaviors within and between the sexes in nature. The results of this study formed the basis for questions addressed in subsequent chapters.

Chapter 3 concerns a laboratory study designed to determine the influence of numbers of matings on lifetime female fecundity and fertility. The purpose of the Chapter 3 study was to investigate potential benefits of multiple matings for females. Based on the results of this study, I designed the semi-natural observational study presented in Chapter 4 to determine how many times male and female R. pomonella would mate in a 14 day period when confined on a host tree. Chapter 4 also discusses the mating system of the apple maggot fly in relation to current sexual selection theory.

In Chapter 5, I present a laboratory study, using starch gel electrophoresis of enzymes, designed to determine paternity of offspring when a female was mated to more than one male. The purpose of this study was to investigate potential benefits, in terms of sperm competition, for a male mating with a non-virgin female.

The final research project, Chapter 6, was designed to integrate findings obtained from laboratory matings (Chapters 3 and 5), observations of interactions of males and females in nature (Chapter 2) and in a field cage (Chapter 4), and previous studies concerning foraging behavior of female R. pomonella searching for oviposition sites (Roitberg et al., 1982). This chapter discusses the influence of female mating status and male density on female apple maggot flies foraging for oviposition sites on a host tree. To my knowledge, no previous studies have directly addressed non-mate resource foraging behavior of an animal in relation to sexual interactions.

Thus, although the studies presented in this dissertation do not involve any new or novel techniques, the questions are asked and the experiments designed to provide unique insights into behavioral and ecological aspects of the mating system of the apple maggot fly. Furthermore, I have attempted to ask questions from both male and female perspectives to elucidate potential conflicts between the sexes due to the operation of sexual selection.

# CHAPTER 2

## SEASONAL CHANGES IN RESIGHTINGS OF MARKED, WILD *RHAGOLETIS POMONELLA* FLIES IN NATURE

### 2.1 Introduction

The apple maggot fly (AMF), *Rhagoletis pomonella* (Walsh), is a well-known pest of apples in northeastern North America and, in recent years, has been detected in many western regions, including California (Joos et al., 1984). This fruit-parasitic tephritid fly has attained its pest status primarily due to expansion of its host range from the native host, hawthorn (*Crataegus* spp.), to fruits more desirable for human consumption, such as apple, pear, and sour cherry (Boller and Prokopy, 1976).

Scientific interest in this fly extends beyond the realm of immediate pest control to include empirical studies of physiology, behavior, and ecology (Boller and Prokopy, 1976; Dean and Chapman, 1973; Prokopy and Roitberg, 1984). The AMF has proven to be an excellent subject for studies of foraging behavior (Prokopy and Roitberg, 1987), visual ecology (Owens and Prokopy, 1986), resource utilization (Averill and Prokopy, 1987; Reissig, 1979), and sexual selection (eg. Prokopy and Bush, 1973). Nevertheless, large gaps in our knowledge of the behavior and ecology of this fly in its natural environment still exist. For instance, we have yet to determine details of dispersal in relation to food, oviposition site, and mate foraging behaviors. In addition, we know little about individual variation in fly behavior over the host fruiting season in nature.

We undertook this study of marking and releasing wild AMF in nature to attempt to answer such basic questions as: How long will an individual fly remain on the same host tree? Does this residence duration differ between the sexes and change over the fruiting season of the host? Does the onset of reproductive maturity following eclosion affect residence duration?

## 2.2 Materials and Methods

### 2.2.1 Site

In early June, 1984, we chose a small, Early MacIntosh variety apple tree in an unsprayed apple orchard naturally infested with AMF on the campus of the University of Massachusetts, Amherst (Fig. 2.1). We pruned the tree, thinning the leaves so that all branches were clearly visible to an observer standing either on the ground or on a 2.3m ladder. By mid-July, 1502 apples were ripening on this tree, whose canopy was ca 5m tall X 5m diam. The two Early MacIntosh variety apple trees in closest proximity to the pruned tree (canopies within 2m) bore few or no fruit that season. In addition, trees of a later fruiting variety (MacIntosh) in the adjacent row (canopies ca 5m away) bore few or no fruit that season. The closest fruiting trees (Cortland) that season were located two rows away (ca 12m).

### 2.2.2 Marking Individuals

The observation tree was checked daily until the first newly eclosed adult AMF was sighted on June 24. Then, using mouth aspirators, we collected flies daily from the tree for 12 days (until July 5).

These flies were brought to the laboratory for sex determination, measurement, and marking. Size was determined by measuring the length of the dorsal mesothorax using an eyepiece micrometer on a dissecting microscope. Each fly was immobilized briefly on ice and was marked individually with dots of one or two colors of Liquid Paper$^{TM}$ on the dorsum of the thorax. A symbol was then written on the Liquid Paper with a waterproof black felt pen (see Walker and Wineriter, 1981). Preliminary laboratory studies had indicated that marks applied in this manner were non-toxic to the flies, yet were waterproof and durable. By using four colors singly and in two color combinations along with 49 different symbols, we were able to develop over 300 unique marks. Although we marked and released 327 female and 272 male AMF, not all flies seen on the observation tree over the course of the experiment were marked, either because they eluded capture or because they emerged or flew to the observation tree following the 12-day period of collection and marking. We released all marked flies on leaves of the observation tree at dusk on the day of collection.

2.2.3 Observations

For 24 days after the first day on which flies were captured and released (i.e. until July 18), we censused the tree for marked flies. Censuses were conducted at one hour intervals between 0900 and 1700 hours when ambient temperature was above 21$^{o}$C and below 33$^{o}$C (the approximate activity thresholds of the flies) (Johnson, 1983; Prokopy et al., 1972), except during periods of heavy rain. During the census periods, we also recorded the numbers of pairs of unmarked AMF in copula

on the observation tree. We accumulated 148 census-hours over the 24-day period for an average of 6.2 census-hours per day.

To ensure that all portions of the observation tree were evenly censused for flies, we divided the tree into 8 approximately equal-area sections based on the natural limb structure of the tree. Leaves, fruit, and branches were examined for 5 min per section. With this method, we were confident that all areas of the tree were inspected each hour except the top sides of leaves located in the top 10% of the canopy.

### 2.2.4 Statistics

To test for differences in resighting frequencies between the sexes and over the season, we used G-tests with Yate's correction for continuity on frequencies (see Sokal and Rohlf, 1981). We used t-tests for unequal variances to assess both the differences in total numbers of days in which flies of each sex were sighted and the influence of fly size on mating and resighting.

## 2.3 Results

Of the 599 marked AMF which were released, we saw 183 (30.6%) at least once during the 24 days of census. The great majority of these flies (137 of 183; 74.9%) were seen only during the first week of observation. The remainder (46 of 183; 25.1%) were seen during the first week but then were absent for an intervening period of 1-2 weeks before resighting. We did not see equal proportions of marked male and female flies; significantly more marked males were seen (100 of 272; 36.8%) than marked females (83 of 327; 25.4%) (G=8.52 with Yate's

correction; $p<0.001$). Multiple sightings of males over time were also more common than of females; whereas only 4% of females were seen on more than two consecutive days, 24% of males were seen on more than two consecutive days. Thus, on average, individual males were seen over more days (mean=2.18 days; S.E.=0.27) than females (mean=1.37 days; S.E.=0.09) ($t=2.85$; $p<0.05$; $df=181.0$). The maximum number of consecutive days over which we saw an individual male or female was 14 and 7, respectively.

The oviposition and mating behaviors of male and female flies changed over the season. Early in the census season, before July 7, we did not observe either marked or unmarked females ovipositing into apples in the orchard. The apples were sufficiently ripe to allow oviposition because when apples from our observation tree were brought into the laboratory, our wild, laboratory-maintained AMF readily attempted oviposition (D. R. Papaj, Dept. Entomology, University of Massachusetts, Amherst, personal communication). Thus, we hypothesize that the flies observed in the field prior to July 7 were not ovipositing because they were not reproductively mature. This contention is supported by the fact that no flies were observed mating prior to July 7.

Prior to July 7, we detected no significant difference between the number of male or female flies observed on only one day versus the number of flies observed on more than one day ($G=1.13$; $p>0.05$). After July 7, the pattern of sightings of males and females differed (Fig. 2.2), though not significantly ($G=1.00$; $p>0.05$), probably due to low sample sizes ($n=17$ females; $n=33$ males). The primary difference in sighting frequency between males and females resulted not from a change

in the frequency of seeing females (both before and after July 7, most marked females were seen on only one day; $G=0.95$; $p>0.05$), but was due to a change in the pattern of male sightings. Following the onset of oviposition, males were more likely to be seen for many days ($G=5.80$; $p<0.05$). The maximum time span over which a male was periodically resighted was 22 days, and the maximum time span for a female was 24 days.

The peak time of day in which marked flies were seen also differed between males and females. During the 1500 h census, we saw slightly more marked females than at any other time (mean=1.08 females/census hour), whereas the greatest mean number of males were seen during the 1600 h census period (mean=2.89 males/census hour) (Fig. 2.3). In addition, the latter census period, during which we saw the greatest numbers of males, was one of the periods in which the fewest females were seen. For each census period, a greater mean number of males than females were seen. The pattern of sightings of unmarked mating pairs corresponded more closely with the pattern of sightings of marked males than of marked females; most were seen at 1600 h, with a considerable decrease during the 1700 h census period (Fig. 2.3).

We observed very few marked flies in copula. Only 12 marked males (12% of all sighted marked males) and only 6 marked females (7.2% of all sighted marked females) were observed copulating, in every case with an unmarked partner. Only 1 marked female was seen to mate more than once (2 matings); 4 marked males (33% of all marked males observed mating) mated multiply during census periods. One marked male mated 6 times and the other 3 marked males mated twice.

Sighted, marked females did not differ in size from females which were not seen ($t= -0.44$, $p>0.05$, $df=175.1$). Marked males which were sighted were significantly larger than marked males which were not sighted ($t=-2.00$, $p<0.05$, $df=224.9$). Marked males and females which we observed mating did not differ in size from flies which were not observed to mate (males: $t=-0.98$, $p>0.05$, $df=19.4$; females: $t=-0.84$, $p>0.05$, $df=6.6$).

## 2.4 Discussion

Dispersal prior to reproduction is fairly common in adult insects and is sometimes accompanied by the loss of flight ability once reproduction begins (Harrison, 1980). In AMF, many pre-reproductive adults dispersed away from the site of emergence. Approximately 75% of the newly emerged AMF we marked left the host tree after being seen within the first week and were not seen again. The remaining 25% apparently left the host tree shortly after emergence but returned when reproductively mature, 1-2 weeks later. Using radiolabelled AMF, Neilson (1971) also found that many flies which dispersed outside of a naturally infested orchard early in the season later returned.

Similar dispersal behaviors of the immature adults of a close relative of the AMF, Dacus tryoni (Froggatt), the Queensland fruit fly, have been reported (Fletcher, 1973; 1974). Using mark-recapture methods in a naturally infested orchard, Fletcher (1973; 1974) found that 75% of D. tryoni left the orchard in their first week and did not return. In later weeks, as flies became mature, many re-entered the orchard. Although D. tryoni are larger and capable of longer dispersal flights than AMF (Fletcher, 1974; Neilson, 1971), the same general pattern of

dispersal away from hosts prior to reproduction followed by return when reproductively mature occurs as we observed in the AMF. This pattern likely corresponds to the change from primarily food foraging behavior when reproductively immature to mate and host foraging behaviors when mature (Harrison, 1980).

We detected distinct differences between wild male and female AMF in the tendency to remain on a host tree, with those differences magnified following the onset of fly reproductive maturity. In general, we resighted many more males than females, but the most striking differences between the sexes occurred after oviposition began. Following oviposition, female AMF deposit a marking pheromone on the surface of fruit that deters further egglaying (Prokopy, 1972). Previous studies using field-caged flies showed this marking pheromone elicits female emigration from host trees (Roitberg et al., 1982; 1984). Just the opposite behavior, arrestment of activity, occurs in male AMF when they contact marking pheromone on fruit (Prokopy and Bush, 1972).

Both before and after the onset of oviposition and deposition of marking pheromone, we found that female AMF were not likely to remain on a single host apple tree for more than one day. Although this effect may have been heightened by our thinning of tree leaves, we could not detect any increase in female emigration from the host tree which might have been due to contact with marking pheromone. The lack of fruit on immediately adjacent host trees may have caused females to remain on our observation tree longer than if suitable host fruit were available nearby, or may have resulted in longer dispersal flights by females to find new oviposition sites (see also Neilson, 1971). Fletcher (1973) found that the length of time D. tryoni remained in an orchard was in

great part determined by the quantity of fruit available for oviposition. Hendrichs and Reyes (1987), however, felt that the length of time D. longistylus (Wied.) females spent on a host was influenced by encounters with patrolling males which were continually attempting forced copulations.

Male AMF tended to remain on the tree longer and were seen for more consecutive days in the latter than in the earlier part of the season. We hypothesize that once females had commenced oviposition, males frequently were contacting female marking pheromone on fruit. Contact with marking pheromone would arrest male activity on fruit (Prokopy and Bush, 1972). Similarly, Johnson (1983) found that male AMF responded more strongly to the mating-oviposition stimulus of a red sphere trap than to the feeding stimulus of a yellow panel trap. By remaining on fruit, males increase the probability of encountering females arriving on the fruit to oviposit, and thus increase their opportunities to mate, since over 90% of matings occur on fruit (Prokopy et al., 1987) and most occur when females are in some phase of oviposition behavior (Prokopy and Bush, 1973; Smith and Prokopy, 1980). This observation is consistent with the hypothesis of Thornhill and Alcock (1983) that when females of a species multiply mate, males would be expected to search for mates near sites of female oviposition. Although we were not able to document multiple female mating in this study, we expect multiple mating to occur in nature because laboratory studies have shown that female AMF benefit from multiple copulations in terms of increased fecundity and fertility (Chapter 2).

The peak time of mating by unmarked AMF corresponded more closely with time of observation of peak male presence than peak female presence

(see also Prokopy et al., 1972). Since most matings on fruit are male rather than female initiated (Smith and Prokopy, 1980), peak time of male abundance on fruit might be one of the primary factors governing diel mating patterns.

Using the maximum time span over which marked male and female AMF were sighted (24 days for females and 22 days for males), we conservatively estimate that, as adults, some flies may live up to 4 weeks in nature. Neilson and Wood (1966) estimated from field and laboratory cage studies that AMF adults may live up to 1 month when supplied with aphid honeydew. Although female size appeared to have no influence on longevity in our study, body size may have affected longevity of males because more large males were resighted over time than small males. We detected no interaction between body size and mating success for either sex, although body size is known to influence mating success in other dipteran species (Borgia, 1981; Burk and Webb, 1983; Sivinski, 1984).

This study provides information on individual fly activities in nature but raises many questions concerning AMF behavior. For example, although we know that AMF are not likely to remain on the host tree early in the season for more than one day, we do not know where these pre-reproductive individuals go. Furthermore, we do not have comprehensive information concerning the natural food of these flies and their food foraging behavior, although we know that protein is necessary to attain reproductive maturity (Webster et al., 1979). Finally, many questions remain concerning male-female interactions, especially the average numbers of times individuals mate on host plants. We know that most matings occur on fruit and are male initiated but we have no

estimate of variance in individual mating success. We plan to address these and many more questions in future studies.

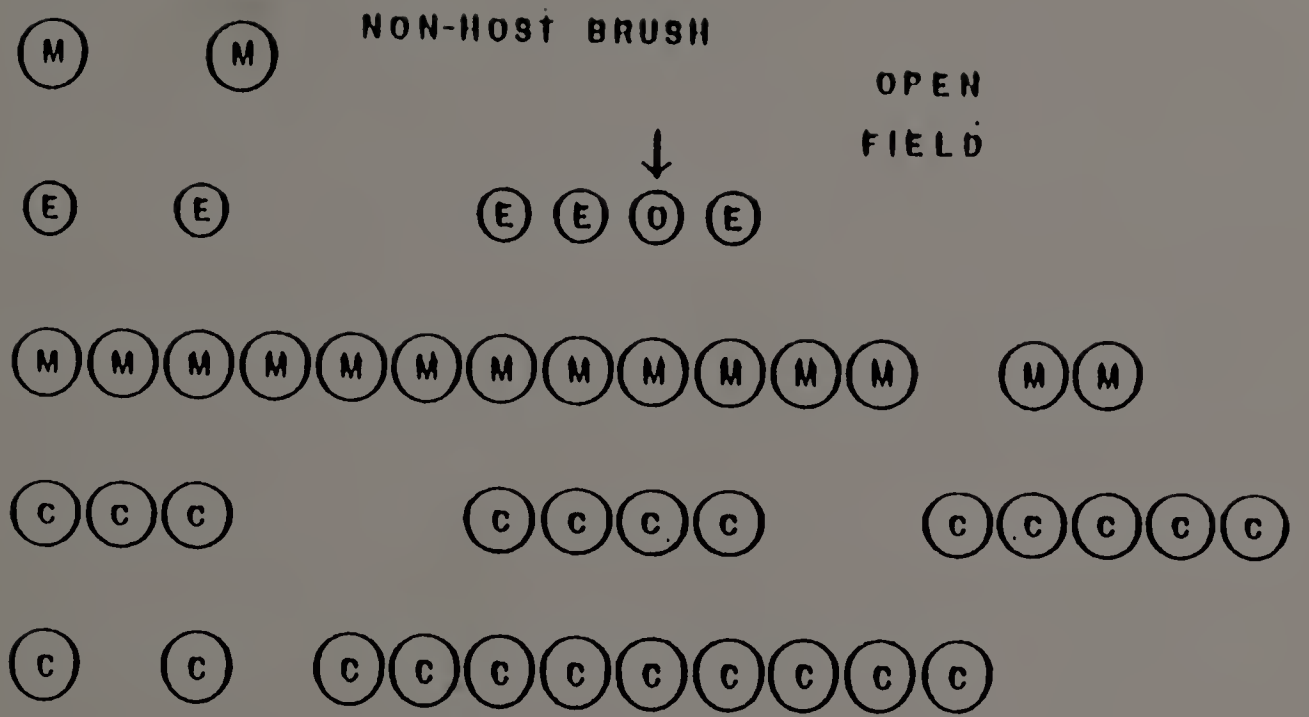


Figure 2.1
Arrow denotes location of observation tree (O) in relation to other Early MacIntosh (E), MacIntosh (M), and Cortland (C) variety apple trees at Orchard Hill, University of Massachusetts, Amherst.

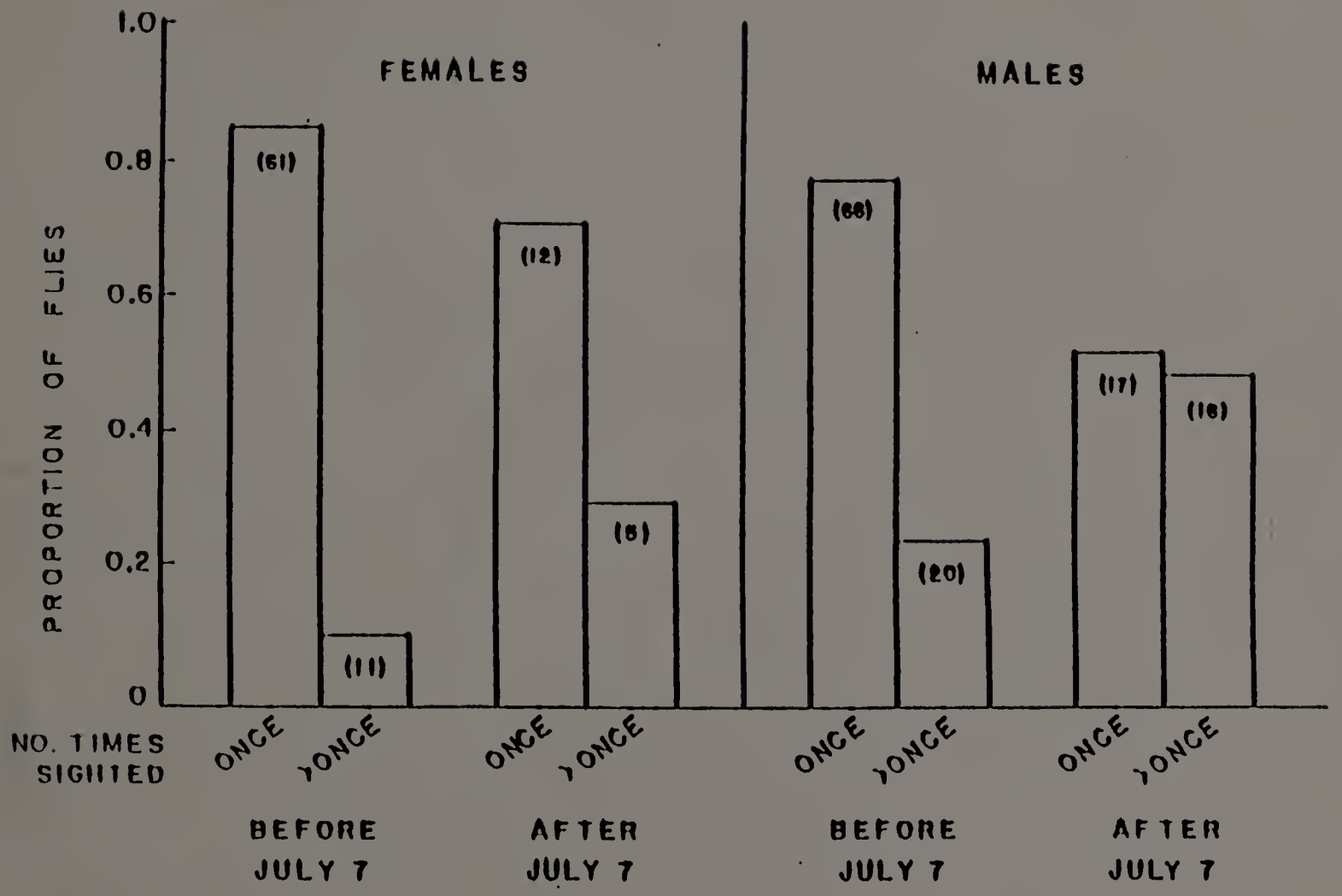


Figure 2.2
Proportion of marked female and male AMF seen once or more than once in relation to the onset of reproductive maturity on July 7. (Numbers of individuals.)

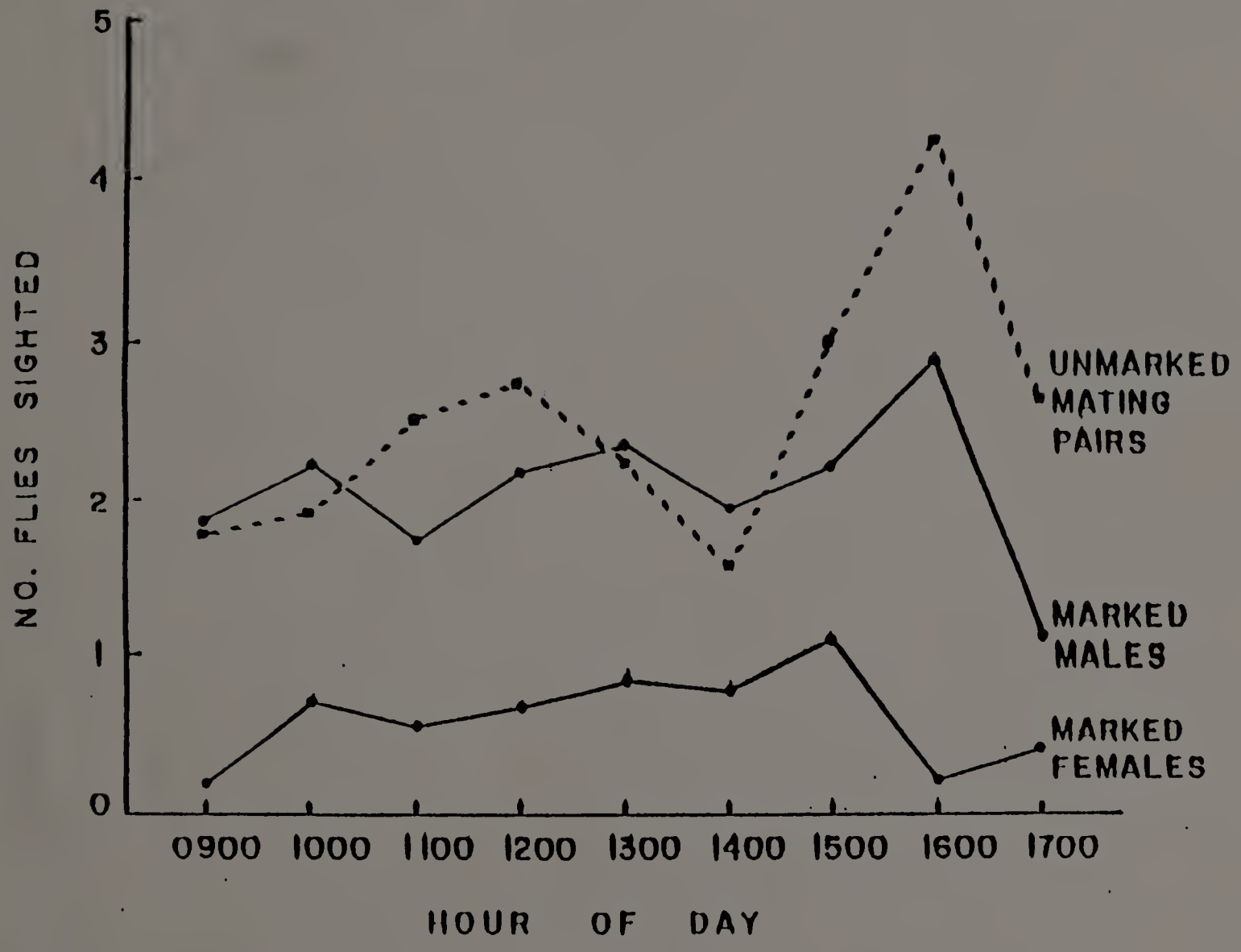


Figure 2.3
Relationship between hour of day and average numbers of marked individual male and female and unmarked mating pairs of AMF seen on the observation tree over the 24 day observation period.

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# CHAPTER 3

## VARIATION IN LABORATORY OVIPOSITION BY *RHAGOLETIS POMONELLA* IN RELATION TO MATING STATUS

### 3.1 Introduction

Sexual selection involves the differential ability of individuals to acquire mates and results in conflicts between and within the sexes. In general, male animals produce smaller, more motile gametes at a faster rate and contribute less in parental investment than females (Baylis, 1978). According to theory, the sex whose average parental investment is greater will become a limiting resource for the opposite sex (Trivers, 1972). Thus, females tend to be a limiting resource for males, resulting in competition among males for access to females. In addition, because most males have the potential to fertilize many females, polygyny tends to be the most common mating system in animals (Thornhill and Alcock, 1983). Although several scenarios have been proposed in which polyandry may benefit a female (Thornhill and Alcock, 1983), few instances of polyandry have been observed or investigated except in the Hymenoptera (Page and Metcalf, 1982). Furthermore, polygamy is a mating system rarely encountered in either theoretical or empirical studies of animal mating sytems.

Both laboratory and field observations suggested that polygamy occurs in the apple maggot fly (AMF), *Rhagoletis pomonella* (Walsh) (Neilson and McAllan, 1965; unpublished data). The average total number of matings per fly in nature is unknown, but many, if not all, matings involving nonvirgin females are thought to result from male-forced

copulations (Prokopy and Bush, 1973c; Smith and Prokopy, 1980). Beyond a minimal number of matings needed to ensure female fertility, these forced copulations may do nothing to increase female reproductive output. Multiple copulations, in fact, may represent a loss of fitness to females because of time wasted or increased risk of predation (Thornhill, 1980).

We undertook this study to determine the effects of multiple copulations on female AMF fertility and fecundity. Previous studies had indicated that multiple copulation may have negative effects on female AMF fecundity even though proportional egg hatch (fertility) increases with multiple copulations (Neilson and McAllan, 1965). This result was later expanded by Prokopy and Bush (1973b) who hypothesized that copulation provided neurohormonal stimulation of oogenesis, based on their observations of increased oviposition with mating. Laboratory studies, such as these, may have been confounded by effects of grouping female flies; when held in groups, both virgin and mated females exhibit increased oviposition (Prokopy and Bush, 1973b). Thus, the relationship between multiple mating, on one hand, and fecundity and fertility, on the other, has been unclear with this fly.

## 3.2 Materials and Methods

The studies were conducted in the laboratory where external factors could be controlled and lifetime female reproductive output could be measured directly. The terms "mating" and "copulation" will be used interchangeably here, but are not necessarily synonymous with insemination -- i.e., sperm transfer (see Page, 1986).

In nature, AMF eggs are laid under the skin of a number of rosaceous fruits including apple (Malus spp.) and hawthorn (Crataegus spp.). In the laboratory, flies will insert eggs beneath the surface of artificial fruit that provides proper size, shape, color, and texture cues for oviposition (Prokopy, 1966; 1967; Prokopy and Boller, 1971; Prokopy and Bush, 1973a). Mating is not a prerequisite for oviposition in the laboratory, but mating is necessary to ensure egg fertilization and hatch (Prokopy and Bush, 1973a,b; Neilson, 1975; Webster et al., 1979).

Apples infested with apple maggots were field-collected from a naturally infested unsprayed orchard located at the University of Massachusetts, Amherst. Puparia were collected and stored in moist vermiculite at 5°C for at least 6 months. They were warmed as needed at 23 $\pm$ 2°C to stimulate adult eclosion. Before eclosion, individual pupae were weighed and placed in 30-ml plastic cups with damp vermiculite to ensure lack of contact with other flies upon eclosion. Adult females were maintained at 55 $\pm$ 5% RH under a photoperiod of (L:D) 16:8 in individual 0.27 liter plastic cup cages supplied with water and a mixture of yeast hydrolysate and sugar as food. Adult males were placed in groups of 15-20 individuals in 16-cm Plexiglas and screen cages similarly supplied with water and food.

For 10-12 days following eclosion, when flies were reproductively mature (i.e., capable of oviposition (Webster et al., 1979)), females were subjected to one of five mating treatments. A female was either: 1) virgin: remained unmated and was confined individually in a cup cage (n = 21); 2) once-mated: was allowed to mate once, then was confined individually in a cup cage (n = 27); 3) twice-mated: was allowed to mate

once, then was allowed to mate a second time 10-14 days later, but following each mating was confined individually in a cup cage (n = 19); 4) multiply-mated: was continually confined with a healthy male fly in a cup cage (n = 15); or 5) sham-mated: was continually confined with an emasculated male fly in a cup cage (n = 16). Emasculated males were rendered incapable of successful transfer of sperm or other substances through surgical removal of the entire aedaegus. For the treatments consisting of females mated once or twice, five females were confined in a cage with five males for 4 h or until copulation occurred. The duration of each copulation was timed and recorded and each pair was removed from the mating cage following natural termination of copulation. Individual females were then placed again in cup cages. Females which refused a second mating following two 4-h mating periods that occurred 10-14 days following the first mating (n = 16) were also placed in individual cup cages. All females confined continually with healthy male flies were observed to copulate more than twice, although the exact numbers of copulations were not determined. Each emasculated male was observed to exhibit normal copulatory behavior -- i.e., mounting of a female fly for an average copulatory duration of 30 min.

Each female fly was supplied daily with a dome-shaped artificial fruit made of black ceresin wax (Prokopy and Boller, 1971; Prokopy and Bush, 1973a) for oviposition. For the lifetime of a fly, all eggs found daily on the inside of the wax dome were transferred carefully to a petri dish using a sable paintbrush. The eggs were maintained on three layers of moistened filter paper and one layer of moistened black construction paper for 7 days to allow hatching. Any eggs laid on the outside of the wax dome (which occurred often when flies lived >60 days)

were recorded but were not saved for hatching assessment because these eggs desiccated rapidly and died.

Total fecundity, rate of egg laying, egg hatch, and female longevity were compared among the treatments using one-way analysis of variance (ANOVA) procedures and Tukey's w procedure for multiple comparisons with unequal sample sizes (Steel and Torrie, 1980). Females which refused a second mating were not included in ANOVA procedures because these females were not considered in the original experimental design. These females were compared with females that were chosen to mate only once for differences in total fecundity, rate of egg laying, and egg-laying longevity using t tests. The relationship between duration of copulation and the proportion of hatching eggs was investigated for females allowed only one copulation using a least-squares linear regression procedure (Ryan et al., 1976).

## 3.3 Results

Lifetime fecundity differed significantly among females of the five mating treatments (ANOVA: $F = 6.39$; $df = 4,93$; $P < 0.001$). Virgin females and females mated once laid fewer eggs than females that were twice-mated, multiply-mated, or sham-mated (Fig. 3.1).

The effects of mating on the components of lifetime fecundity -- i.e., egg-laying rate and egg-laying longevity -- were less clear. Both egg-laying rate and egg-laying longevity were significantly affected by the five mating treatments (egg-laying rate ANOVA: $F = 5.57$; $df = 4,93$; $P < 0.001$), but in different ways. Multiply-mated females had significantly higher oviposition rates than virgin and once-mated females, and substantially (but not statistically significant) higher

rates than twice-mated and sham-mated females (Fig. 3.2). Thus, an upward trend in oviposition rate occurred with increasing numbers of matings, with sham-mated females falling between twice-mated and multiply-mated females. In contrast, twice-mated females oviposited for a significantly greater number of days than females from any of the other four treatments (Fig. 3.3).

Although the trend toward increased percent egg hatch with greater numbers of matings was not statistically significant (ANOVA on arcsine transformed percentages: $F = 2.91$; $df = 2,58$; $P > 0.05$), multiply-mated females were significantly more fertile than once- or twice-mated females (Fig. 3.4). In addition, the combined effects of mating on fecundity and on percent fertility resulted in significant effects of the mating treatments on total numbers of hatching eggs (Fig. 3.5) (ANOVA: $F = 6.43$; $df = 2,57$; $P < 0.005$). Sham-mated females remained essentially virgin as evidenced by the lack of egg hatch (Fig. 3.4).

Biweekly percent egg hatch per female and biweekly female mortality patterns were different for females mated different numbers of times (Fig. 3.6). Females mated once showed greatest average egg hatch (fertility) in the first 2 weeks following onset of oviposition, with declining egg hatch and increasing mortality in the ensuing weeks. With two matings, females maintained relatively high levels of egg hatch (>40%) through the 6th week of oviposition and did not begin to suffer mortality until 9 weeks following initiation of oviposition. Females that were allowed unlimited matings, however, showed increasing average percent egg hatch through the 8th week of oviposition, although mortality began in the 5th week. Both virgins and sham-mated females failed to hatch any eggs, and showed early mortality (within 3 weeks)

followed by greatly increased mortality after the 6th week (numbers surviving at 2-week intervals following onset of oviposition: virgins: 20, 19, 17, 11, 7; sham-mated females: 16, 16, 15, 11, 4).

Females which refused a second mating (therefore, were only mated once and comprised 46% of the females originally chosen to mate twice) demonstrated fecundity, rate of egg laying, and egg-laying longevity effects intermediate to females mated once or twice (Figs. 3.1-3.3). Although those females which refused a second mating oviposited significantly longer than females predestined to mate only once ($t = -3.00$; $df = 26$; $P < 0.001$), no significant differences were detected in lifetime fecundity ($t = -1.29$; $df = 31$; $P > 0.05$) or rate of egg laying ($t = -0.22$; $df = 38$; $P > 0.05$) between once-mated and refused-second mating females. The most important effect, however, concerned fertility; females which refused a second mating laid a greater percentage of hatching eggs than once-mated females ($t = -2.35$; $df = 20$; $P < 0.05$) (Fig. 3.4).

Duration of copulation in once-mated females was not significantly correlated with percent egg hatch ($r = 0.105$; $df = 26$; $P > 0.05$), possibly indicating that beyond a minimal amount of time necessary to ensure sperm transfer, amount of time spent in copula was not related to quantity of sperm transferred.

## 3.4 Discussion

Multiply-mated females of R. pomonella produced more potential offspring (hatching eggs) than females limited in numbers of matings (Fig. 3.5) due to effects of mating on both fecundity and fertility.

Our findings indicate that one mating does not significantly increase the egg output of female AMF compared with virgin females (Fig. 3.1). When total fecundity was broken down into components of rate of egg laying and egg-laying longevity, no differences were detected between virgin females and singly-mated females.

Previous studies of the effects of mating on reproduction in this fly did not quantify the effect of only a single mating (Neilson and McAllan, 1965; Neilson, 1975). Nevertheless, the authors concluded that mating had no stimulatory effect on oviposition because both virgin and mated females oviposited readily (Neilson and McAllan, 1965; Neilson, 1975) and because females which mated frequently did not lay significantly greater numbers of eggs than those mated only a few times (Neilson and McAllan, 1965). In contrast, Prokopy and Bush (1973b) found that mated females always laid more eggs over a 20-day period than virgin females whether the females were caged singly or in groups and whether the nonvirgin females mated only once or mated unlimited times. We found that more than one mating was necessary for females to attain maximal reproduction in terms of both fecundity and fertility. Furthermore, although two matings did lead to some increase in egg-laying rate, more than two matings were necessary for a significant increase in egg-laying rate compared with that of virgin females.

In another polygamous insect, the milkweed beetle, Tetraopes tetraophthalmus (Forster), similar relationships between multiple mating and fecundity and fertility have been reported (McCauley and Reilly, 1984). Female beetles mated only once showed lower fecundity and fertility compared with multiply-mated females. These beetles, however, demonstrated no measurable increase in fertility with frequent matings

compared with a few matings early in adult life. Thus, the adaptive significance of multiple mating throughout life in these female beetles was unclear (McCauley and Reilly, 1984).

Our data indicate that more than one mating increases female AMF reproductive success when females are confined individually and are ovipositing into artificial fruit in the laboratory. Because females with more than two matings did not lay significantly more eggs than females mated twice, as with the milkweed beetle (McCauley and Reilly, 1984), we have no evidence to support the hypothesis that female AMF need to mate at intervals throughout their lives to maintain a high level of oviposition, as proposed by Neilson and McAllan (1965). In fact, because females that mated twice oviposited significantly longer and suffered lower mortality compared with females mated either fewer or greater numbers of times, we conclude that two matings may achieve the highest reproductive longevity with the least time spent mating. We caution, however, that reproductive longevity, as measured in the laboratory, may not be relevant to natural field situations.

A strong trend existed toward increased egg hatch with greater numbers of matings. The greatest differences in total average percent egg hatch were between once- or twice-mated females and multiply-mated females. Furthermore, decreased fertility began after 2 weeks of oviposition in once-mated females and after 6 weeks of oviposition in twice-mated females. Females allowed unlimited matings had increasing fertility up to the 8th week of oviposition. These data agree with the conclusion of Neilson and McAllan (1965) that unlimited matings increase female fertility. We conclude that sperm depletion probably occurred

over time in females mated twice or less, but did not occur to any extent in females allowed unlimited matings.

Sham-mated female AMF were physiologically unmated (uninseminated) because they laid no fertilized eggs, but were behaviorally multiply-mated because each emasculated male was observed to copulate with a female at least twice. The sham-mated female treatment permits us to partition the behavioral effects of multiple mating from the physiological effects of insemination. Because the females in this treatment did not differ significantly from multiply-mated females in overall fecundity, rate of egg laying, or egg-laying longevity, many of the observed effects of multiply matings on fecundity were behaviorally rather than physiologically based. In contrast, since egg hatch depends on sperm transfer, the effects of multiple mating on fertility were indeed physiological. The behavioral component of increased fecundity is not limited to interactions with males alone because virgin females caged in groups likewise lay more eggs per female than virgin females caged individually (Prokopy and Bush, 1973b). This situation is similar to that reported in Drosophila mercatorum Patterson and Wheeler, in which females housed in groups with other females or with sterile or fertile males produced more eggs than females housed individually (Crews et al., 1985).

Female AMF that did not readily mate a second time had fecundity slightly greater than that of females chosen to have only one mating. More importantly, average fertility of females that refused a second mating was much greater than that of females mated once and somewhat greater than that of females mated twice (Fig. 3.4). These results lead us to hypothesize a situation similar to that found in Drosophila

melanogaster Meigen (Newport and Gromko, 1984) exists in R. pomonella: those females accepting a second mating had lower initial sperm loads than those refusing to remate. This hypothesis is supported by the high variability in average percent egg hatch (Fig. 3.4), which was evident in females of all treatments and may have resulted from many of the females receiving low sperm loads in at least one mating.

Four possible benefits of multiple mating from the female perspective have been proposed by Thornhill and Alcock (1983): 1) sperm replenishment, 2) provision of nutrients and/or hormones by the male, 3) increased genetic diversity of offspring due to multiple paternity, and 4) energy and time conservation if the avoidance of unnecessary copulations is costly. In R. pomonella, multiple matings increase female fecundity, fertility, and egg-laying longevity. We have reason to believe that multiple matings may result in sperm replenishment, but as yet we have no evidence of nutrient and/or hormone transfer.

As a final note, we caution that, as pointed out by Newport and Gromko (1984), the outcome of multiple mating experiments may depend on experimental design, particularly when the number of sperm transferred during a single copulation is highly variable. In such studies, females that refuse to mate a second time may be physiologically different from females which are allowed to mate only once. Although we found considerable variation in fertility of mated females in these experiments, egg hatch is, at best, an indirect quantification of sperm transfer.

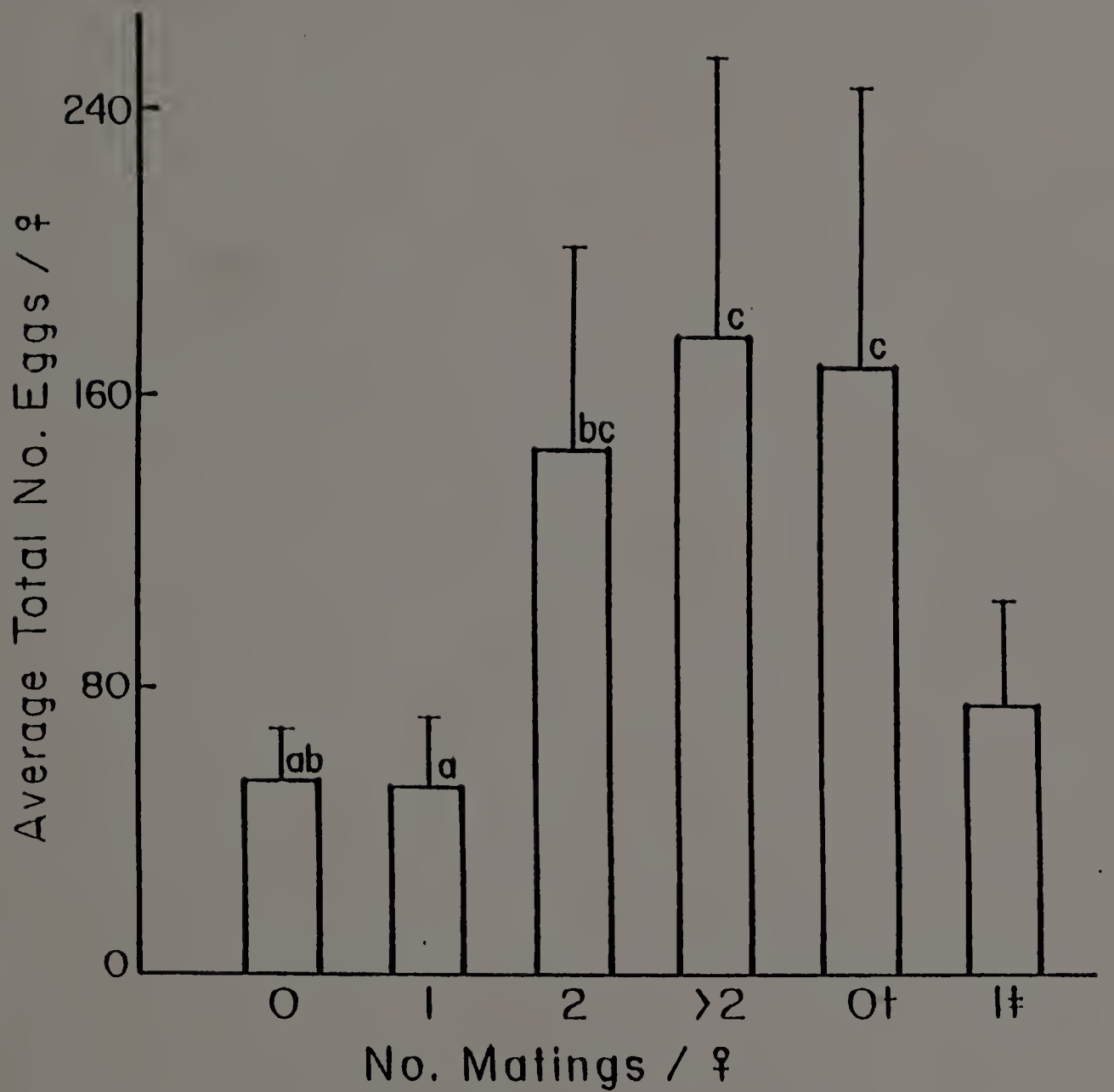


Figure 3.1
Average lifetime fecundity (±95% CL) of virgin females (0), once-mated females (1), twice-mated females (2), multiply-mated females (>2), sham-mated females (0+), and females which refused a second mating (1‡). Bars with the same letter do not differ significantly from each other (Tukey's w procedure: w=436.1; $P < 0.05$).

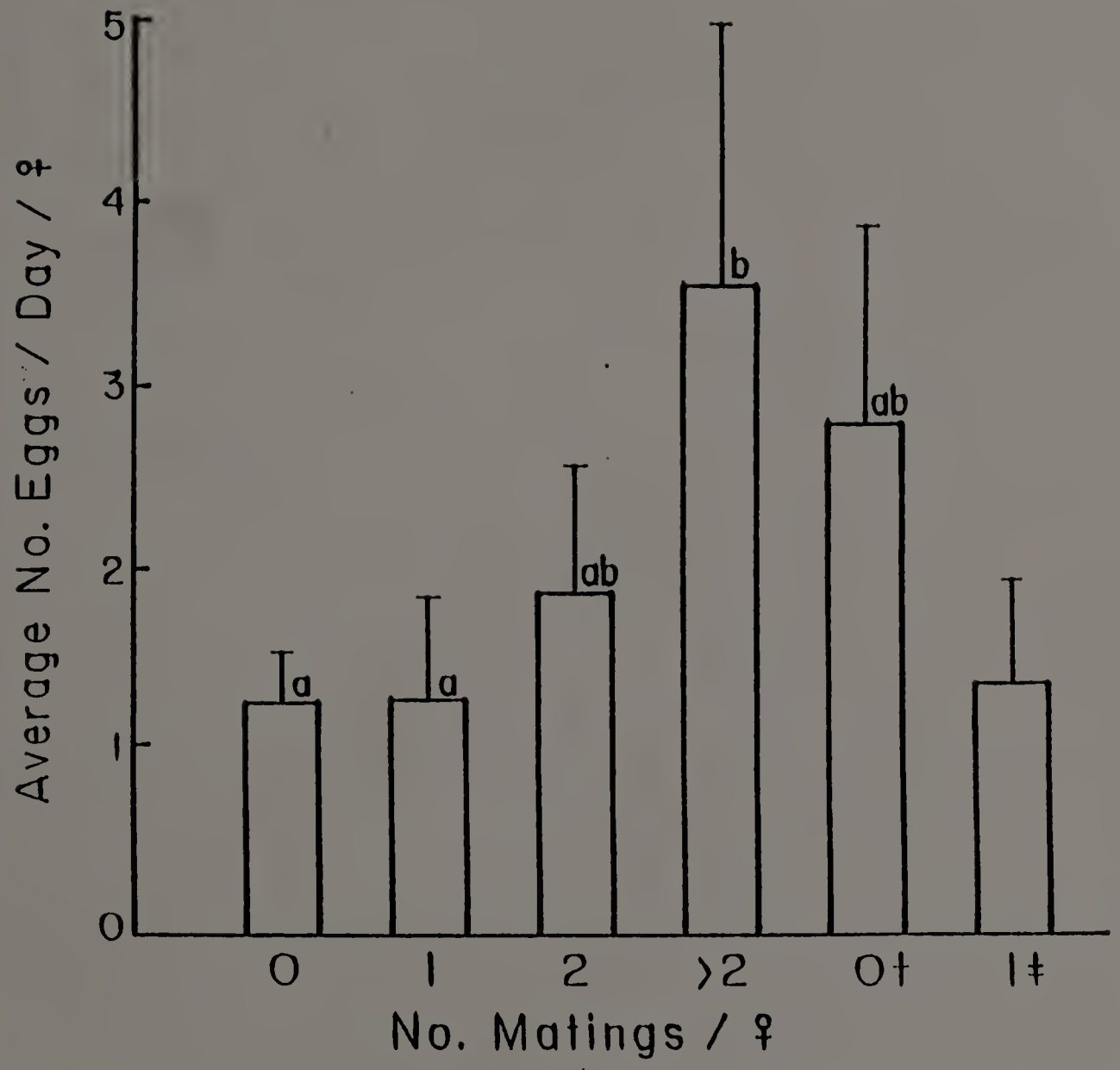


Figure 3.2
Average rate of egg laying (±95% CL) of virgin females (0), once-mated females (1), twice-mated females (2), multiply-mated females (>2), sham-mated females (0+), and females which refused a second mating (1‡). Bars with the same letter do not differ significantly from each other (Tukey's w procedure: w=7.0; $P < 0.05$).

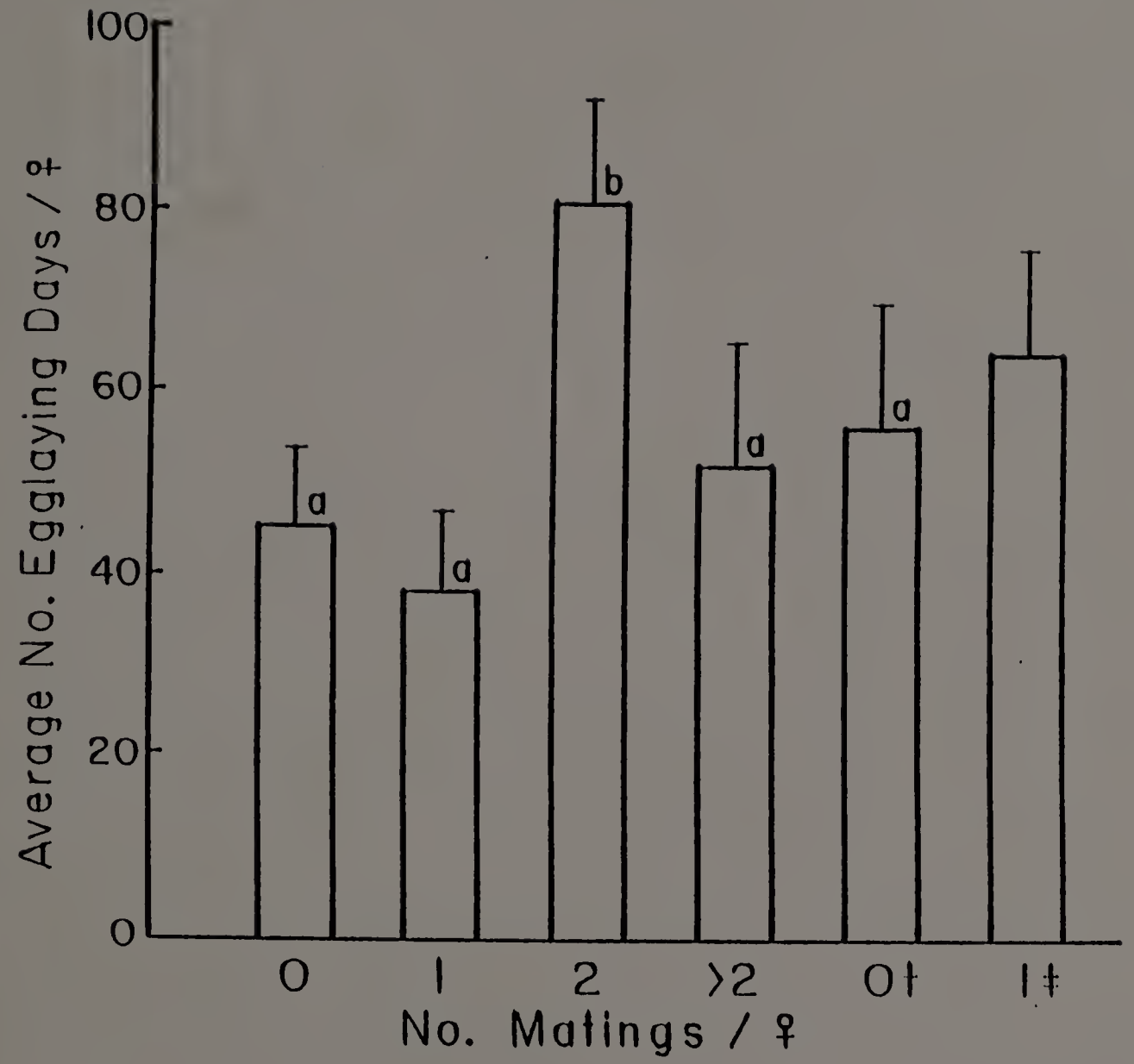


Figure 3.3
Average egg-laying longevity (±95% CL) of virgin females (0), once-mated females (1), twice-mated females (2), multiply-mated females (>2), sham-mated females (0+), and females which refused a second mating (1≠). Bars with the same letter do not differ significantly from each other (Tukey's w procedure: w=96.5; $P < 0.05$).

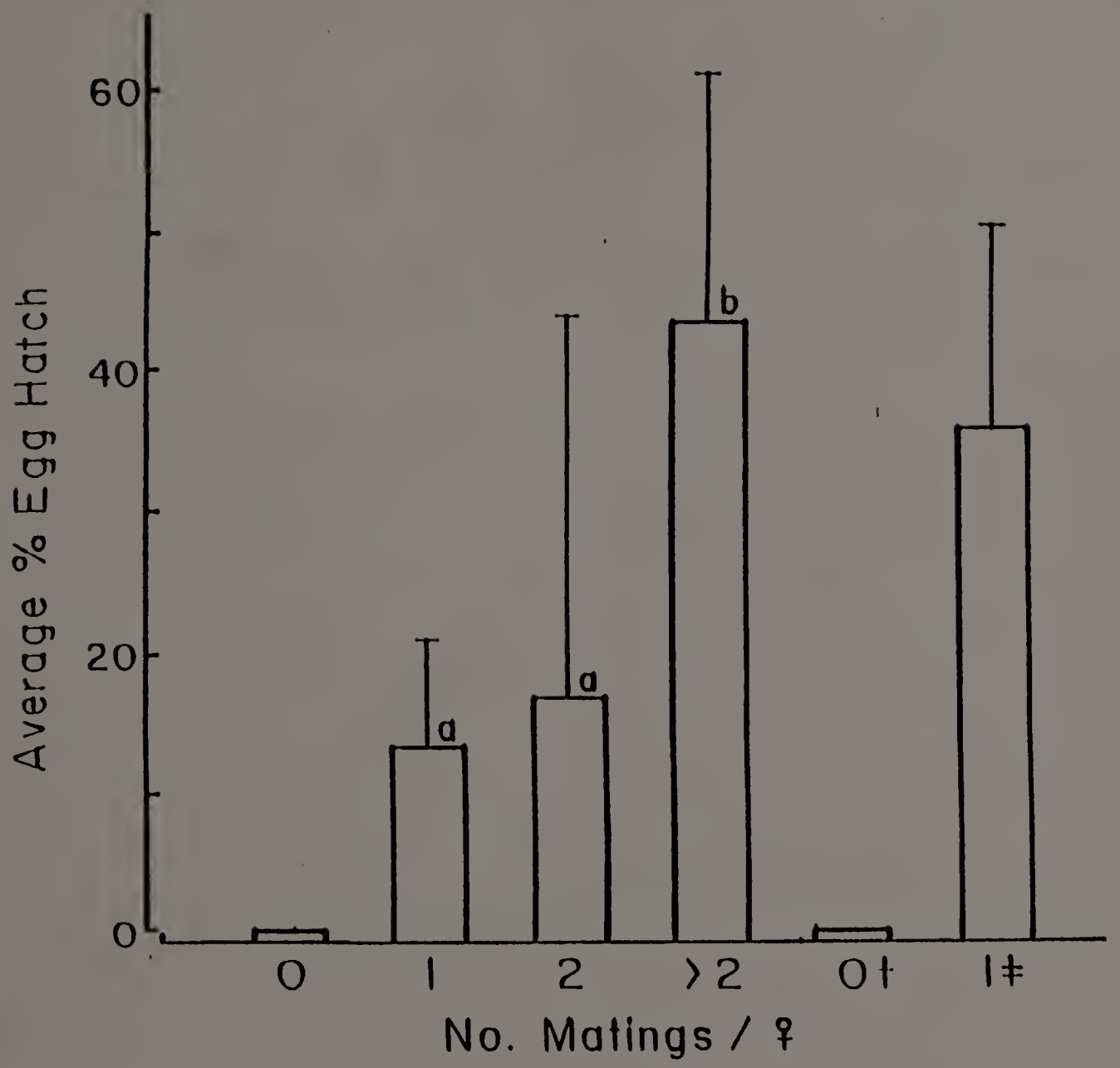


Figure 3.4
Average percent fertility (±95% CL) of virgin females (0), once-mated females (1), twice-mated females (2), multiply-mated females (>2), sham-mated females (0+), and females which refused a second mating (1‡). Bars with the same letter do not differ significantly from each other (Tukey's w procedure on arcsine transformed percentages: $w=1.4$; $P < 0.05$).

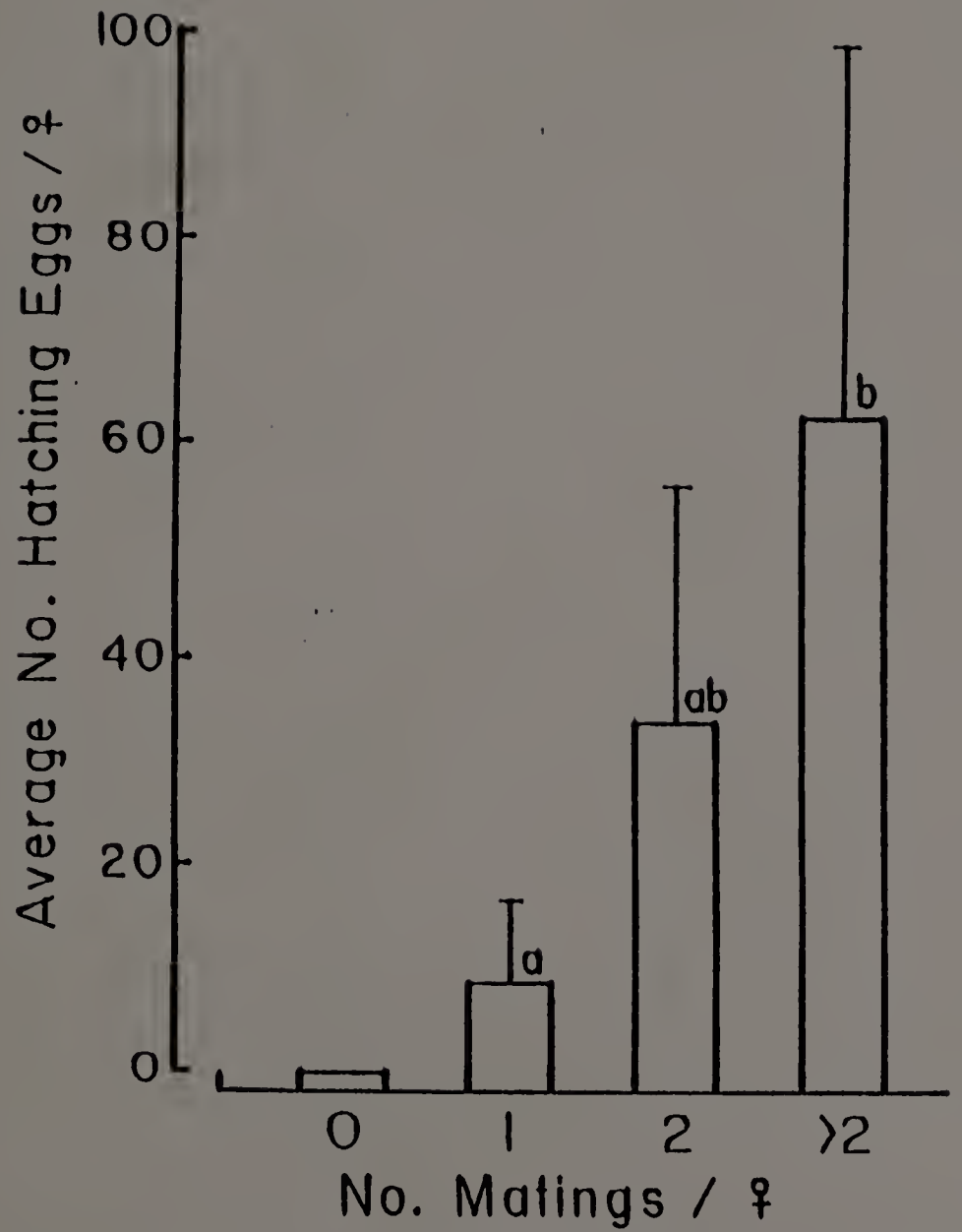


Figure 3.5
Average total egg hatch (±95% CL) of virgin (0), once-mated (1), twice-mated (2), and multiply-mated (>2) females. Bars with the same letter do not differ significantly from each other (Tukey's w procedure: $w=139.5$; $P < 0.05$).

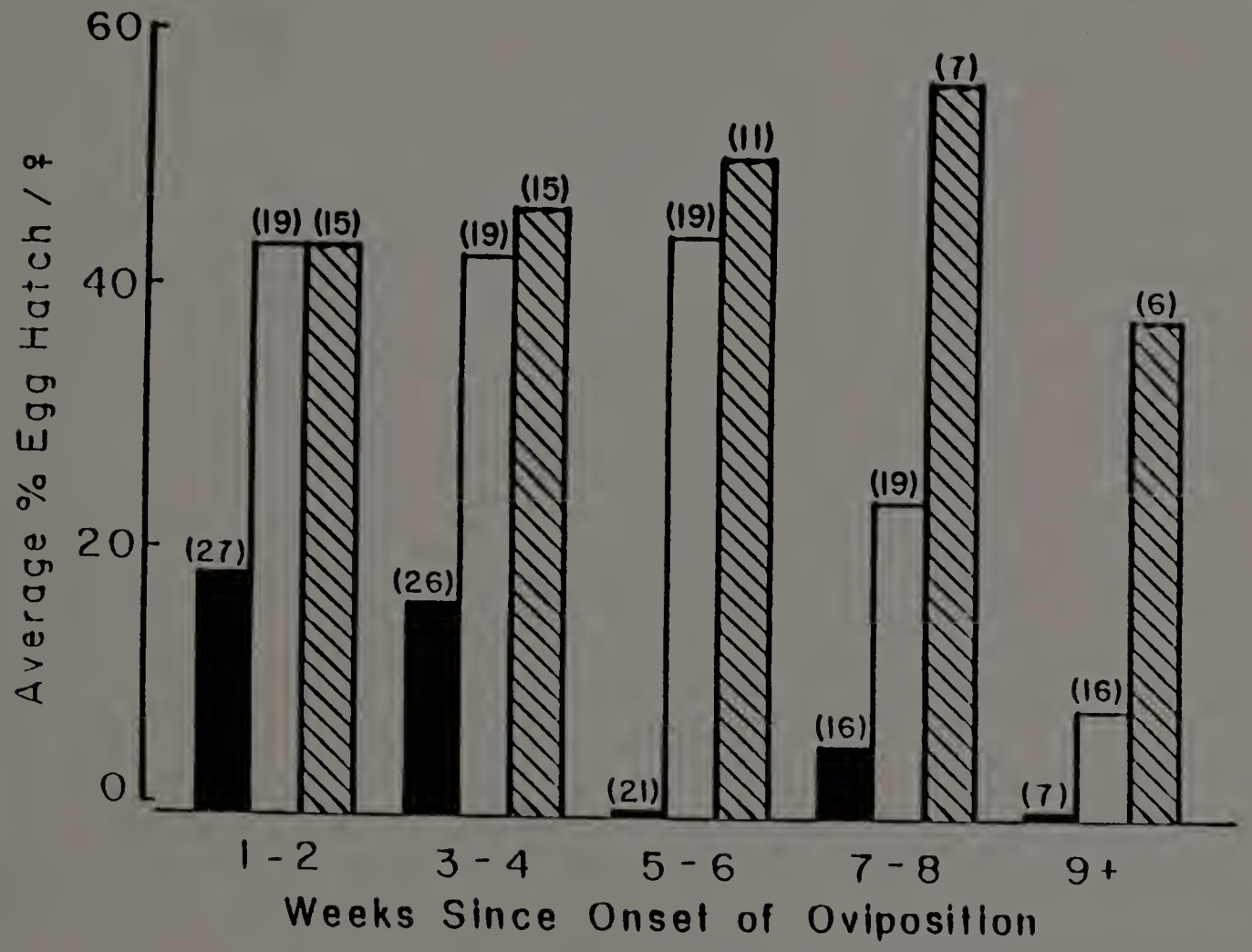


Figure 3.6
Average fertility over 2-week intervals following onset of oviposition of: once-mated females (dark bars); twice-mated females (open bars); and multiply-mated females (hatched bars). Numbers in parentheses indicate numbers of females of each mating treatment alive at the onset of each 2-week interval.

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# CHAPTER 4

# DUAL POLYGAMY IN A TEPHRITID FRUIT FLY, RHAGOLETIS POMONELLA: BEHAVIORAL AND ECOLOGICAL FACTORS

## 4.1 Introduction

A common notion perpetuated in sexual selection literature is that multiple mating is generally a more adaptive strategy for males than for females (Halliday and Arnold, 1987; Parker, 1979; Thornhill and Alcock, 1983). This dichotomy between the sexes exists because of the potential ability of males to fertilize many females and because female parental investment usually exceeds that of males (Bateman, 1948). Generally, polygyny (multiple mating by males) is considered the most common animal mating system with monogamy (single matings by both sexes) and polyandry (multiple mating by females) occurring less commonly (Thornhill and Alcock, 1983). The premise is that when polyandry does occur, some reversal of sex roles (i.e. greater male parental investment) also occurs (Trivers, 1972). A third multiple mating system (referred to by Pianka (1978) as no mating system at all) where each member of each sex has an equal opportunity to mate, i.e. where mating occurs at random, is sometimes called promiscuous, and might occur in animals such as marine invertebrates which shed their gametes at sea. In spite of empirical data refuting these generalizations within many vertebrate and invertebrate species (eg. Smith, 1984), notions of male competition and female choice as predominant avenues for the operation of sexual selection have been perpetuated since the time of Darwin (1871).

Particularly in insects, owing to their often unique mechanisms for long-term sperm storage in females and also to lifestyles requiring little or no parental investment following egg-laying (except in the Hymenoptera (Page and Metcalf, 1982)), multiple mating may occur frequently in, and potentially to the benefit of, both sexes. Empirical evidence supports this contention for more than a few species of insects (eg. odonates (see Waage, 1984), lepidopterans (see Drummond, 1984), coleopterans (eg. Dickinson, 1986; McCauley, 1982), hemipterans (eg. Evans, 1987; Wood et al., 1984), solitary bees (eg. Alcock et al., 1977) and Drosophila spp. (eg. Dobzhansky and Pavlovsky, 1967; Fuerst et al., 1973; Richmond and Ehrman, 1974; Turner and Anderson, 1983)), including at least one tephritid fly (eg. Dacus longistylus (Hendrichs and Reyes, 1987)). In fact, we hypothesize that a phenomenon we term "dual polygamy," in which both males and females mate multiply and benefit from multiple matings, may be a mating system which is as common as polygyny in insects and in much of the vertebrate animal kingdom as well. Although many descriptive studies exist of insect multiple mating systems, researchers tend to discuss these mating systems only in terms of the more well-known polygynous, polyandrous, and promiscuous mating systems, none of which may be appropriate. We have chosen to investigate the occurrence of multiple mating, and the behavioral and ecological factors which influence this type of mating system, in an insect in which males and females have been shown to benefit from multiple matings (Myers et al., 1976; Chapters 3 and 5).

The apple maggot fly, Rhagoletis pomonella (Walsh), is a fruit parasitic fly in which females demonstrate increased fecundity and fertility from multiple matings (Chapter 3). Most matings in these

flies last an average of about 30 min, occur on the host plant (Prokopy et al., 1971), and result from males attempting copulation with females engaged in oviposition behavior on fruit (Prokopy and Bush, 1973; Smith and Prokopy, 1980). Considering all that is known about behavioral and temporal trends of mating in apple maggot flies in nature (Hendrichs and Prokopy, unpub; Prokopy and Bush, 1973; Prokopy et al., 1971; Prokopy et al., 1972; Smith and Prokopy, 1980) and effects of multiple matings in the laboratory (Chapter 3), it is surprising that nothing is known about mating frequency and the variance in mating success among individual males and females in the field.

Variance among individuals in mating success is often a primary factor used to categorize animal mating systems (Thornhill and Alcock, 1983; but see Sutherland, 1985). In polygynous animals, variance among males in mating success exceeds variance among females because competition for access to females is keen and because males contribute little in parental care. Though most females become mated at least once, not all males participate in these matings. Female mating success exceeds that of males in polyandrous mating systems, with females frequently producing offspring fathered by more than one male. In a dually polygamous mating system, then, we expect male and female mating success to be essentially equal in mean and variance among individuals. The goal of this study was to gather information on the behavioral and ecological correlates of mating success in the apple maggot fly that would allow us to characterize the mating system and provide a framework for investigating similar multiple mating systems in other animals (Burk, 1981; Emlen and Oring, 1977).

4.2 Materials and Methods

All flies were wild, collected as larvae from naturally infested hawthorn trees (Crataegus mollis) planted on campus at the University of Massachusetts, Amherst. Adult R. pomonella emerged in the laboratory, where individuals were separated within 1 day of emergence and held in individual vented Solo$^{TM}$ cup cages provided with water and a 4:1 mixture of sucrose and enzymatic yeast hydrolysate as food. When 6-8 days old, 31 males and 32 females were individually marked on the dorsum of the thorax with a spot of Liquid Paper$^{TM}$ upon which was written an identifying symbol in waterproof felt pen (Chapter 2). Because previous studies (Chapter 2) had indicated that, when reproductively immature in nature, R. pomonella flies emigrate from host trees under which they emerged (presumably in search of food sources), we used only reproductively mature flies (6-8 days old) which had been given ample food and water while maturing in the laboratory.

We placed a single, potted, non-fruiting hawthorn tree (Crataegus sp.) having a canopy approximately 1.5 m diameter into a Saran$^{TM}$ screen field cage (2.5 m x 2.5 m x 2.5 m) bearing a cloth sun shade. We divided the tree into 10 approximately equal-area sections which we mapped and labeled using the natural branching structure of the tree. Hawthorn fruit (C. mollis), picked the previous year and held in controlled atmosphere cold storage, were sorted to ensure a lack of R. pomonella infestation damage and were washed in spring water in preparation for the study. Each tree section received 2 clusters of 3 hawthorn fruit hung on wires, for a total of 60 fruit in the tree. Fruit were replaced with fresh fruit every 4 days during the 14 day

observation period. Fruit clusters were always re-hung on the same permanent, labeled hangers on the tree.

In the morning on the first day of observations, marked flies were released into the cage where they remained for 14 days. Water and food sources were naturally available from overnight dew and aphid honeydew on leaves. Censuses of individual fly activities and locations were conducted for 14 consecutive days at 1/2 h intervals between 0900 and 1700 hrs (when the majority of matings have been shown to occur in nature (Prokopy et al., 1972; Chapter 2)), except during heavy rain (which occurred on 1 day). Maximum daytime temperatures inside the cage ranged from 25 to 35.5$^{o}$C. The fly activities which were particularly noted were mating (male mounted on female and in contact with female ovipositor with his claspers), fighting (both sexes will rear back on their hind legs while "boxing" with their front legs (Prokopy and Bush, 1972)), resting (including feeding), and oviposition. We also recorded fly location (including tree section) and, within a tree section, whether a fly was on a fruit or non-fruit plant structure (leaves, stems and branches). If a fly was seen during 2 consecutive censuses (i.e. twice within 1 h) on the cage wall, ceiling or floor, it was assumed to be attempting to emigrate from the tree. To avoid unrealistically high estimates of mating frequency due to confinement, flies attempting to emigrate were removed to individual cup cages (as described previously) and were re-released into the field cage the following morning. Flies which died or escaped were not replaced with new flies.

4.3 Results

In all, 187 censuses were conducted over 14 consecutive days for an average of 13.4 censuses per day.

Multiple mating by females was very common. Twenty-three of the 32 females (72%) mated more than once in 14 days. Out of the 14 females which lived the entire 14 days, 13 (93%) mated more than once and 10 (71%) mated more than 10 times. The mean number of matings per female over the entire 14 days was 15.5 (s.e. = 2.5) (Fig. 4.1). The maximum total number of matings per female was 30 and the minimum was 1 (however, 3 females did not mate). The number of matings per female was not randomly distributed as evidenced by a significant difference from a Poisson distribution ($G = 14.85$, $p<0.01$, d.f. = 2).

On a daily basis, multiple female matings were likewise very common. Twenty females (63%) were observed to multiply mate on at least one of the observation days. Nine females (19%) were observed to mate only once per day (however, 3 (33%) of these single-mating females were observed for only one day). The mean number of matings observed per female per day was 1.0 (s.e. = 0.1) (Fig. 4.1), while the maximum number of matings per day per female was 8.

Males also mated multiply. Twenty of the 31 males (65%) released mated more than once in 14 days. Only 8 males lived the entire 14 days, but all of them mated more than once, and 7 (88%) mated more than 10 times. The mean number of matings observed per male for the entire 14 days was 18.6 (s.e. = 2.6) (Fig. 4.1). The maximum number of matings seen per male was 31 and the minimum was 1 (excluding the 6 males which were not observed to mate). As with females, the distribution of number

of matings per male differed significantly from random when tested in relation to a Poisson distribution ($G = 15.89$, $p<0.01$, d.f. = 2).

Nineteen of the 31 males (61%) were observed to mate more than once a day. While 4 of the 31 males (13%) were observed to mate a maximum of once per day, 2 of these 4 were only seen for one day. The mean number of matings observed per male per day was 1.0 (s.e. = 0.1) (Fig. 4.1), and the maximum number of matings per day per male was 6.

Males and females did not differ significantly in mean number of daily matings per fly ($t = 0.13$, $p>0.05$, d.f. = 31, 30) nor in mean number of total matings for 14 days per fly ($t = 0.85$, $p>0.05$, d.f. = 13, 7) (Fig. 4.1). In addition, no difference was found between males and females in variance in daily copulation success (Bartlett's test for homogeneity of variances: $X^2 = 0.68$, $p>0.05$, d.f. = 1), or in copulation success totalled over 14 days (Bartlett's test for homogeneity of variances: $X^2 = 0.46$, $p>0.05$, d.f. = 1) (Sokal and Rohlf, 1981). Males and females also did not differ in their propensity to remate with the same fly. Eight of 29 mating females, and 12 of 26 mating males remated with the same partner at least once in the same day ($G = 2.05$, $p>0.05$, d.f. = 1). In one day, a female mated twice each with 4 males, while in one day, a male mated twice each with 3 females. In two instances, a pair of flies mated three times in one day.

With the exception of the first day, the proportion of females emigrating from the tree always equalled or exceeded the proportion of males emigrating, a significant difference between the sexes (Sign test, $p<0.05$) (Fig. 4.2). Generally, on days in which fruit were replaced with fresh fruit (days 5, 9, and 13) decreases in fly emigration were noted. The proportion of flies emigrating from the tree bore no

apparent relationship to the maximum daily temperature for either sex (males: $r = 0.36$, $p>0.05$, d.f. = 13) (females: $r = 0.09$, $p>0.05$, d.f. = 13). (Proportions were used for these analyses because the number of flies in the cage decreased over time, resulting in fewer flies available for emigration.)

A significant positive relationship existed between mating and movement by males ($G = 28.27$, $p<0.01$, d.f. = 1). A male was categorized as moving if, on any particular day, he was seen in more than one section of the tree and as mating if he was observed to mate at least once that day. No significant relationship was found between fruit residence (defined as being seen on a fruit at least once that day) and mating when each fly was categorized on a daily basis ($G = 0.39$, $p>0.05$, d.f. = 1). Similarly, out of the 11 males seen mating on at least 10 of the 14 observation days, only 2 individuals exhibited a significant positive correlation between number of mates acquired and number of observations on fruit per day (Table 4.1). Agonistic encounters (defined as engagement in at least one episode of "boxing" that day) and mating were likewise not related when totaled over the entire 14 days ($G = 0.25$, $p>0.05$, d.f. = 1) for males. In only 1 male out of the 11 seen for 10 days or more was a significant positive correlation found between number of mates and number of fights per day (Table 4.1).

As in males, movement and mating in females were significantly related ($G = 17.38$, $p<0.01$, d.f. = 1). Because females rarely engaged in agonistic encounters, this parameter was not tested in relation to female mating. In females, a significant positive relationship existed between fruit residence and mating when totaled over the entire 14 days ($G = 5.49$, $p<0.05$, d.f. = 1). For 13 of the 14 females seen mating on

each of 10 or more days, a significant positive correlation existed between number of sightings on fruit and number of matings per day (Table 4.2). In contrast, no significant relationship was found between oviposition and mating over the entire 14 days ($G = 0.57$, $p>0.05$, d.f. = 1). Stated differently, for only 4 out of 14 females did a significant positive correlation exist between number of ovipositions and number of matings (Table 4.2).

Because contingency table analyses do not lend themselves to assignments of cause and effect but merely show relationships, we chose to analyze further the positive relationships we found between movement and mating in each sex. We categorized each fly for each day as to whether movement among tree sections preceeded or followed the first mating. In other words, we asked: Did the fly begin moving about in the tree and then mate, or did the fly mate and then commence movement? In males, movement preceeded mating in the majority of cases; on 11 out of 14 of the observation days, mating most often followed the onset of movement ($n = 142$ observations; Sign test, $p<0.05$). In contrast, in females, mating usually preceeded movement; on 10 out of 14 days, females were most often seen mating first and then moving ($n = 110$ observations; Sign test, $p<0.05$).

## 4.4 Discussion

From a previous study, we estimated that male and female apple maggot flies may live up to 4 weeks in the field (Chapter 2). Although in this field cage study the initial ratio of fly to fruit density (1:1) exceeded what we would expect to find in nature, we feel the results are generally applicable to the field situation because we allowed flies to

emigrate from the tree and because we used a 1:1 male:female sex ratio. Thus, the maximum number of matings we observed in this 14 day study, 30 and 31 for a single female and male, respectively, may be a conservative estimate of copulation potential in the apple maggot fly even though flies were in a confined situation. More importantly, the great majority of females and males participated in multiple matings, and a non-random pattern of mating among members of each sex was found. In addition, females and males did not differ from one another in either mean or variance in mating success and were equally likely to remate with the same individuals. Based on these findings, combined with previous results indicating that female (Chapter 3) and male (Myers et al., 1976; Chapter 5) apple maggot flies benefit from multiple matings, we propose the adoption of a new term, dual polygamy, to describe this type of mating system.

Sutherland (1985) has criticized the use of variance in mating success to indicate the operation of sexual selection. According to Sutherland (1985), when little time is invested in mating by one sex, that sex is likely to demonstrate a large variance in mating success simply due to chance. Because we have measured variance in mating success in a species in which both males and females invest approximately equal and potentially great amounts of time (up to 4 h per day) in mating, we feel we have not fallen prey to this criticism. Furthermore, we have shown that non-random mating patterns occur in each sex, a comparative method which Sutherland (1985) suggests as a more direct means of testing for the operation of sexual selection. Thus, we are compelled to conclude that dual polygamy is a robust

characterization of the mating system of the apple maggot fly based on the criteria suggested by Sutherland (1985).

Dual polygamy differs from classical polygyny in that not only males, but also females, multiply mate and benefit from multiple matings. In certain respects, however, the mating system of the apple maggot fly appears consistent with notions of resource defense polygyny in that males appear to dominate resources necessary for female reproduction (Hendrichs and Reyes, 1987). We do not agree with the use of the term polygyny to denote mating systems in which females also multiply mate, as has been suggested in the apple maggot fly (Hendrichs and Reyes, 1987), for we feel this leads to confusion regarding the effects of multiple mating on female reproductive success.

Male apple maggot flies often attempt copulation with females arriving on fruit to oviposit (Prokopy et al., 1988; Smith and Prokopy, 1980); yet in our study we found no correlation between male residence on fruit and male mating success. Although our study did not directly address this question, it seems unlikely, based on previous studies (Prokopy and Bush, 1973), that males are equally successful at mating when they reside on leaves and other non-fruiting structures as when they reside directly on fruit. Instead, we feel that the vagility of males in relation to our frequency of census may have resulted in a misleading lack of correlation between fruit residence and mating success. To address this paradox, additional studies need to be undertaken in which the movements of individually marked males are observed in relation to mating success. We agree with the general observation of Burk (1981) for some acalyptrate flies that males may be searching resource areas for females and interacting aggressively with

other males, when encountered, without defending any particular area. These and our observations correspond more closely to the model of Courtney and Anderson (1986) in which males have unstable distributions and often abandon encounter sites than to the sometimes stringent criteria used to define true territories (see Baker, 1983). Further experiments are necessary to investigate the possibility of territoriality in male apple maggot flies.

Recently, the concept of sexual dimorphism in dispersal behavior among insects has received some attention, although results tend to differ dependent upon species. In milkweed bugs, Evans (1987) found that males but not females tended to remain in the host plant area where mated. In contrast, male milkweed beetles were more likely than females to move between host plant patches (Lawrence, 1982), and dispersal provided an alternative mating tactic for smaller males dependent on local sex ratio (Lawrence, 1987). In this study and previously (Chapter 2), we found that female apple maggot flies exhibited a greater tendency to disperse (i.e. emigrate) than males. In this highly visually-oriented fly, the presence of other individuals on fruit, while eliciting copulation attempts by males, may actually discourage arrival on fruit by foraging females (Prokopy and Bush, 1973). Furthermore, intra-tree movements differed between the sexes, with most female movements occurring after copulation and most male movements preceeding copulation. We hypothesize that females begin to move to avoid male harassment during oviposition attempts on fruit, as has been hypothesized to occur in another tephritid fly, Dacus longistylus (Hendrichs and Reyes, 1987). Male harassment of ovipositing females is not an uncommon attribute of multiple mating systems in insects (eg.

Alcock et al., 1977; Fincke, 1984; Hough-Goldstein et al., 1987; Svard and Wiklund, 1986; Waage, 1984). In the apple maggot fly, because most matings occur at the oviposition site and because males tend to restrict inter-tree movements following the onset of oviposition (Chapter 2), the potential for conflict between female oviposition attempts, on the one hand, and male mating attempts, on the other hand, is great. We cannot, however, conclusively argue that male harassment is an important attribute of this mating system until detailed behavioral observations of the foraging paths of individual females in relation to encounters with males are undertaken. Furthermore, the hypothesis of male harassment does not negate our proposal of a dual polygamous mating system. Male harassment, in this case, does not result in a polygynous mating system where male mean and variance in mating success exceed that of females, as is sometimes found in other insects (eg. Hughes, 1981; Hughes and Hughes, 1985). On the contrary, female apple maggot flies show increased fecundity and fertility with multiple matings, at least under laboratory conditions of unlimited access to oviposition sites (Chapter 3).

It has also been proposed that when males control mating decisions, as in the case of resource-based polygyny, females end up multiply-mated primarily because they make multiple visits to the resources (see Burk, 1981). Although our results indicate a strong relationship between visits to fruit and mating by female apple maggot flies, we do not feel this pattern necessarily results from a resource-based polygynous system. Because females engage in and benefit from multiple matings in R. pomonella, this mating system does not appear consistent with the general concept of polygyny in which males multiply

mate. Nor does the mating system of the apple maggot fly correspond to more specific concepts of resource-based polygyny in which males have primary control over mating decisions. In contrast, we feel the mating system of R. pomonella may more closely conform to notions of foraging theory which take into account risk-balancing trade-offs as in cases of predator avoidance (Pitcher et al., 1988). Female apple maggot flies may be balancing the benefits of multiple mating and access to oviposition sites against the risk of male harassment.

Dual polygamy, with equal male and female mating success, obviously also differs greatly from polyandry, i.e. multiple mating among females, in which female mating success is typically greater and more variable than male mating success and in which male parental investment is as great as or greater than that of females in non-social insects (eg. giant water bugs (Smith, 1979); see also Page and Metcalf (1982) for social insects). Finally, in contrast to promiscuous mating systems in which gametes unite at random (Pianka, 1978), we have found non-random mating patterns among male and female apple maggot flies.

We assert that dual polygamy is a mating system heretofore overlooked as being distinct from other multiple mating systems. The adoption of the term dual polygamy in studies of sexual selection could help to clarify a somewhat confusing and often contradictory array of terminology and usage surrounding studies of multiple mating. We encourage the use of the terms polygyny and polyandry to denote multiple mating systems in which males and females have unequal mating success considered both in terms of mean and variance. Furthermore, we agree with Sutherland (1985) that unless patterns of mating success for each sex are found to deviate from randomness, observed variation in mating

success might be due to chance. We also encourage more studies that investigate potential costs and benefits of multiple mating from both the male and female perspectives for only such balanced studies will give us the complete picture necessary to categorize accurately animal mating systems.

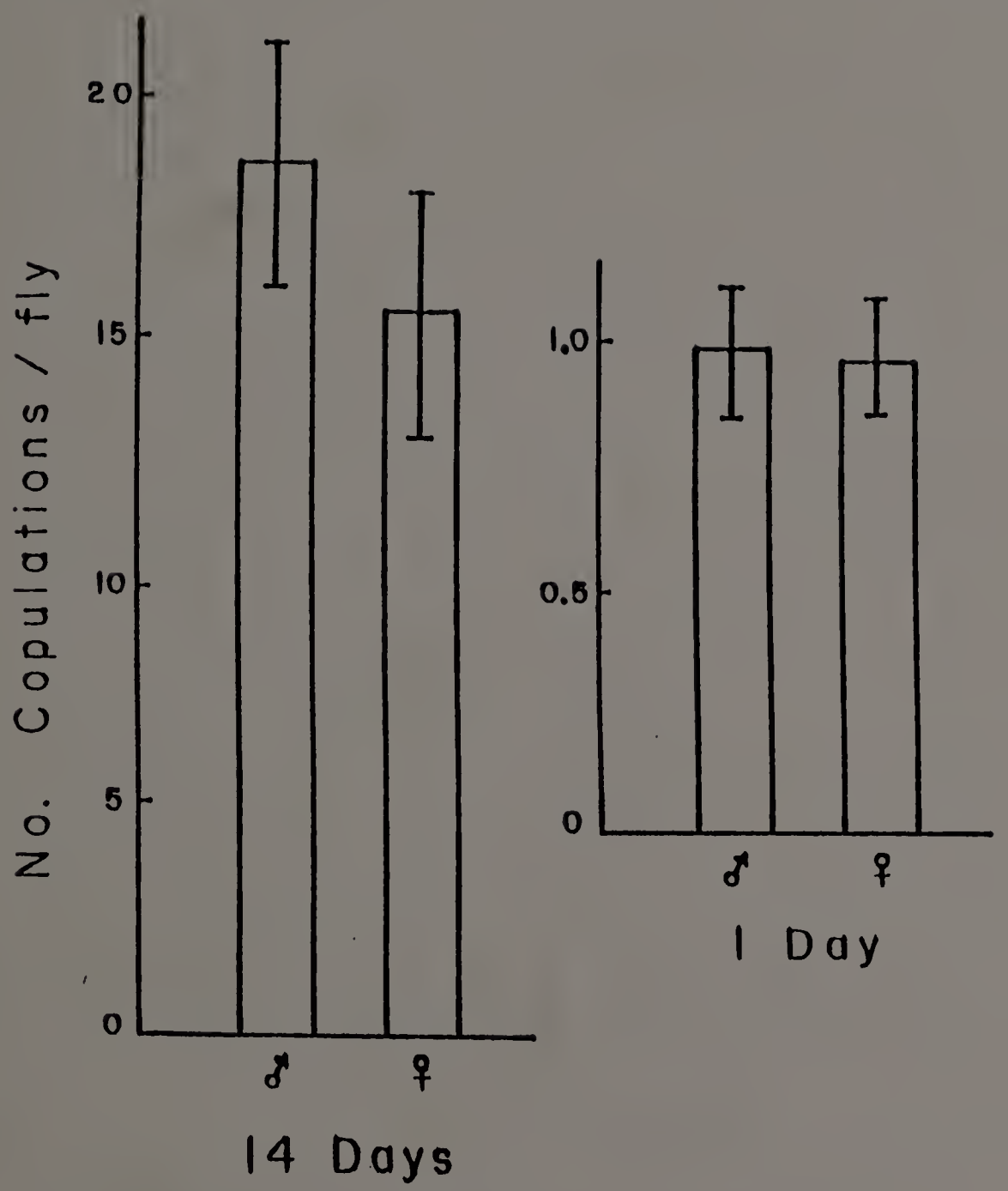


Figure 4.1
Mean number of copulations per fly (±s.e.) for male and female apple maggot flies over the entire 14 days of observation and on a daily basis.

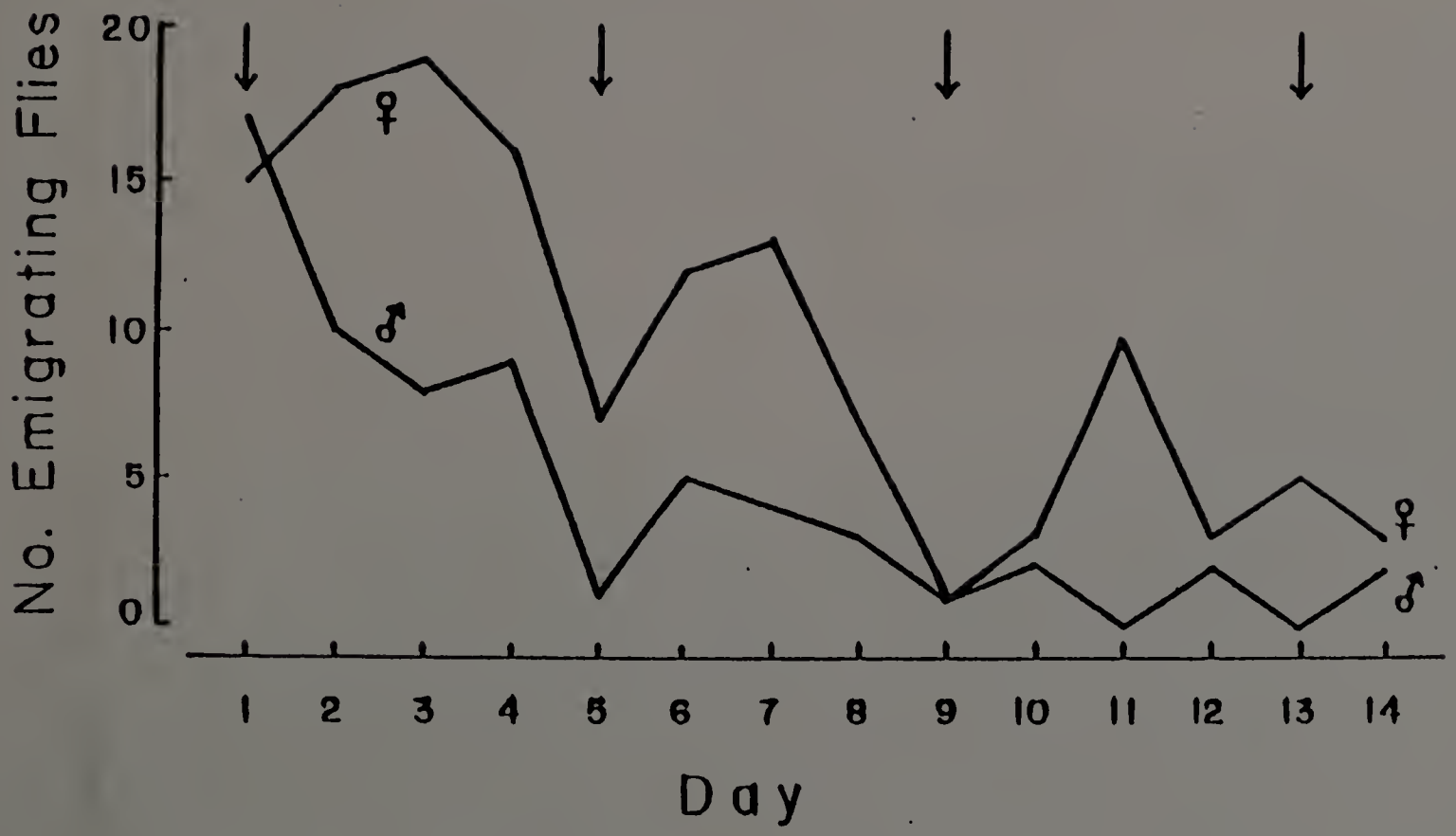


Figure 4.2
Proportion of male and female apple maggot flies emigrating from the observation tree for each day of observation.

Table 4.1
Correlations between number of mates acquired and number of times seen on fruit or between number of mates acquired and number of times seen fighting per day for each male observed mating on each of at least 10 days (square root transformed counts).

| | | R values | |
|---|---|---|---|
| Male # | No. days | Mates vs fruit | Mates vs fights |
| 204 | 11 | 0.391 | 0.083 |
| 208 | 14 | 0.329 | 0.101 |
| 213 | 11 | 0.390 | 0.391 |
| 215 | 12 | 0.161 | 0.233 |
| 216 | 14 | 0.529 | 0.620* |
| 217 | 13 | 0.545 | 0.026 |
| 222 | 14 | 0.085 | 0.115 |
| 228 | 11 | 0.904** | 0.502 |
| 229 | 14 | 0.521 | 0.265 |
| 231 | 13 | 0.513 | 0.035 |
| 232 | 14 | 0.737** | 0.422 |

* $p<0.05$
** $p<0.01$

Table 4.2
Correlations between number of mates acquired and number of times seen on fruit or between number of mates acquired and number of times seen ovipositing per day for each female observed mating on each of at least 10 days (square root transformed counts).

| | | R values | |
|---|---|---|---|
| Female # | No. days | Mates vs fruit | Mates vs ovipositions |
| 102 | 10 | 0.810** | 0.628 |
| 103 | 12 | 0.813** | 0.387 |
| 105 | 14 | 0.688** | 0.680** |
| 108 | 13 | 0.464 | 0.481 |
| 112 | 13 | 0.751** | 0.266 |
| 113 | 13 | 0.934** | 0.681* |
| 116 | 10 | 0.760** | 0.583 |
| 120 | 14 | 0.699** | 0.564* |
| 121 | 14 | 0.755** | 0.136 |
| 126 | 14 | 0.782** | 0.020 |
| 130 | 13 | 0.707** | 0.273 |
| 132 | 12 | 0.648* | 0.245 |
| 133 | 11 | 0.753** | 0.634* |
| 135 | 14 | 0.677** | 0.046 |

*p<0.05
**p<0.01

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# CHAPTER 5

## FACTORS INFLUENCING ESTIMATES OF SPERM COMPETITION IN THE APPLE MAGGOT FLY, RHAGOLETIS POMONELLA

### 5.1 Introduction

Sperm competition is a form of sexual selection which is particularly intense in insects due to the ability of females to store and maintain living sperm for long periods of time in the spermatheca, the female sperm storage organ (Parker, 1984). Because more than one ejaculate may be stored concurrently by a female who mates more than once, sperm may compete for fertilization of eggs. Sperm competition has been viewed by some researchers as an entension of intermale competition in which selection favors a male's adaptations to preside over ejaculates of other males while protecting his own sperm from subsequent rival males (Parker, 1970). More recently, sperm competition has been considered from the female perspective, with the outcome of sperm competition not only dependent on female anatomy and behavior but also of potential benefit to females (Walker, 1980).

Studies of insect sperm competition from mechanistic, ecological, and behavioral perspectives have become relatively common (eg. Dickinson, 1986; Fincke, 1984; Saul et al., 1988; Simmons, 1987; Turner, 1986; Waage, 1979; Wood et al., 1984). Techniques for investigating the outcome of sperm competition in insects fall into 3 main categories: 1) studies using morphological markers (eg. Gromko and Pyle, 1978; Saul et al., 1988; Schlager, 1960; Sims, 1979; Smith, 1979), 2) studies using irradiated males (eg. Backus and Cade, 1986; Economopoulos, 1972;

Economopoulos et al., 1976; Fincke, 1984; McVey and Smittle, 1984; Myers et al., 1976; Parker and Smith, 1975; Sakaluk, 1986; Simmons, 1987; Woodhead, 1985), and 3) studies employing electrophoresis for comparison of parent and offspring alleles (Dickinson, 1986; Turner, 1986; Turner and Anderson, 1984; Wood et al., 1984; Zouros and Krimbas, 1970). Each technique has potential drawbacks. Use of morphological markers generally requires extensive laboratory breeding of insects, and markers may be genetically linked to traits which reduce fitness (Saul et al., 1988; Turner, 1986). Irradiated insects may produce sperm which are not as competitive as normal sperm in fertilizing eggs (Economopoulos et al., 1976; Parker and Smith, 1975), thereby altering estimates of sperm competition. Development of electrophoretic systems of buffers and stains may take years of work for a particular insect species, but given that linkage disequilibrium does not occur between the allozymes being analyzed and given that sufficient polymorphism exists, electrophoresis as a technique to investigate sperm competition in insects has few drawbacks (Turner, 1986).

The apple maggot fly, Rhagoletis pomonella (Walsh), is a tephritid fruit fly which lends itself well to studies of sperm competition using electrophoresis of allozymes. Not only does multiple mating occur frequently in both sexes of this fly (Chapter 3), but electrophoretic methods have been developed extensively in this fly to study questions of population genetics (Berlocher, 1980; Smith and Berlocher, 1983). This study was undertaken to investigate paternity of offspring following multiple matings in R. pomonella. Specifically, this study addresses the outcome of sperm competition analysis using electrophoresis in relation to: duration of egg collection from twice-

mated females, duration of each of two copulations per female, male mating status, and statistical methods of paternity estimation.

## 5.2 Materials and Methods

### 5.2.2 Mating and Rearing *R. pomonella*

Wild apple maggot fly adults, collected as larvae from naturally infested apples the previous year, were separated by sex and maintained in the laboratory at 24 $\pm$ 2°C and 60% RH with a 16 h photoperiod. Flies were held in 25 $cm^3$ Plexiglas and screen cages provided with water and a 4:1 mixture of sucrose and enzymatic yeast hydrolysate as food for 14-16 days to allow for reproductive maturation (Webster et al., 1979).

Male flies were marked individually on the wings with felt pen prior to testing. Preliminary tests indicated no negative effects of wing marking on mating ability. On Day 1, 5 mature females together with 5 mature marked males were placed in 16 $cm^3$ Plexiglas and screen cages for mating in the laboratory. Two spring-water-washed hawthorn fruit (*Crataegus mollis*) were hung in each mating cage because mating encounters between the sexes most commonly occur on fruit in nature (Smith and Prokopy, 1980). Cages were observed continuously, and matings were timed from onset (male clasping of female ovipositor) to completion (natural separation of male aedaegus and female ovipositor). Following mating, females were removed to individual vented Solo$^{TM}$ cup cages (see Chapter 3) provided with food and water, as described previously. Males were either frozen in liquid nitrogen immediately after mating or were returned to a mating cage for copulation with a second female that same day. Following a male's second copulation, he

was frozen in liquid nitrogen and his mate was placed in a cup cage. Thus, on Day 1, females mated once with either a virgin or non-virgin male. All mated females were given 3-4 C. mollis hawthorn fruit for egglaying on Day 1 to assess the success of sperm transfer with the first mating.

On Day 2, 3 virgin, wing-marked 14-16 day old males were placed in each Solo$^{TM}$ cup cage with a female who was mated the previous day to a virgin male. Durations of matings were timed, then males were removed from the cages. As on Day 1, following one mating, a male was either frozen in liquid nitrogen or was returned to a cup cage with a female for a second mating that same day (as a non-virgin male). On Day 2, non-virgin males that had mated that same day were placed only in those cup cages with females mated to non-virgin males on the previous day.

In all, 2 mating treatments were created: 1) females mated once each on Days 1 and 2 to a virgin male, or 2) females mated once each on Days 1 and 2 to a non-virgin male, i.e., a male that had mated once previously that same day. Daily from Day 2, all twice-mated females were given 3-4 C. mollis for oviposition. In addition to the 2 mating treatments, females mated to virgin males received one of two oviposition duration treatments: 1) V2-10 females = mated to two virgin males and allowed to oviposit for 10 days (n = 5), or 2) V2-20 females = mated to two virgin males and allowed to oviposit for 20 days (n = 6). Females mated to non-virgin males received only one oviposition duration treatment: NV2-20 females = mated to two non-virgin males and allowed to oviposit for 20 days (n = 9).

Fruit were removed daily from the cup cages and were maintained in groups according to female and by date of oviposition at 27 $\pm$ 2$^{o}$C and

75% RH with constant light. After 10-18 days, larvae emerged from fruit and dropped through screen into cups where they were collected daily and frozen in liquid nitrogen. All females were frozen in liquid nitrogen following the 10 or 20 days of oviposition, or at death, whichever came first.

5.2.2 Electrophoretic Methods

Using horizontal slab starch gel electrophoresis, we examined the following 4 polymorphic enzymes (abbreviations, subunit structure, and enzyme commission number in parentheses): phosphoglucomutase (PGM, monomer, EC 2.7.5.1), NADP-dependent cytosol isocitrate dehydrogenase (IDH, dimer, EC 1.1.1.42), *beta*-hydroxybutyrate dehydrogenase (HBDH, dimer, EC 1.1.1.30), and glucose phosphate isomerase (PGI, dimer, EC 5.3.1.9). All gels were prepared at 12% starch according to the methods of Berlocher (1980) and Berlocher and Smith (1983). Genetic nomenclature follows that of Berlocher and Smith (1983) for R. pomonella, in which letters are used as an abbreviation for each allele.

Each female and her 2 mates were electrophoretically analyzed on the same slab gel to ensure correct identification of allozymes and to determine whether sufficient polymorphism between males existed to allow progeny assignment. In those cases (14 of 20) in which a female's two mates did not have unambiguously different alleles for the 4 enzymes, a maximum likelihood ratio method (McCulloch and Dickinson, 1988) was used to estimate proportion of larvae assignable to each father. A minimum of 13 larvae was analyzed per family ($x = 48.2$, s.e. = 3.2). A total of 964 larvae was analyzed from 20 families (each family = a female + her 2 mates + resultant larvae).

Paternity estimates were compared between treatments using G-tests for independence. To test for values that differed significantly from complete sperm mixing, G-tests of goodness of fit to a 50:50 ratio of offspring were conducted on proportions of larvae estimated to have been fathered by each male for each treatment. Effects of durations of copulations on paternity estimates were tested within each treatment by correlating mating durations with arcsine transformed (angular transformed) proportions of larvae fathered by the second male. Durations of copulations were not recorded for 1 female mated to 2 non-virgin males, thus reducing the sample size from 9 to 8 for the NV2-20 treatment.

## 5.3 Results

Each of the 5 V2-10 female (mated to 2 virgin males and allowed to oviposit for 10 days) demonstrated paternity which differed significantly from sperm mixing (50:50 ratio of offspring) (Table 5.1). In these females, paternity was unambiguous based on parental allozymes, and precedence of the second male's sperm ranged from 79% to 98%. The overall mean level of sperm precedence among females of this treatment was 93% precedence of the second male's sperm, a significant deviation from equal sperm use ($G = 280.02$, $p < 0.01$).

Allowing females to oviposit for twice as long (20 days) did not change the pattern of sperm use. Four of the six V2-20 females exhibited paternity patterns differing significantly from sperm mixing (Table 5.2). Paternity of the second male was estimated to range from 44% to 100% in these families, none of which had unambiguous paternity based on parental allozyme patterns. The overall pattern was one of

significant precedence of the second male's sperm ($x = 79\%$, $G = 104.46$, $p < 0.01$).

Although in both treatments in which females mated with virgin males a significant pattern of sperm precedence was found, the patterns for these two treatments differed significantly from each other (V2-10: 93% precedence; V2-20: 79% precedence; $G = 25.35$, $p < 0.01$). This difference was not due to the effects of the second 10 days of oviposition, contrary to what one might expect. In only 1 V2-20 family (female #2: first 10 days: 33% of offspring from second male; second 10 days: 67% of offspring from second male; $G = 4.29$, $p < 0.05$) did the second 10 days of oviposition yield a pattern of sperm precedence differing significantly from the first 10 days. The other difference between these two treatments was that no estimation methods were necessary to determine paternity for the V2-10 families (due to unambiguous parental allozymes), whereas the maximum likelihood ratio method (McCulloch and Dickinson, 1988) was used to estimate paternity for the V2-20 families.

Precedence of the second male's sperm was also found in the families of females mated with non-virgin males (Table 5.3) ($x = 82\%$, $G = 174.10$, $p < 0.01$). In eight of the nine NV2-20 families, a significant proportion of the offspring was fathered by the second male, with paternity by the second male estimated to range from 31% to 100%. In the one family in this treatment exhibiting unambiguous paternity based on family allozymes (female #3), the second male fathered 88% of the offspring.

Although a significant overall deviation from sperm mixing was found with females mated to non-virgin males, the level of sperm

precedence differed significantly from that of V2-10 females (NV2-20: 82% precedence; V2-10: 93% precedence; $G = 19.21$, $p < 0.01$). On the other hand, NV2-20 families (82% precedence) did not differ significantly in estimated sperm precedence from V2-20 families (79% precedence) ($G = 1.01$, $p > 0.05$). Furthermore, in four of the six families of flies in which females mated with non-virgin males and continued to oviposit for the full 20 days (three females were terminated in less than 20 days), no significant difference was found between the first 10 and second 10 days of oviposition in terms of estimated paternity. In the two families in which significant differences were found between the first and second 10 days of oviposition, one family (female #8) exhibited a pattern of increasing precedence of the second male's sperm over time (from 56% to 100%) ($G = 12.68$, $p < 0.01$), while the second female (#9) exhibited a pattern of decreasing precedence of the second male's sperm over time (from 100% to 51%) ($G = 22.90$, $p < 0.01$).

Durations of the first and second matings were not correlated significantly with proportion of offspring fathered by the second male for any of the three mating and rearing treatments (Table 5.4). In each case, however, durations of second matings were more strongly correlated with paternity estimates than were durations of first matings. Low sample sizes likely contributed to the lack of statistical significance (Table 5.4). No significant correlation was found between proportion of offspring fathered by the second male and duration of the second male's previous mating when males were non-virgin ($r = 0.66$, $p > 0.05$). In other words, assuming that duration of mating is positively correlated with amount of sperm transferred (as found in

C. capitata (Saul et al., 1988)) there was no evidence that non-virgin males had become sperm-depleted by mating twice within one day. Yet, in 13 out of the 16 cases in which a male mated twice (8 families of flies), his second mating was of shorter duration than his first (first matings: $x = 2484$ s, s.e. = 250; second matings: $x = 1735$ s, s.e. = 85; $t = -2.53$, $df = 15$, $p < 0.05$).

On a per female basis, no significant difference was found between the duration of a female's first and second matings (first matings: $x = 2019$ s, s.e. = 126; second matings: $x = 2118$ s, s.e. = 145; $t = -0.51$, $df = 18$, $p > 0.05$). Furthermore, no differences were found in mating durations between thoses females mated with virgin ($x = 2044$ s, s.e. = 170) or with nonvirgin males ($x = 1735$ s, s.e. = 85) ($t = 1.63$, $p > 0.05$).

## 5.4 Discussion

In a previous study using the irradiated male technique, Myers et al. (1976) found incomplete sperm precedence in the apple maggot fly, similar to our results. However, based on two criteria, we wished to expand the results of Myers et al. (1976). First, these researchers found female R. pomonella which mated twice laid fewer eggs than females mated once (Myers et al., 1976), in direct contrast to our results (Chapter 3) in which females mated twice laid greater numbers of eggs than females mated once. A possible cause of this discrepancy lies in a difference in egg collection method and duration; Myers et al. (1976) collected R. pomonella eggs in apples for only 9 days, while in our previous study (Chapter 3), we collected eggs in wax domes over the lifetime of a female. This methodological difference was somewhat

alleviated in the current set of experiments in which we allowed females to oviposit in fruit (hawthorn) for less than their entire lives (10 or 20 days). A second difficulty with the findings of Myers et al. (1976) is that their results were not reciprocal between females mated with a sequence of normal-irradiated versus a sequence of irradiated-normal males. Thus, as reported with other studies using irradiated males (Economopoulos et al., 1976; Parker and Smith, 1975), we were concerned that irradiated R. pomonella sperm might not be as competitive as normal sperm.

The degree of sperm precedence we found in R. pomonella agreed with or exceeded that found by Myers et al. (1976). While they reported average precedence of second-male sperm ranging from 66-78%, we found average precedence of second-male sperm to range from 79-93%, dependent on treatment. Thus, we agree with the conclusion of Myers et al. (1976) that there is a limited amount of sperm competition from the first mating, with sperm from the second mating predominating. We found this to be the case regardless of the period of time over which eggs were collected and regardless of male mating status.

Our results for R. pomonella differ in many ways from those reported for a close relative, the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). In C. capitata, the duration of the first male's mating had significant positive effects on the proportion of offspring fathered by that male compared to the second male (Saul et al., 1988). These researchers felt that as the duration of the first male's mating increased, his paternal (fertilization) contribution also increased. Yet, the proportion of offspring attributable to the first male varied widely (from 1-84%), dependent on both duration of copulation and male

genotype (Saul et al., 1988). Thus, although these authors concluded that second-male sperm precedence occurs in C. capitata, their results were not nearly as clearcutas ours for R. pomonella. We do not find this surprising, because it is unknown whether multiple mating in C. capitata occurs commonly in nature (Saul et al., 1988). Further, we do not expect multiple mating, if it does occur, to reach the levels found in R. pomonella (see Chapter 3).

The maximum likelihood ratio estimation method, based on expected mendelian ratios (McCulloch and Dickinson, 1988), appears to provide conservative estimates of sperm precedence in R. pomonella. The estimates of precedence for V2-20 and NV2-20 treatments were significantly lower than the unambiguous measures of precedence for the V2-10 treatment. We do not find fault with the estimation method, however, because we could have improved our estimations by the addition of more polymorphic enzymes per family. We feel confident, from our use of both unambiguous measures and the estimation method, in stating that two matings by R. pomonella females will on average result in 80-90% offspring fathered by the second male.

Although, as pointed out by Myers et al. (1976), R. pomonella exhibits incomplete sperm precedence, 80-90% precedence is highly significant from the viewpoint of sexual selection studies. For females, the outcome of sexual selection is usually a straightforward measure: number of offspring produced. For males, particularly male insects, with such complications as sperm removal (Waage, 1979) and sperm competition, number of matings can be a very inaccurate measure of number of offspring produced. Yet, some researchers (eg. Sutherland 1985) continue to ignore the potential effects of sperm competition in

discussions of sexual selection and measures of mating success. If one were to ignore the effects of sperm competition in R. pomonella, in which both males and females may mate more than 5 times a day (Chapter 4), very unrealistic estimates of male mating success would result. In the future, we hope to incorporate the effects of sperm competition into a comprehensive picture of the factors which determine male mating success in nature in R. pomonella.

Table 5.1
Segregation of parental enzyme alleles and proportion of larvae attributable to each father for V2-10 females (n = number of larvae examined). Significant G-values indicate significant deviation from a 50:50 paternity ratio.

| | | Allozymes | | | | Proportion | |
|---|---|---|---|---|---|---|---|
| Parent | (n) | PGM | IDH | HBDH | PGI | of larvae | G-value |
| Female #1 | (48) | bb | aa | aa | aa | | |
| Male #1 | | bb | bb | ab | ab | 0.06 | |
| Male #2 | | ab | aa | ab | aa | 0.94 | 44.47** |
| Female #2 | (53) | ab | aa | -- | aa | | |
| Male #3 | | bb | bc | -- | aa | 0.21 | |
| Male #4 | | bb | aa | -- | aa | 0.79 | 19.34** |
| Female #3 | (52) | ab | bb | aa | aa | | |
| Male #5 | | aa | aa | bb | aa | 0.02 | |
| Male #6 | | aa | aa | aa | aa | 0.98 | 62.20** |
| Female #4 | (55) | bb | ab | aa | aa | | |
| Male #7 | | ad | aa | bb | aa | 0.02 | |
| Male #8 | | bb | ab | aa | aa | 0.98 | 66.25** |
| Female #5 | (60) | ab | bb | aa | aa | | |
| Male #9 | | bb | ac | aa | aa | 0.03 | |
| Male #10 | | bb | bb | aa | aa | 0.97 | 65.64** |

**p<0.01 *p<0.05

Table 5.2
Segregation of parental enzyme alleles and proportion of larvae attributable to each father for V2-20 females (n = number of larvae examined). Significant G-values indicate significant deviation from a 50:50 paternity ratio.

| | | Allozymes | | | | Proportion | |
|---|---|---|---|---|---|---|---|
| Parent | (n) | PGM | IDH | HBDH | PGI | of larvae | G-value |
| Female #1 | (67) | bb | ab | ab | aa | | |
| Male #1 | | bb | ab | aa | aa | 0.39 | |
| Male #2 | | ab | aa | aa | aa | 0.61 | 3.39 |
| Female #2 | (39) | -- | ab | ab | ab | | |
| Male #3 | | aa | aa | aa | aa | 0.56 | |
| Male #4 | | ab | bb | aa | aa | 0.44 | 0.64 |
| Female #3 | (39) | ab | ab | ab | aa | | |
| Male #5 | | ab | ab | aa | ab | 0.10 | |
| Male #6 | | aa | bc | ab | ab | 0.90 | 28.27** |
| Female #4 | (53) | ab | ab | ab | aa | | |
| Male #7 | | bb | cc | ab | aa | 0.17 | |
| Male #8 | | ab | bc | aa | aa | 0.83 | 25.18** |
| Female #5 | (29) | bb | ab | aa | aa | | |
| Male #9 | | bb | bb | ab | aa | 0.0 | |
| Male #10 | | ab | aa | aa | aa | 1.0 | 40.20** |
| Female #6 | (71) | ab | bb | aa | ab | | |
| Male #11 | | ab | aa | aa | aa | 0.06 | |
| Male #12 | | aa | aa | ab | aa | 0.94 | 67.65** |

**p<0.01 * p<0.05

Table 5.3
Segregation of parental enzyme alleles and proportion of larvae attributable to each father for NV2-20 females (n = number of larvae examined). Significant G-values indicate significant deviation from a 50:50 paternity ratio.

| | | Allozymes | | | | Proportion | |
|---|---|---|---|---|---|---|---|
| Parent | (n) | PGM | IDH | HBDH | PGI | of larvae | G-value |
| Female #1 | (13) | aa | aa | bb | aa | | |
| Male #1 | | aa | aa | ab | aa | 0.0 | |
| Male #2 | | ab | ab | aa | ab | 1.0 | 18.02** |
| Female #2 | (55) | bc | aa | bb | aa | | |
| Male #3 | | bc | ab | aa | ab | 0.09 | |
| Male #4 | | ab | aa | aa | aa | 0.91 | 42.74** |
| Female #3 | (59) | aa | aa | ab | aa | | |
| Male #5 | | aa | bb | ab | aa | 0.12 | |
| Male #6 | | aa | aa | aa | aa | 0.88 | 38.81** |
| Female #4 | (30) | aa | ab | aa | aa | | |
| Male #7 | | aa | aa | aa | aa | 0.69 | |
| Male #8 | | aa | bb | aa | aa | 0.31 | 4.94* |
| Female #5 | (29) | aa | bb | aa | ab | | |
| Male #9 | | aa | bb | ab | aa | 0.09 | |
| Male #10 | | aa | ab | aa | aa | 0.91 | 20.91** |
| Female #6 | (48) | bc | aa | ab | aa | | |
| Male #11 | | bb | aa | ab | aa | 0.0 | |
| Male #12 | | ab | aa | aa | aa | 1.0 | 66.54** |
| Female #7 | (58) | aa | bb | aa | ab | | |
| Male #13 | | aa | aa | ab | aa | 0.14 | |
| Male #14 | | aa | aa | bb | aa | 0.86 | 33.87** |
| Female #8 | (53) | ac | ab | ab | ab | | |
| Male #15 | | bc | ab | ab | aa | 0.38 | |
| Male #16 | | bc | aa | aa | ab | 0.62 | 3.22 |
| Female #9 | (53) | aa | bb | ab | aa | | |
| Male #17 | | aa | aa | aa | aa | 0.13 | |
| Male #18 | | ab | aa | ab | aa | 0.87 | 32.10** |

** $p<0.01$ * $p<0.05$

Table 5.4
Correlations of mating duration (seconds) and proportion of larvae fathered by the second male (angular transformed proportions) for families of R. pomonella from V2-10, V2-20, and NV2-20 females (n = number of families of flies examined per treatment).

| Male | Oviposition duration | (n) | r-values First mating | Second mating |
|---|---|---|---|---|
| Virgin | 10 days | (5) | 0.20 | 0.47 |
| Virgin | 20 days | (6) | 0.07 | 0.75 |
| Non-virgin | 20 days | (8) | 0.23 | 0.63 |

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# CHAPTER 6

# EFFECTS OF FEMALE MATING STATUS AND MALE DENSITY ON OVIPOSITION SITE FORAGING BEHAVIOR OF *RHAGOLETIS POMONELLA*

## 6.1 Introduction

Behaviors of animals foraging for resources may be influenced by a variety of factors, including resource quantity, quality, and distribution (see Hassel and Southwood, 1978; MacArthur and Pianka, 1966; Pyke, 1984). In general, foraging behavior theory assumes that foragers are attempting to maximize rate of gain of some resource, often in terms of energy intake (Charnov, 1976; MacArthur and Pianka, 1966; Pulliam, 1974). A confounding factor may exist when foragers encounter risks such as predators while foraging. Such risks have been found to influence greatly foraging behavior (Fraser and Huntingford, 1986; Milinski and Heller, 1978; Pitcher et al. 1988; Sih, 1980). Another type of risk to foragers may be due to conspecific mating attempts which, although not life threatening, may result in time wastage, increased predation hazard, unnecessary energy expenditure, and/or loss of access to resources. Although numerous studies have shown that male harassment of females may affect female behavior (Alcock et al., 1977; Hough-Goldstein et al., 1987; Thornhill, 1980; Zalucki and Kitching, 1984), these studies have not quantified effects of male harassment on female foraging behavior such as search persistence and resource acquisition.

One purpose of this study was to determine the effects of female mating status on propensity of female *Rhagoletis pomonella* (Diptera:

Tephritidae) to forage for oviposition sites in a host tree under semi-natural conditions. In nature, eggs are laid by this fly beneath the surface of host fruit where larvae grow to maturity. In the laboratory, female flies will lay eggs beneath the surface of ceresin wax artificial oviposition substrates (Prokopy, 1966, 1967). In a previous laboratory study, we found that females given unlimited access to artificial oviposition sites showed an increased tendency to lay eggs (increased fecundity) with increased numbers of matings (Chapter 3). This effect was not limited to inseminated females because sham-mated females, which were behaviorally multiply-mated but physiologically uninseminated, also demonstrated higher fecundity than virgins. We wished to determine whether this mating effect would extend to a field situation where females would be forced to search for egglaying sites.

A second purpose of this study was to investigate the effects of male density on female oviposition site foraging behavior. Because most matings in nature occur on fruit while females are engaged in some aspect of oviposition behavior (Smith and Prokopy, 1980; Chapter 2) and because males tend to reside on fruit to await female arrival (Prokopy et al., 1988), the potential for encounters between foraging females and males is high. We had reason to believe that males might be harassing foraging females, potentially limiting female access to oviposition sites (Chapter 3). Furthermore, we were interested in the potential interaction effects of male density and female mating status. Because in the laboratory females which were multiply-mated showed fecundity and fertility increases over females which were only mated once (Chapter 3), we felt that the effects of male encounters on female foraging behavior might vary with female mating status. To our knowledge, studies of

female oviposition site foraging behavior which concurrently assess the effects of female mating status and male harassment have not been undertaken previously.

## 6.2 Materials and Methods

### 6.2.1 Fly Preparation

Apple maggot puparia were formed from larvae collected from unsprayed hawthorn (Crataegus mollis) naturally infested with R. pomonella in Northampton, Mass. Puparia were held in moist vermiculite at 5°C for 9 mos, then were warmed as needed at 23±2°C to stimulate adult eclosion. Within 2 days of emergence, adults were separated by sex into groups of 15-20 individuals held in 16-cm screen and Plexiglas cages at 23±2°C and 55±5% RH with 16 h photoperiod. Each cage was supplied with water and a mixture of yeast hydrolysate and sugar as food.

When 12-18 days old, females were given spring-water-washed, uninfested C. mollis fruit for oviposition (ca. 1 fruit per 5 females) and were subjected to one of four mating treatments: 1) Virgin - females maintained in female-only group cages; 2) Singly-mated - females observed to mate once with a virgin male after which all males were removed and females maintained in female-only group cages; 3) Multiply-mated - after two observed matings, females held in group cages of males and females; or 4) Sham-mated - females maintained in group cages with emasculated males. Males were emasculated by removal of the entire aedaegus, rendering males incapable of insemination but capable of normal copulatory behaviors (see Chapter 3). After 3 days, hawthorn

fruit were replaced with fresh spring-water-washed, uninfested fruit. Females were tested when 18-24 days old, i.e. following 6 days of oviposition.

Males for testing were marked individually with Liquid Paper and a waterproof felt pen (see Chapter 2) and were maintained as virgins in 16-cm screen and Plexiglas cages with water and food, as with females. One day prior to testing, spring-water-washed, uninfested C. mollis fruit (ca. 1 fruit per 3 males) were hung in the cages to familiarize males with hawthorn fruit. Males were tested when 12-16 days old.

### 6.2.2 Experimental Protocol

Tests were conducted in a 2.5 m saran screen field cage into which was placed a single potted, non-fruiting hawthorn tree (Crataegus sp.). Thirty spring-water-washed, uninfested C. mollis hawthorn fruit were hung in the tree in 10 clusters of 3 fruit each. Fruit were hung on permanent, labelled wire hangers in the tree to ensure consistent fruit placement in the tree on different test days. Each fruit which received an egg during testing was replaced with a fresh, uninfested hawthorn fruit before proceeding with the next test. All fruit were replaced with fresh specimens daily.

Each female was tested at one of three male densities in the field cage: 1) zero males (n = 22 virgin, 24 singly-mated, and 22 multiply-mated females); 2) low density = 10 males (average of 1 male per fruit cluster) (n = 26 virgin, 22 singly-mated, and 24 multiply-mated females); or 3) high density = 30 males (average of 1 male per fruit) (n = 24 virgin, 25 singly-mated, and 28 multiply-mated females). Sham-mated females were tested only with zero males present (n = 26 females).

On any particular day, only one density of males was tested over all female mating treatments. The order of testing female mating treatments was randomized within a day, and the order of male density treatments was randomized over testing days.

One-half hour prior to testing, the appropriate number of males for that day's density treatment was released into the hawthorn test tree to allow males to become familiarized with the fruit and tree. An individual female was released on a particular leaf in the lower, center portion of the tree. All female movements and behaviors were followed and recorded verbally by a single observer using a hand-held cassette tape recorder. Behaviors of interest included: walking, resting, flying between leaves and/or fruit, turning to face males, wing-waving toward males, searching on fruit (head held low to fruit while female walks in a zig-zag manner), probing with ovipositor on fruit, dragging ovipositor following egg laying (to deposit fruit marking pheromone), and successful and unsuccessful male mating attempts. A mating attempt, which began when a male mounted a female, was considered successful if the male grasped the female ovipositor with his claspers and succeeded in aedaegus insertion (copulation), and was considered unsuccessful if the male and female separated before copulation could occur. A test was terminated when a female left the tree, became mated, or when 30 min had elapsed, whichever came first.

### 6.2.3 Statistical Analysis

Effects of female mating status, male density, and interaction of female mating status and male density were evaluated in relation to aspects of female foraging behavior using 2-way analysis of variance

(ANOVA) procedures. Relatively uniform variances and robust sample sizes per treatment (n = 22 - 28 females per treatment) rendered data transformations unnecessary for these simple comparisons. Relationships between male density and female mating status in regard to frequency of mating were determined using G-tests of independence on counts of numbers of females from each treatment category becoming mated.

## 6.3 Results

When males were not present on the host tree, female oviposition site foraging behavior was not significantly affected by female mating status. Total host tree residence time bore no significant relationship to female mating status (ANOVA: $F = 1.50$, $df = 93$, $p = 0.22$) (Fig. 6.1, male density = 0). Similarly, female mating status alone did not significantly affect number of fruit clusters visited (ANOVA: $F = 0.56$, $df = 93$, $p = 0.64$) (Fig. 6.2, male density = 0), although a trend existed toward increased fruit visitations with more matings. A related measure, number of ovipositions, likewise was not significantly affected by female mating status (ANOVA: $F = 0.79$, $df = 93$, $p = 0.50$) (Fig. 6.4, male density = 0), yet number of ovipositions tended to increase with number of matings.

The presence of males on the host tree significantly affected female residence time (Table 6.1). In the presence of males, virgin and multiply-mated females decreased host residence time, but singly-mated females showed no effect (Fig. 6.1). Unlike male density, neither female mating status nor interaction of male density and female mating status significantly affected residence time (Table 6.1).

Number of fruit clusters visited (a measure of propensity of females to forage for oviposition sites) was significantly affected by both female mating status and male density, while interaction effects were not significant (Table 6.2). Presence of males at low density (10 males per tree) tended to decrease number of fruit visited, regardless of female mating status (Fig. 6.2). Yet, at high male density (30 males per tree), number of fruit clusters visited per female neared or exceeded the number of clusters visited without males present (Fig. 6.2). The latter effect was most pronounced in singly-mated females, wherein number of fruit visited when 30 males were present exceeded the number visited when no males were present.

Female foraging rate (number of fruit clusters visited divided by residence time) did not show the same pattern as number of fruit clusters visited per female (Figs. 6.2 and 6.3). Only male density and not female mating status significantly affected foraging rate (Table 6.3). Except in the case of multiply-mated females at low male density (10 males), the addition of males to the field cage (from zero, to 10, to 30 males per tree) successively increased female foraging rate (rate of fruit visitation) (Fig. 6.3). Singly-mated females showed the greatest net increase in foraging rate with increasing male density.

Number of ovipositions per female likewise was affected (but not significantly) by male density (Table 6.4). Female mating status was the only significant factor influencing number of ovipositions per female (Table 6.4). In the presence of males, number of ovipositions decreased in virgin and multiply-mated females, but not in singly-mated females (Fig. 6.4).

Rate of oviposition was significantly affected by female mating status, but not by male density or the interaction of these two factors (Table 6.5). While singly-mated females demonstrated a steady increase in oviposition rate with increasing male density, both virgin and multiply-mated females showed decreased followed by increased oviposition rate in response to increasing male density (Fig. 6.5).

Male density and female mating status both significantly influenced number of males encountered per female (Table 6.6). This effect was most pronounced for singly-mated females where, at high male density, females averaged between 2 and 3 encounters with males per test (Fig. 6.6).

Male density and female mating status also significantly influenced the propensity of females to become mated on the host tree (Fig. 6.7). At low male density, no significant difference in propensity to mate was seen among females of the three mating treatments (G-test of independence: $G = 2.61$, $df = 3$, $p > 0.05$). But at high male density, far fewer multiply-mated females became mated than either virgin or singly-mated females (G-test of independence: $G = 6.92$, $df = 3$, $p < 0.05$).

Because female mating status influenced probability of females alighting on fruit (Table 6.2) and because most mating attempts occur on fruit (Chapter 4), we subdivided the data such that only those females finding fruit were analyzed. Again, significant differences among females were found at high male densities (Fig. 6.8). Multiply-mated foraging females (i.e. those alighting on fruit) were far less likely to become mated than either virgin or singly-mated foraging females (G-test for independence: $G = 8.24$, $df = 3$, $p < 0.05$). At low male densities,

multiply-mated foraging females were less likely than virgin or singly-mated foraging females to be mated, although the effect was not significant (G-test for independence: $G = 0.88$, $df = 3$, $p > 0.05$).

## 6.4 Discussion

No significant influence of female mating status on foraging behavior was found among females foraging without males present (Figs. 6.1, 6.2, 6.4). However, without males present, all aspects of foraging behavior showed trends similar to those expected based on laboratory findings in which fecundity and fertility increased with numbers of matings (Chapter 3). Thus, under semi-natural conditions in a field cage, females foraging alone on a host tree for oviposition sites demonstrated increased fruit-finding and egglaying when multiply-mated compared to when virgin or singly-mated.

Sham-mated females also exhibited a greater likelihood to visit fruit and to lay eggs compared with virgin or singly-mated females (Figs. 6.2, 6.4). Again, this was similar to the situation found in the laboratory where sham-mated females, which were behaviorally multiply-mated but physiologically uninseminated, laid more eggs than virgin or singly-mated females (Chapter 3).

The addition of males to the foraging arena altered many aspects of female foraging behavior. Female search persistence (measured as host residence time) decreased in virgin and multiply-mated females but not in singly-mated females (Fig. 6.1); the effect of male density on female residence time was significant (Table 6.1). Male density was also a significant factor along with female mating status influencing the number of fruit clusters visited, a measure of foraging propensity

(Table 6.2). When viewed graphically, however, the results appeared quite variable (Fig. 6.2). Compared to when no males were present, low male densities appeared to decrease fruit finding by females, while, at high male densities, fruit-finding by all females increased (but this increase was most pronounced among singly- and multiply-mated females).

Foraging rate demonstrates the combined effects of number of fruit visited and residence time in relation to male density and female mating status (Fig. 6.3). With the exception of multiply-mated females at low male density (i.e. 10 males), the addition of males to the female foraging arena functioned to increase the rate of fruit-visitation (foraging rate) in females, regardless of female mating status. The implication is that, due to male harassment in the form of mating attempts, females 1) leave fruit more quickly to avoid males residing on fruit, and/or 2) forage more quickly to compensate for time lost in male avoidance behaviors. In contrast, in studies of fish foraging in the presence of predators, foraging rate (food intake rate) decreased in the prey species when predators were abundant (Fraser and Huntingford, 1986; Milinski and Heller, 1978). One possible explanation was that confusion occurred as a fish attempted to divide its attention between feeding and avoiding predators (Milinski and Heller, 1978). In a study of male copulatory guarding in a water strider insect (Gerridae), Wilcox (1984) found that a female's foraging, i.e. prey capture rate, was enhanced when she carried a copulating male because her mate apparently repelled other males, thereby reducing male harassment.

Because in R. pomonella egglaying can occur only following fruit-finding, one might expect effects of male density and female mating status to be similar on both fruit-finding and egg-laying. In our

study, however, neither number of ovipositions nor oviposition rate were significantly affected by male density (Tables 6.4, 6.5), unlike number of fruit found and rate of fruit finding (Tables 6.2, 6.3). Only female mating status significantly affected number of eggs laid or egglaying rate. By examination of Figs. 6.3 and 6.5, it is clear that while foraging rate increased with greater male density, oviposition rate did not increase among females of each mating status. Singly-mated females showed increased rates of foraging and of oviposition when more males were present. Multiply-mated females demonstrated increased rates of foraging and slightly decreased rates of oviposition in the presence of increased numbers of males. Virgin females slightly increased foraging rates in the presence of males, but showed varying effects of male density on rates of oviposition. Thus, increased rate of foraging did not translate into increased oviposition in any but singly-mated females.

Using the scenario of possible responses of foragers to predation hazard discussed by Fraser and Huntingford (1986), we may make some generalizations regarding foraging behavior of R. pomonella females of different mating status. Multiply-mated females may be "risk adjusters" because they make greater adjustments to foraging and oviposition rate as the "hazard" (male density) increases (Fraser and Huntingford, 1986). Singly-mated females, on the other hand, may be "risk reckless" because they ignore hazards (males) or respond to hazards by increasing foraging and oviposition rate (Fraser and Huntingford, 1986). Virgin females demonstrate foraging and oviposition rates which are low and variable under all conditions, making generalizations or predictions of their behavior difficult.

The fact that females of different mating status show differing degrees of compensation to potential hazards of male harassment might also indicate differing perceptions of the severity of the hazard among females mated different numbers of times. Not surprisingly, both female mating status and male density significantly affected the probability of female encounter with males (Table 6.6). Nevertheless, since most mating encounters occur on fruit (Smith and Prokopy, 1980; Prokopy et al., 1988), if probability of male encounter was a simple function of females landing randomly on fruit and of male density, one would expect those flies landing on fruit the most often to encounter the most males. This was clearly not the case; while multiply-mated females at high male density exhibited the highest rate of fruit visitation (foraging rate; Fig. 6.3), singly-mated females encountered the most males at high male density (Fig. 6.6). Therefore, male encounter was not a random process determined by rate of females landing on fruit and male density. Rather, singly-mated females were either preferentially landing on male-occupied fruit, or multiply-mated females were actively avoiding male-occupied fruit, or both. Multiply-mated females were also significantly less likely than virgin or singly-mated females to become mated while foraging (Fig. 6.8). Thus, multiply-mated females appeared capable not only of avoiding males but, once encountered, of resisting mating attempts by males on fruit. The mechanisms by which they accomplish this are unknown, although wild R. pomonella females have been found in nature to respond to the visual stimulus of flies on fruit by emigrating from the fruit, exhibiting aggressive behavior, or remaining motionless (Prokopy and Bush, 1973c).

Harassment of females by males attempting to mate has been reported in a number of insect species (Alcock et al., 1977; Hough-Goldstein et al., 1987; Shapiro, 1970; Thornhill, 1980; Ubukata, 1984; Zalucki and Kitching, 1984). In two butterfly species (Pieris protodice (Shapiro, 1970) and Danaus plexippus (Zalucki and Kitching, 1984)) and one species of solitary bee (Anthidium maculosum (Alcock et al., 1977), male harassment often results in female dispersal or emigration. In Panorpa scorpionflies, male forced copulation is an alternative mating tactic for males without a nuptial (food) offering, and males attempting this tactic are avoided by females (Thornhill, 1980). In a dragonfly, Cordulia aenea amurensis, females avoid male harassment and unnecessary matings by ovipositing at hidden spots where they are unlikely to be found by patrolling males (Ubukata, 1984). As mentioned previously, male copulatory guarding enhances foraging in a water strider, Gerris remigis, because copulating males repel the advances of competing males (Wilcox, 1984). It is likely that male-female interactions influence resource foraging behavior in numerous species of insects and other animals, but few studies have focused on the integration of sexual selection and foraging behavior for other resources.

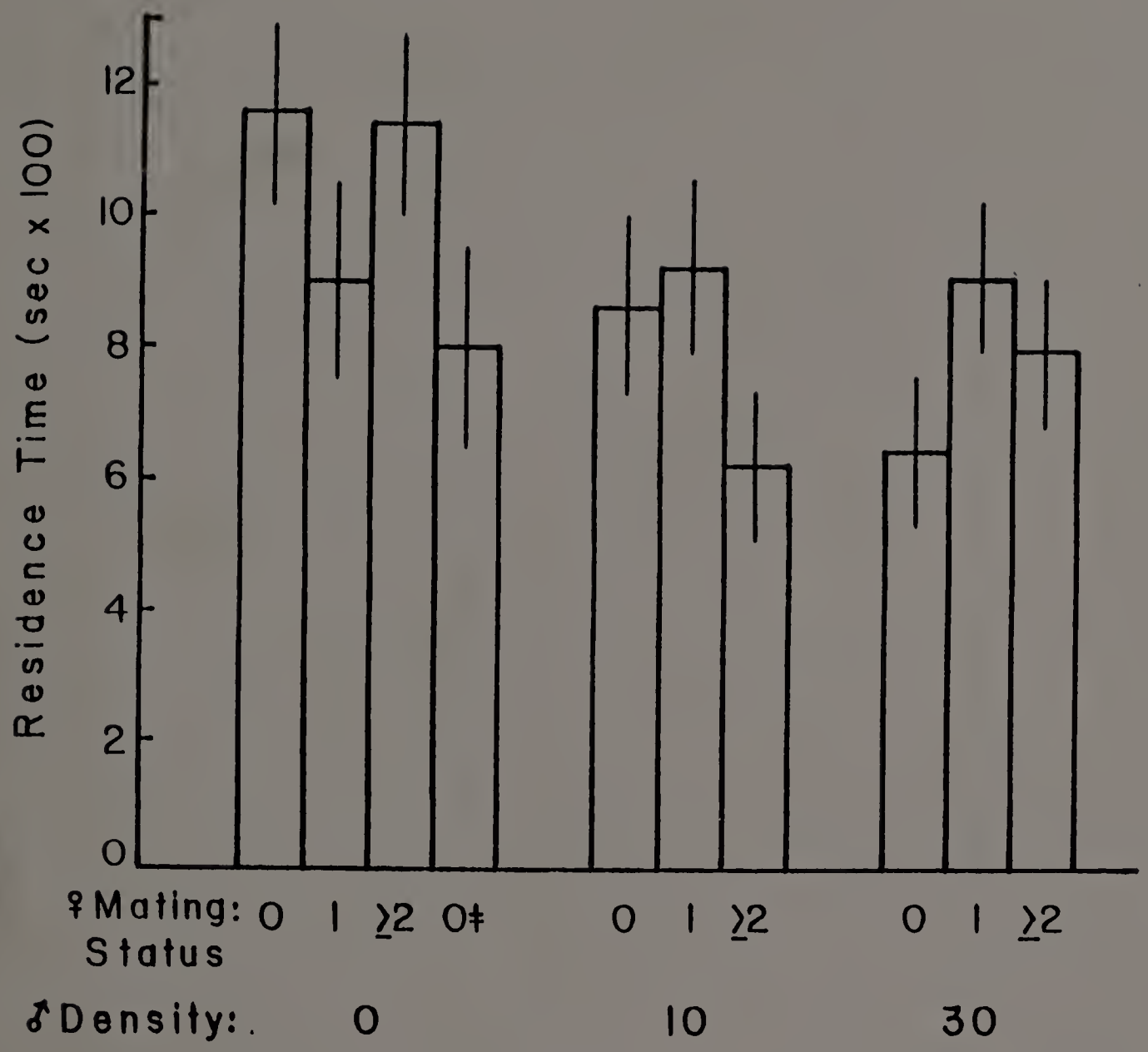


Figure 6.1
Female residence time (± s.e.) on the host tree in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated, and 0♯ = sham-mated) and male density on the tree (0 males, 10 = low density, 30 = high density).

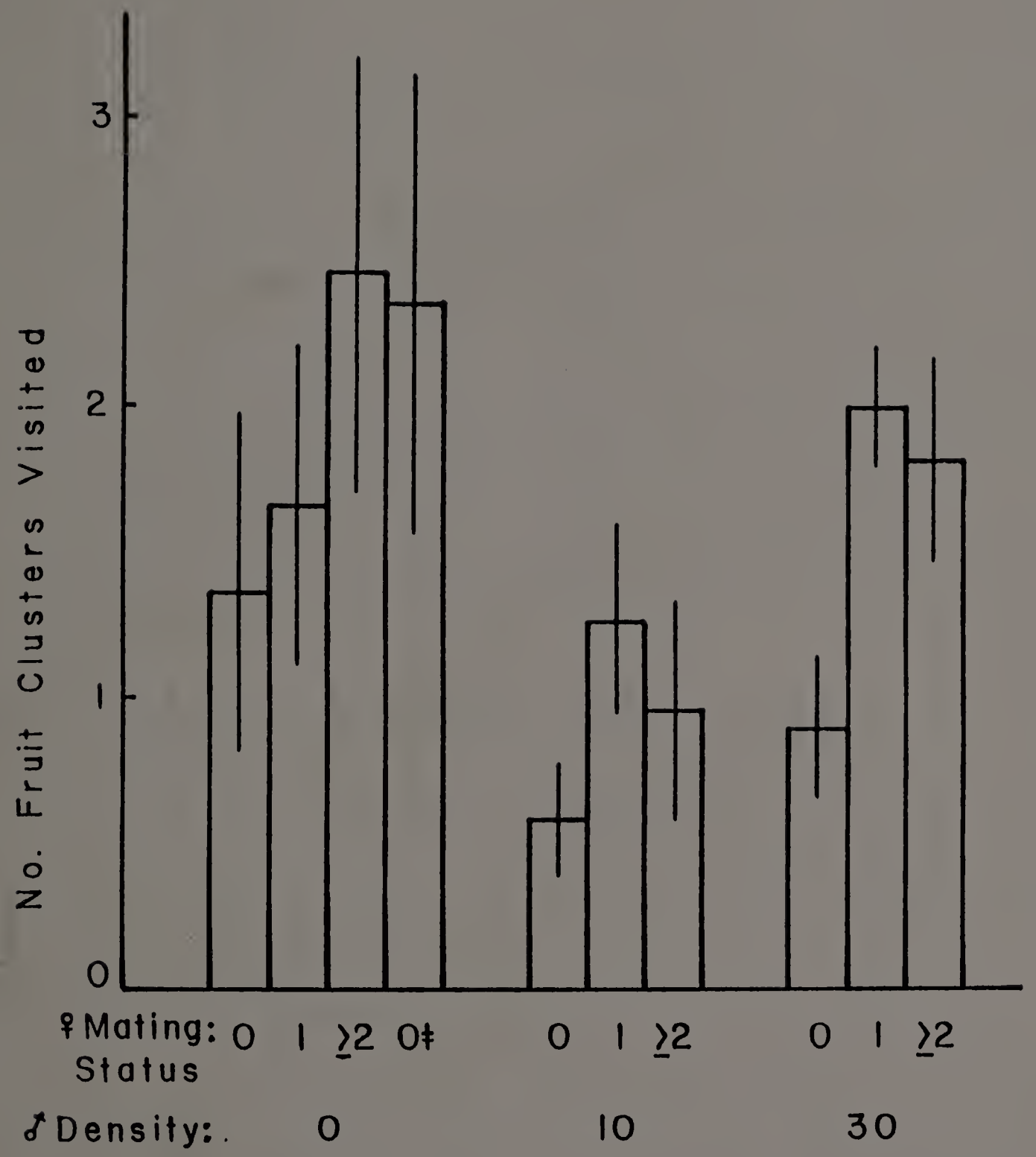


Figure 6.2
Number of fruit clusters visited per female (± s.e.) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated, 0‡ = sham-mated) and male density on the tree (0 males, 10 = low density, 30 = high density).

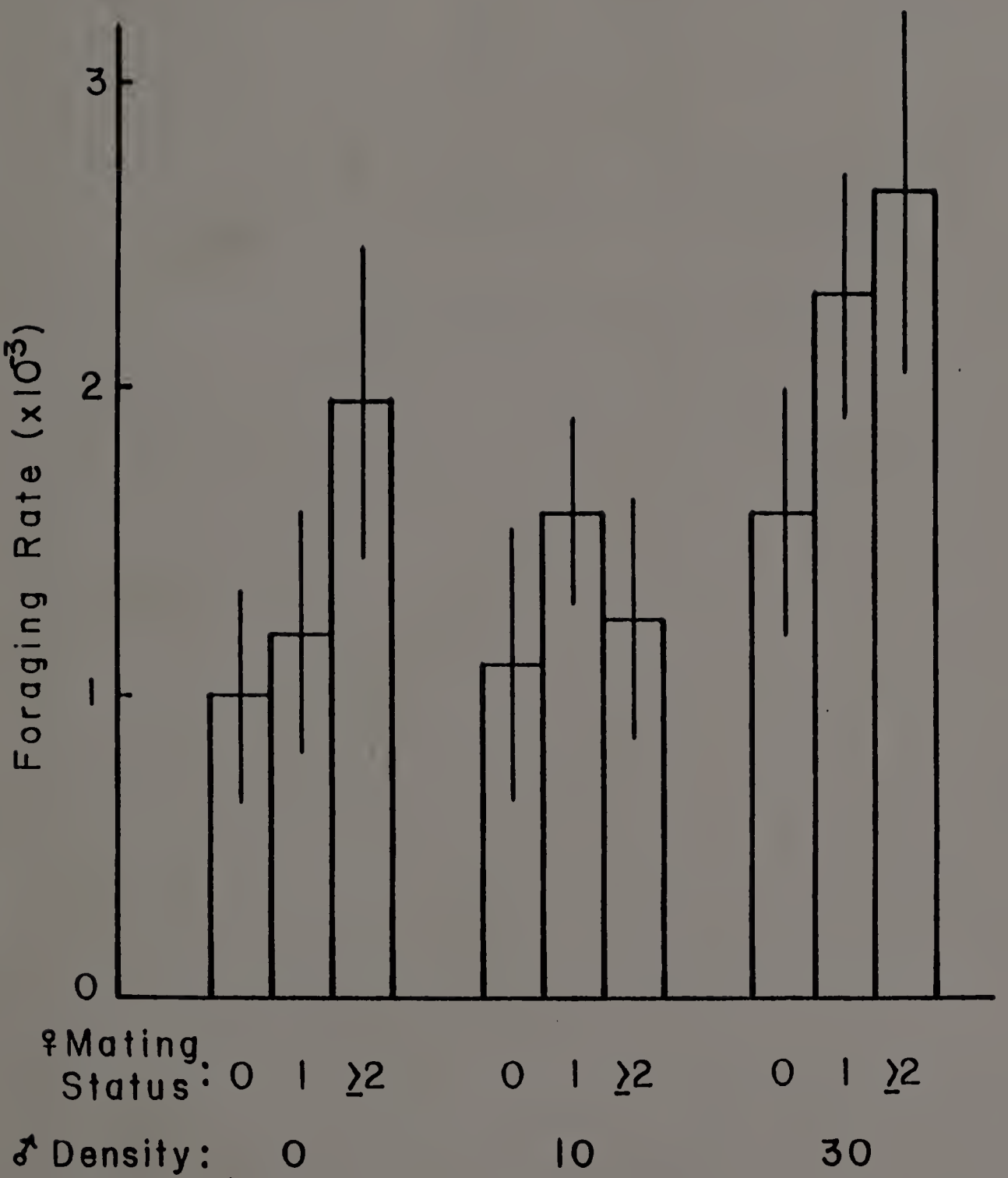


Figure 6.3
Foraging rate (number of fruit clusters visited per second of residence time) (± s.e.) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply mated) and male density on the tree (0 males, 10 = low density, 30 = high density).

Table 6.1
Two-way analysis of variance (ANOVA) of female residence time on the host tree in relation to female mating status, male density, and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 0.17 | 0.84 |
| Male density | 2 | 4.33 | 0.01 |
| Interaction | 4 | 1.81 | 0.13 |
| Error | 208 | | |

Table 6.2
Two-way analysis of variance (ANOVA) of number of fruit clusters visited per female in relation to female mating status, male density, and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 3.10 | 0.05 |
| Male density | 2 | 3.44 | 0.03 |
| Interaction | 4 | 0.61 | 0.66 |
| Error | 208 | | |

Table 6.3
Two-way analysis of variance (ANOVA) of foraging rate (number of fruit clusters visited per second of residence time) in relation to female mating status, male density, and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 1.88 | 0.15 |
| Male density | 2 | 4.06 | 0.02 |
| Interaction | 4 | 0.51 | 0.73 |
| Error | 208 | | |

Table 6.4
Two-way analysis of variance (ANOVA) of number of ovipositions per female in relation to female mating status, male density, and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 4.17 | 0.02 |
| Male density | 2 | 1.72 | 0.18 |
| Interaction | 4 | 0.61 | 0.65 |
| Error | 208 | | |

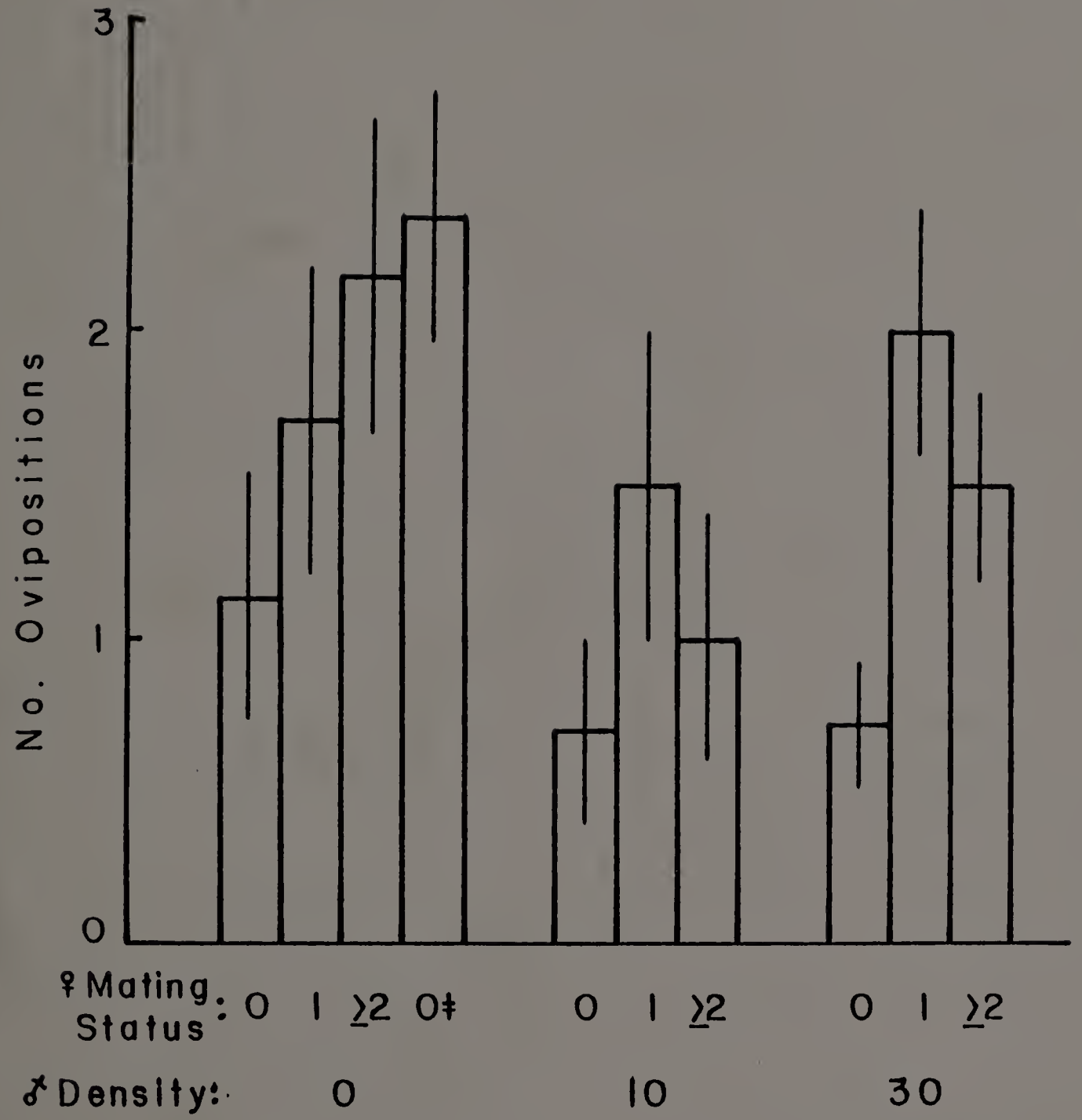


Figure 6.4
Number of ovipositions per female (± s.e.) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated, 0‡ = sham-mated) and male density on the tree (0 males, 10 = low density, 30 = high density).

Table 6.5
Two-way analysis of variance (ANOVA) of rate of oviposition (number of ovipositions per second of residence time) in relation to female mating status, male density, and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 4.24 | 0.02 |
| Male density | 2 | 1.42 | 0.24 |
| Interaction | 4 | 1.10 | 0.36 |
| Error | 208 | | |

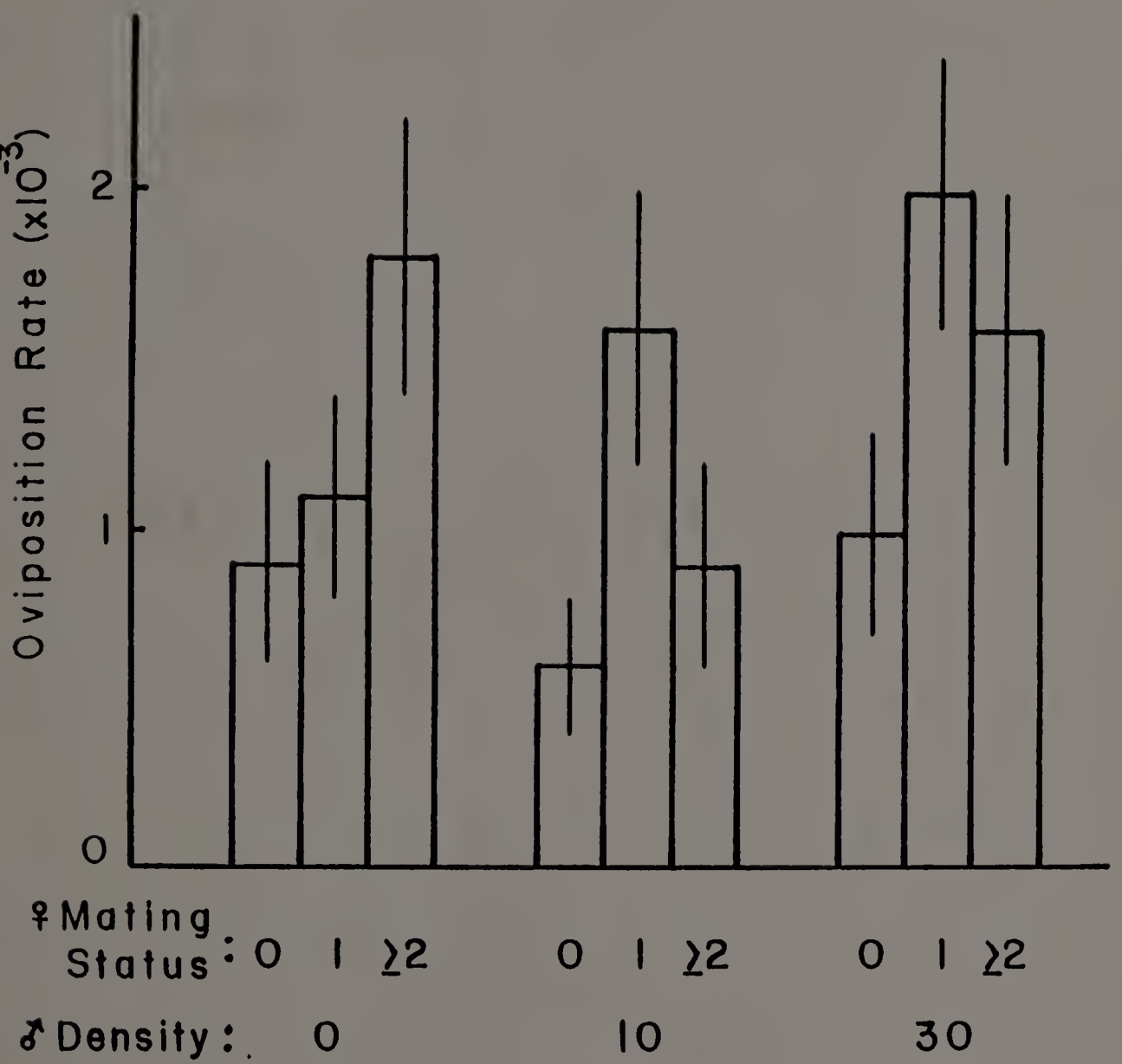


Figure 6.5
Oviposition rate (number of ovipositions per second of residence time) (± s.e.) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated) and male density on the tree (0 males, 10 = low density, 30 = high density).

Table 6.6
Two-way analysis of variance (ANOVA) of number of males encountered per female in relation to female mating status, male density (either low or high), and the interaction of female mating status and male density. Significant effects indicated by $p<0.05$.

| Source: | df | F-value | p |
|---|---|---|---|
| Female mating status | 2 | 3.83 | 0.02 |
| Male density | 1 | 10.49 | 0.001 |
| Interaction | 2 | 1.51 | 0.22 |
| Error | 143 | | |

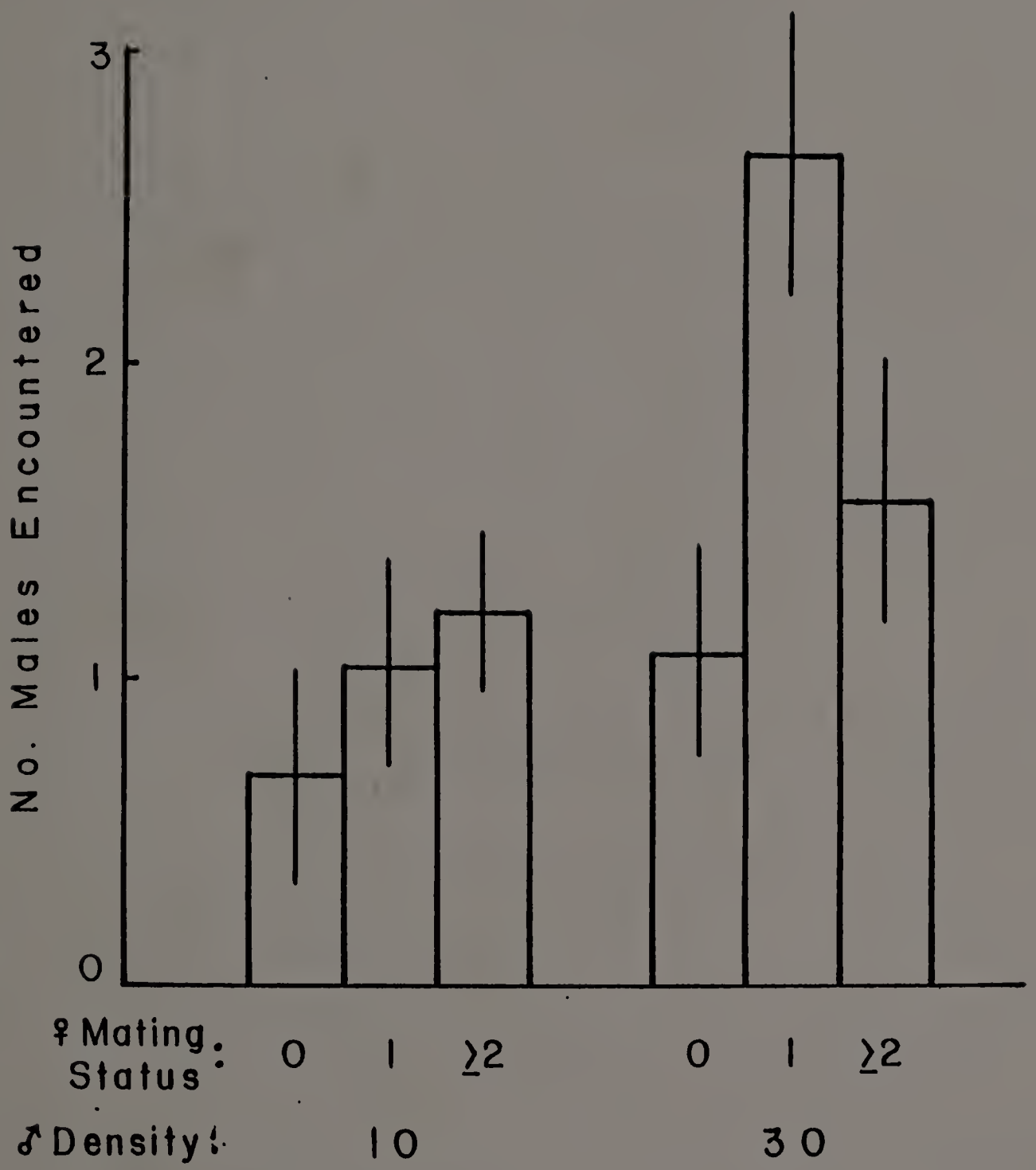


Figure 6.6
Number of encounters with males per female (± s.e.) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated) and male density (10 = low density, 30 = high density).

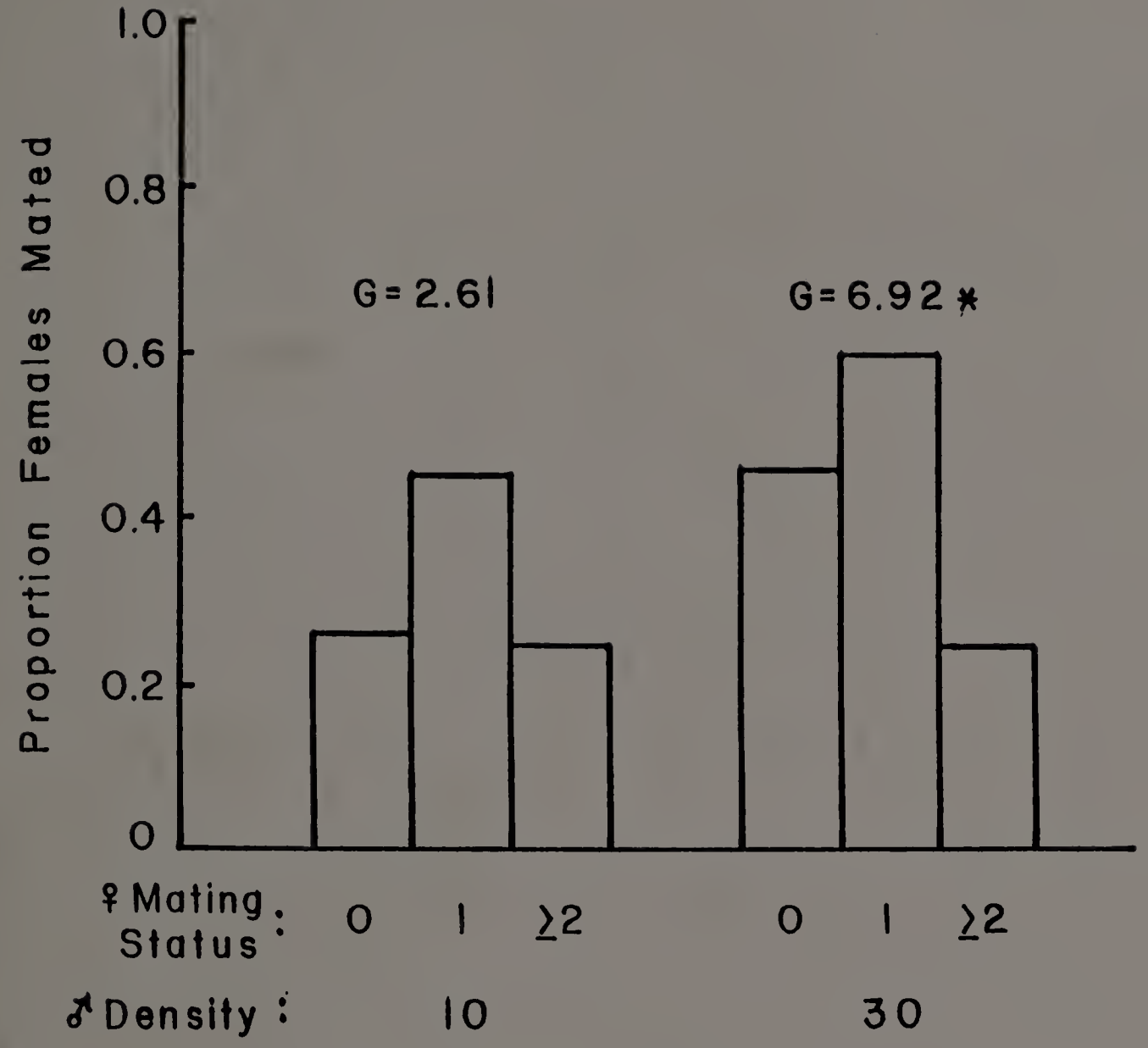


Figure 6.7
Proportion of females tested which became mated during the test period (30 min maximum) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated) and male density (10 = low density, 30 = high density). Significant G-value indicates significant effect of female mating status on likelihood of mating at a particular male density (*$p<0.05$).

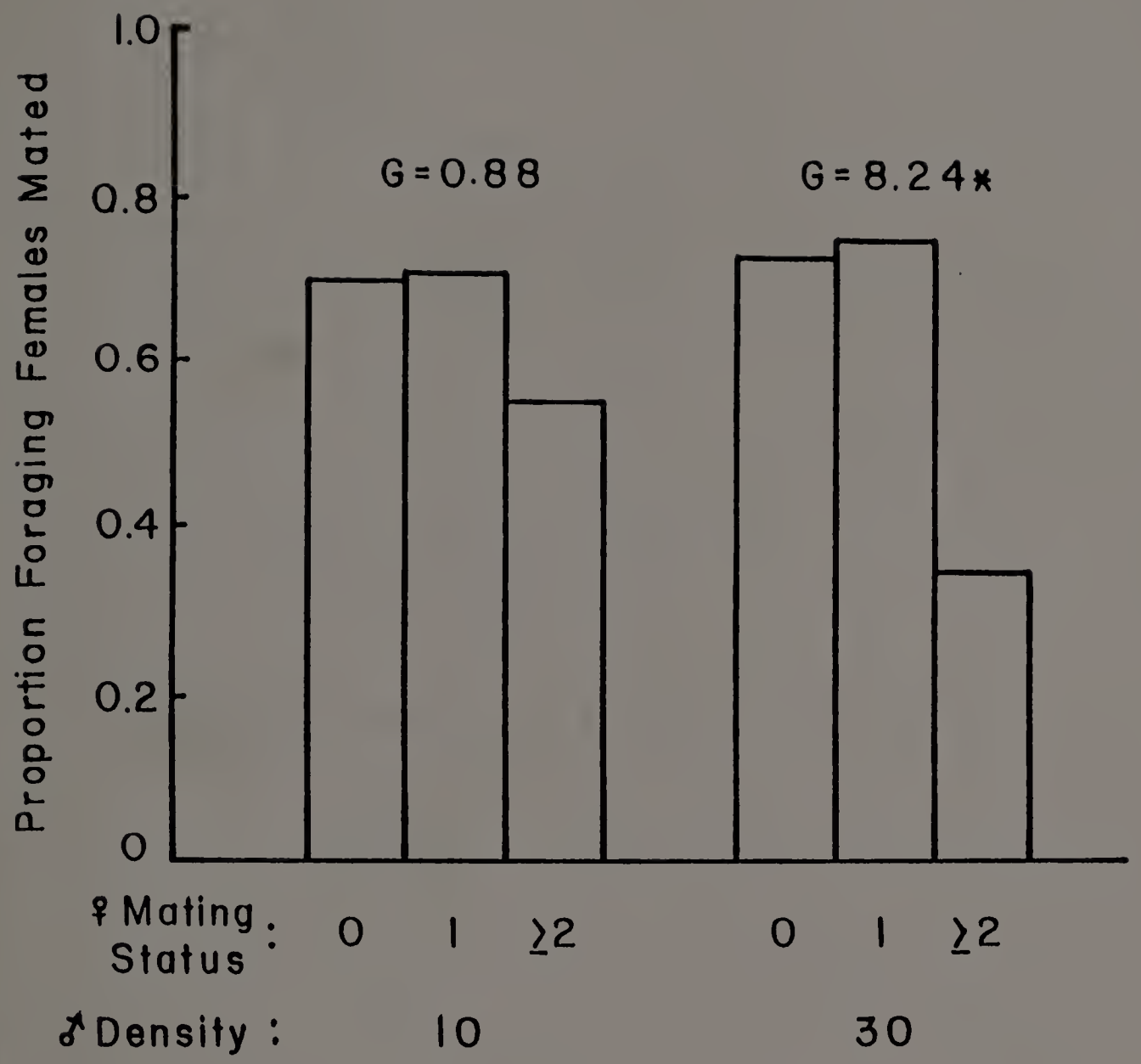


Figure 6.8
Proportion of foraging females (i.e. females finding fruit) which became mated during the test period (30 min maximum) in relation to female mating status (0 = virgin, 1 = singly-mated, ≥2 = multiply-mated) and male density (10 = low density, 30 = high density). Significant G-value indicates significant effect of female mating status on likelihood of mating at a particular male density (*$p<0.05$).

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# CHAPTER 7

# CONCLUSIONS, PRACTICAL IMPLICATIONS, AND FUTURE STUDIES

## 7.1 Introduction

The apple maggot fly, Rhagoletis pomonella, is a unique study animal because it is amenable to applied, basic, and theoretical investigations. In fact, almost any study with this fly may have importance in a number of disciplines. Such is the case with the behavioral-ecological research presented in this dissertation. Although each research project was conceived and executed as a basic, empirical study, the results and conclusions drawn point the way for future research in numerous diverse areas. This concluding chapter is divided into sections based on the 5 primary research chapters of this dissertation. In each section, I discuss major conclusions in empirical, theoretical, and applied terms, and point out some avenues for future studies.

## 7.2 Movements in Nature

In Chapter 2, we found that 25% of pre-reproductive adult R. pomonella dispersed away from the site of emergence (host apple tree) only to return when reproductively mature, 1-2 weeks later. The remaining 75% dispersed and were not seen again. Although much is known about behavior of reproductively mature R. pomonella, comparatively little is known about behavior of immature flies. It is thought that fly dispersal immediately following emergence is linked to food

foraging, and this possibility is currently under investigation (Hendrichs and Prokopy, unpub.).

When reproductively mature, male and female apple maggot flies differed in their tendency to remain on the host tree. Males were seen for more consecutive days than females. Apparently, host marking pheromone deposited by female flies following oviposition served to arrest males on fruit while it elicited female dispersal. From empirical and theoretical viewpoints, many questions remained regarding estimates of male and female mating success and other aspects of sexual selection. Many of these questions are addressed by subsequent chapters of this dissertation.

From an applied viewpoint, the implications of this Chapter 2 study of fly movement are numerous. First, production and application of marking pheromone on a commercial basis for use in apple orchards has been proposed to keep females from attacking fruit. In the case of localized infestations, however, application of marking pheromone could enlarge the area of infestation by prompting female dispersal. Second, production and release of sterile male R. pomonella for large scale pest eradication, as in some Medfly programs, appears impractical, at best. Sterile male, like wild male, R. pomonella would probably remain in localized areas on host trees following the onset of reproductive maturity. The sterile insect technique is dependent on equal movement and mixing of sterile individuals with wild individuals (Burk and Calkins, 1983), and this appears unlikely to occur in the apple maggot fly since late in the host season male movements are arrested while female movements are not. Thus, release of sterile male apple maggot flies might reduce widespread pest populations, but overflooding with

high densities of sterile males could also elicit female dispersal into new areas (see also Chapter 6).

## 7.3 Fecundity and Fertility in the Laboratory

In this study, presented in Chapter 3, we found significant positive effects of multiple matings on female lifetime fecundity and fertility in the laboratory. In addition, multiple matings appeared necessary to maintain high levels of fertility throughout a female's life. Females which had mated once had fecundity similar to virgin females and had low, variable levels of fertility. Females mated twice demonstrated fecundity similar to multiply-mated females and lower mortality rates than females of any other mating status. Thus, confinement with males, as in the case of multiply-mated females, may have increased female fertility at the expense of longevity. Females confined with emasculated males were physiologically uninseminated but behaviorally multiply-mated and demonstrated fecundity and longevity similar to multiply-mated females.

Theoretically, the implications of this study are numerous. First, although multiple matings are usually assumed to benefit males more than females (Thornhill and Alcock, 1983), significant benefits from multiple matings accrued for female apple maggot flies. Second, benefits to females were behaviorally as well as physiologically based, indicating that assumptions of male-only benefits from seemingly forced copulations may be in error in some species (Thornhill, 1980; Smith and Prokopy, 1980). Third, the fecundity and fertility effects of multiple matings observed under set laboratory circumstances may have been misleading because females were given unlimited oviposition sites and

food and were not exposed to hazards which might occur during foraging for oviposition sites in nature. Some of these problems are addressed by the foraging behavior investigation presented in Chapter 6.

Finally, in practical terms, this study further diminishes chances that the sterile insect technique could be used to control R. pomonella. Because females benefit from multiple matings, it is likely that females would mate multiply in the field, potentially diluting the effects of sterile male matings. Yet, without knowledge of sperm competition in R. pomonella, we cannot state with certainty what the outcome of multiple sterile and fertile matings might be.

## 7.4 Characterization of the Mating System

From the results of this observational study (Chapter 4), I characterized and developed a new term, dual polygamy, for the mating system of the apple maggot fly and described the criteria necessary for its inclusion in this mating system. Observations of equal male and female variance in mating success and of non-random mating patterns in each sex, together with results indicating that females benefit from multiple matings (Chapter 3), formed the basis for the characterization of dual polygamy. Although polygamy is rarely discussed in sexual selection literature, I feel it is likely a common, but frequently overlooked, type of mating system, particularly in insects.

As is often the case with observational studies which embrace new theoretical ideas, the Chapter 4 study raised more questions than it answered. For example, we do not know what factors contributed to the variance in mating success observed in both sexes. In males, a particularly fruitful avenue of future research would be to investigate

territorial behavior of males which reside on fruit to await female arrival. Poethke and Kaiser (1987) have suggested that high male density and aggressiveness combined with comparatively short female visits to mating sites could favor the evolution of male territoriality. Courtney and Anderson (1986), on the other hand, feel that male distributions which are unstable are likely due to males abandoning encounter sites, a notion inconsistent with criteria used to define true territoriality (Baker, 1983).

Another question raised by this study concerned male harassment of females attempting to oviposit in fruit. This question, along with questions raised in Chapters 2 and 3, formed the basis of the research project presented in Chapter 6 concerning female oviposition site foraging behavior.

## 7.5 Sperm Competition and Multiple Paternity

Using starch gel electrophoresis of whole insects to compare parent and offspring allozyme profiles, we found precedence of second male sperm ranging from 79-93% in the study presented in Chapter 5. Male mating status (virgin or mated twice in one day) and length of egg collection (10 or 20 days) did not significantly affect estimated paternity. A maximum likelihood statistical estimation method based on mendelian inheritance, employed when fathers did not differ unambiguously, appeared to give more conservative estimates of sperm precedence than when no estimation method was necessary due to unambiguous paternity.

Male apple maggot flies clearly may benefit from mating with non-virgin females due to strong precedence of second male sperm. Despite

the purposeful exclusion of sperm competition from some considerations of the forces governing sexual selection (eg. Sutherland, 1985), the operation of sperm competition is obviously an important aspect of animal multiple mating systems. Future studies concerning sperm competition in the apple maggot fly should focus on such factors as intervals between matings in males and females, male sperm depletion, varied copulation durations, and the effects of more than 2 matings.

Practical implications of this sperm competition study relate primarily to the sterile insect technique. A high degree of competition of the last male's sperm could offset much of the negative effect of multiple female mating if sterile male sperm were as competitive as wild male sperm at fertilizing eggs. Obviously, more research is needed in this area before the full implications can be understood.

## 7.6 Female Oviposition Site Foraging Behavior

The Chapter 6 study integrates many questions which arose from previous studies. First, we wished to know whether females of different mating status would forage for oviposition sites alone in a host tree in the manner predicted by results from the laboratory mating study of Chapter 3. As expected, multiple matings increased the tendency of a female to forage for oviposition sites, and, upon finding sites, to subsequently lay an egg. In addition, sham-mated females demonstrated similar effects as multiply-mated females, reinforcing the hypothesis of behavioral effects of multiple mating, as presented in Chapter 3.

Second, we wanted to know whether male presence would affect aspects of female foraging behavior and if some effects would be dependent on female mating status. In general, multiply-mated females

were less inclined to forage and oviposit in the presence of males than were singly-mated females. In fact, singly-mated females increased both foraging and oviposition rates while multiply-mated females increased foraging rates but decreased oviposition rates in the presence of males. I hypothesize that females of different mating status might perceive the "hazard" of encountering males differently. For example, if singly-mated females could benefit from additional matings, then encounters with males might not be perceived as hazardous. Carefully designed future experiments could test for differences in hazard perception by females of different mating status. In addition, future experiments should be designed to test for effects of resource quantity and quality. Based on studies of foraging behavior of animals faced with predation hazards (eg. Fraser and Huntingford, 1986), we might expect that varying resource quantity and quality would change a female's response to male encounters, and that the change in response would depend on female mating status. Such investigations could potentially help us to understand the manner in which natural selection and sexual selection integrate to influence the behavior and ecology of the apple maggot fly and other animals.

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