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Sexual communication in the Oriental fruit fly, *Dacus dorsalis* Hendel (Diptera : Tephritidae)

Poramarcom, Ratana, Ph.D.

University of Hawaii, 1988

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SEXUAL COMMUNICATION
IN THE ORIENTAL FRUIT FLY, *Dacus dorsalis* Hendel
(DIPTERA: TEPHRITIDAE)

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

DOCTOR OF PHILOSOPHY

IN ENTOMOLOGY

DECEMBER 1988

BY

Ratana Poramarcom

Dissertation Committee:

Kenneth Y. Kaneshiro, Chairman
Bruce E. Tabashnik
Donald O. McInnis
Wallace C. Mitchell
Christine R. Boake

ACKNOWLEDGEMENTS

There are numerous agencies and people who deserve grateful acknowledgement for their significant contributions to the success of this research. My sincere gratitude goes to:

The government of Thailand for granting me a leave from my job to pursue this long-term graduate study.

The East-West Center (EWC) for awarding me a 4-year study grant which enabled me to attend the University of Hawaii.

The California Department of Food and Agriculture (CDFA) for providing me a 20-month assistantship and necessary supplies for the research.

The EWC Environment and Policy Institute for providing me a part-time research internship and the American Association of University Women (AAUW) for granting me a Pacific Fellowship, both financial sources helped relieve a financial burden I encountered while completing the last part of my degree requirements.

Dr. Kaneshiro, my committee chairman, for providing an assistantship and supplies for this research through the CDFA grant in addition to other research facilities, for his very helpful comments and advice during the writing of my disssertation, for his brilliant insights and for his own way of prodding me to an intellectual learning procedure which I will carry with me the rest of my life.

Three members of my committee, Dr. Tabashnik, Dr. McInnis, and Dr. Mitchell for their assistance in their various fields of expertise.

Dr. Dickinson and Dr. Dominey from the "far side" of the U.S. for giving me the advice, confidence and inspiration to continue when everything seemed to be at its darkest moment.

Many friends at the Tropical Fruit and Vegetable Research Laboratory, USDA/ARS for sharing with me their insights.

Faculty, secretaries, and several friends in the Department of Entomology especially Dr. Johnson, Aubrey Moore, and Tim Whittier for all sorts of help, answering questions and giving advice when I needed it. Aubrey Moore for providing a computer program of randomization numbers used in some parts of this research.

Dr. Boake for sharing with me her insights, for her extremely useful guidance, professionally and personally, throughout the preparation of my dissertation, for her endless contributions and efforts in guiding, assisting, and inspiring me in both research and career advancement, for being my friend, teacher, confidence, and mentor, and most of all for being there when I needed her most.

My "adopted" father, Alfonso Del Rosario and my understanding friend, Mark for their unselfish and ceaseless assistance which contributed to my survival in Hawaii.

My family in Thailand for being a continual source of moral support and inspiration.

To these agencies and people this dissertation is dedicated.

ABSTRACT

Results of the research on sexual communication in the Oriental fruit fly, Dacus dorsalis were reported in three chapters: 1) description and details of its sexual behavior, 2) determination of the significance of sexual signals for attracting members of the opposite sex and in successful mating, and 3) identification of the behavioral characteristics which make males attractive and successful in mating with females.

Three major components in the sexual behavior included: 1) signal emission through male wing-fanning followed by the arrival of females; 2) courtship behavior involving male wing-fanning followed by attempted copulations; and 3) copulation. Further tests also showed that females that had mated once neither were attracted to males nor accepted subsequent matings as readily as virgin females.

By modifying either the male's ability in emitting sexual signals, i.e. modification of wing fanning, or the various sensory receptors in the females, i.e. the eyes for visual signals, the aristae for acoustic stimuli, and the antennae for olfactory cues, it was possible to investigate the separate modalities of sexual communication during the 1) attraction phase (i.e. the bringing together of the two sexes over a distance of about 50 cm), and the 2) courtship phase (i.e. signal emission at close range of about 6 cm).

The wing fanning in males was found to play a significant role in the production of signals for the attraction of and mating with females. Experiments conducted in the females showed that acoustic as well as olfactory signals were crucial in attracting females to wing-fanning males, but only olfactory stimuli were important for mating acceptance of the females.

Differential sexual success among males and female choice played an important role in the complex mating system in this species. Sexual success in males was measured in terms of ability to attract and mate with females. For the most part, both qualities could be found in the same male. It was also found that sexually successful males had the capability of signaling for a longer duration, and had the top rank in male-male aggressive interactions more frequently than other males. Thus, intermale competition could possibly be another component of sexual selection operating in this species.

The results of this study provide baseline information that will be important for the development of more effective control and/or eradication methods for _D_. _dorsalis_ as well as other related species.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

THE ORGANISM

The Oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), is distributed throughout India, Southeast Asia, Micronesia, and the Hawaiian Islands (Hardy 1973). It was first found in Hawaii in 1945 (van Zwaluwenburg 1947 and others) and soon became established on the major islands. Prior to its establishment, *Ceratitis capitata* (the Mediterranean fruit fly) was widespread throughout the islands. Later, competitive displacement of *C. capitata* by *D. dorsalis* occurred in many areas. At present, *D. dorsalis* occupies the coastal belt areas of the islands formerly occupied by *C. capitata*, while the latter species inhabits areas at higher elevations (Nishida et al. 1980).

D. dorsalis is one of the most destructive crop pests; it attacks more than 120 species of fleshy fruit plants. Major host fruits that are often heavily infested include guava, *Psidium guajava* L.; strawberry guava, *Psidium cattleianum* Sabine; papaya, *Carica papaya* L.; peach, *Prunus persica* (L.) Barsch.; banana, *Musa* spp.; mango, *Mangifera indica* L.; avocado, *Persea americana* Mill.; coffee, *Coffea arabica* L.; ball kamani, *Calophyllum inophyllum* L.; false kamani, *Terminalia catappa* L.; rose apple, *Eugenia jambos* L.; and Surinam cherry, *Eugenia uniflora* L.(Bess and

Haramoto 1961). About 95% of D. dorsalis population on the island of Oahu in Hawaii develops in guava fruits and its population cycle is determined primarily by the fruiting season of guava (Newell and Haramoto 1968). In a few cases, infestation of mangoes or papayas by this species is severe enough to significantly increase localized populations of flies.

D. dorsalis in Hawaii causes three types of economic losses (Bess and Haramoto 1961). First, ovipositional punctures or "stings" may cause various types of surface defects of the fruits including discoloration or formation of abnormal growth around the puncture. Punctures may also provide entrance points for decay organisms resulting in fermentation and decomposition of the fruit. In some species of fruits such as passion fruit, Passiflora edulis f. flavicarpa Degener, stings may cause young fruits to become malformed and prematurely drop off the vine. D. dorsalis will also sting fruits that are not favorable for larval development and survival. Second, the entire fruit can be lost by maggots feeding on and tunneling in its fleshy tissue. Third, an indirect loss comes from the establishment and enforcement of quarantine regulations to prevent the entry of D. dorsalis into uninfested areas of the mainland U.S. These quarantine restrictions obstruct the expansion and development of various Hawaiian fruit crops for export. In the past, post-harvest treatment of fruits for export relied largely on fumigation. However, the only effective chemical fumigant, ethylene dibromide, has been recently banned because of its carcinogenic effect. Other treatments such as heat, cold, or gamma radiation are either unacceptable to consumers or ineffective. Without an effective and acceptable post-harvest treatment, prevention of initial fruit infestations is extremely important and seems to be the best strategy. Recently, the sexual behavior of pest species has been recognized as an important element in developing more effective control effort.

SEXUAL COMMUNICATION

The most general definition of "communication" includes any stimulus produced by one animal that elicits a response in another. From an ethologist's point of view, communicative behavior allows the exchange of information between interacting individuals as they respond to each other (Smith 1977).

These behaviors of communication have evolved in a similar manner as morphological characters (Lorenz 1968, Tinbergen 1951). Primarily, there are two different mechanisms through which animal communication systems may have been shaped. Some animal signals have been derived from an adaptation to the physical environment (Bennet-Clark 1970, Lall et al. 1980, Paul and Walker 1979). For example, the species of fireflies that locate males during twilight produce a yellow flash, while those that call after dark have a green flash. It is thought to be to the advantage of the species to produce a certain flash color to avoid being masked by the environment (Lall et al. 1980). Other animal signals are thought to be derived from social interactions between signalers and receivers (Dawkins and Krebs 1978). For example, the existence in animals of many unusual actions that have functions in achieving matings are thought to be favored by sexual selection.

Darwin's theory of sexual selection (Darwin 1871) describes mechanisms through which differential mating success among males has resulted in sexual dimorphism. As a result, various male traits such as elaborate or bizarre secondary sexual characteristics and sophisticated mating rituals have evolved. The evolution of dimorphism may be the result of one or both of the two primary components of sexual selection, intrasexual selection and intersexual selection. Intersexual selection is the process in which individuals of one sex, females in most cases, exhibit mating preferences for individuals of the other sex possessing particular traits. Intrasexual selection occurs through the competition among members of one sex, usually males, for access to individuals of the other sex.

The mode of communication is conveniently classified by the sense organs. Among insects, major modes of communication include chemical (both airborne substances and chemicals deposited on substrates), auditory, visual, and tactile signals. In most cases, a combination of two or more of these senses contributes to the communication system of a single species. However, many species emphasize one mode of communication more strongly than others. Each mode carries with it some advantages and disadvantages (Alcock 1979). In active diurnal species in open habitats, visual signals provide easy communication between moving individuals. On the other hand, pheromones provide persistent signals over a greater distance than visual signals. Such olfactory signals are important in nocturnal insects whose visual cues may be limited. Among these various modes, auditory signals usually provide detailed communication over a long distance, and are not constrained by wind conditions like pheromones.

Within Diptera, the drosophilids and tephritids are the best known in terms of their sexual communication (Burk 1981). The complexity of signaling in these flies appears to have evolved because individuals compete in producing advertisement signals in order to gain matings. Among various modes of communication signals used in tephritid species, visual signals are suggested to be important in only a few cases. For example, particular movements such as spinning or circling of their shiny gold body, and their clear wings with distinctive brown patterns in the Caribbean fruit fly, Anastrepha suspensa, are described as visual signals used during courtship (Dodson 1978). For very close-range communication, tactile signals produced through licking of the female's genitalia, or probing with the legs during courtship are important in gall-formers of the genus

Valentibulla, Tephritidae (Wangberg 1978). Olfactory and acoustic signals, however, are two major modes of potentially long-range signals that play significant roles in sexual communication of most tephritids and therefore deserve special emphasis.

Olfactory signals, one form of chemical communication, are important in several groups of flies including fruit flies (reviewed by Chambers 1977, and Fletcher 1977). They include both long-distance and contact or short-range pheromones. In some species such as the olive fruit fly, *Dacus oleae*, the female produces a pheromone that attracts the male (Baker et al. 1980, Mazomenos et al. 1985). In other species such as *Dacus tryoni* (the Queensland fruit fly), *C.capitata*, and *A. suspensa* ,the male attracts the female (Chambers 1977, Nation 1972, Pritchard 1967). In these cases, long-distance pheromones which are produced and dispersed by males attract both receptive females and also other males. Among species in which the males release pheromones, long-range pheromones consist of different chemical compounds from those of short-range pheromones. These species include *D. tryoni* (Fletcher 1977), and *Rioxa pornia* (the island fruit fly) (Pritchard 1967).

Several species of Tephritidae produce complicated, stereotyped auditory signals as part of their sexual communication. Males of *A. suspensa* produce characteristic acoustic signals: calling, aggressive and pre-copulatory songs, each with a distinct pulse pattern during rapid wing vibrations (Webb et al. 1976). The calling song increases female activity (Burk 1981) and, with the presence of pheromone, attracts females (Webb 1973). The precopulatory song causes a prolonged copulation and may increase a female's sexual motivation to mate (Burk 1981). Some other tephritid species such as *D. tryoni*, *D. oleae*, and *C. capitata* also produce species-specific sound signals (Monro 1953; Rolli 1976).

MATING BEHAVIOR OF *Dacus dorsalis*

Although various channels of sexual communication have been studied in detail in several species of tephritid flies, there have been only a few relevant reports published for *D. dorsalis* (Kobayashi et al. 1978, Roan et al. 1954). *D. dorsalis* mates only at dusk under low light intensity; i.e., less than 1,000 lux (Arakaki et al. 1984). Decreasing light intensity at twilight is also reported as a stimulus for the initiation of sexual activity. As twilight approaches, sexually active *D. dorsalis* males produce a characteristic high-pitched buzzing sound followed by copulation which lasts for 2 to 12 hrs. (Roan et al. 1954).

It is not clear whether a particular type of sound produced during the wing vibration of males results from stridulation or some other mechanisms. Keiser et al. (1973) noted that the wings of *D. dorsalis* males have deeper and more sharply incised wing margins at the distal tip of Cu1+1A (anterior cubitus + first anal vein, according to the

Comstock and Needham system referred by Borror et al. (1981)) than the females. Two rows of large cilia are found along the abdominal tergites of males only (Hardy 1955). These sexually dimorphic structures were hypothesized to be the stridulatory organs. However, Keiser et al. (1973) failed in their attempt to demonstrate that stridulation exists in D. dorsalis. They failed to see the actual brushing of the wings against the abdominal cilia. Furthermore, they have suggested that male wings and sound are not necessary for successful copulation because wingless males mate with females effectively. Therefore, the role of sound or any supposed stridulatory activity in the mating behavior D. dorsalis has not been determined. However, sound believed to be produced along with the distribution of male pheromones may still be significant in the sexual communication of this species as will be shown in this study.

The presence of male pheromones in D. dorsalis has been suggested in some studies. Live males and the male rectal gland complex were found to be highly attractive to virgin females (Kobayashi et al. 1978, Schultz and Boush 1971). This gland complex was suspected to be the source of an olfactory pheromone. In another study, a substance visible as "smoke" emitted by males at dusk was collected and analysed (Ohinata et al. 1982). It consisted of large amounts of trisodium and potassium phosphates which may act as carriers for the volatile components of the pheromone.

The major chemical compound of the smoke was found to be an amide which is slightly attractive to females in laboratory tests.

Based on available published information related to the mating behavior of D. dorsalis, it appears that its sexual behavior may involve sound as well as a release of pheromone, which are associated with the wing fanning of males. However, the roles of both signal modalities in the two stages of sexual communication, attracting a female or eliciting copulation, are still not clear. It should also be noted that most of the studies done so far have emphasized close-range sexual activities including courtship and mating. To date, the most detailed study of its courtship behavior is largely descriptive (Arakaki et al. 1984). There are three points in that report which should be stressed. First, vibration of male wings exhibited under a low light intensity (a few hundred lux) is accompanied by a rhythmic stroking of the abdomen with the rear legs. Second, the male remains stationary while he is fanning his wings, and the females approach him. Third, physical contact between males and females occurs in wing-fanning males more often than in non-wing fanning males.

SIGNIFICANCE OF STUDY

Due to public awareness of the excessive use of chemical insecticides, entomologists concerned with insect pest control are turning more and more interest toward integrated pest management based on solid biological and ecological knowledge (Clark et al. 1967; Rabb and Guthrie 1970). The goal is to reduce the pest population to a low level so that it seldom reaches an economic threshold level. The economic threshold is the density at which control measures should be applied to prevent an increasing pest population from reaching the density that will cause economic damage (Stern et al 1959). However, for the fruit fly problem, the economic threshold is often near zero due to the acceptance of only perfect fruits by consumers and by those concerned about importing fruits into uninfested areas. As a result, research entomologists currently place great emphasis on the use of the Sterile Insect Release Method (SIRM) for eradication. The SIRM involves the release of laboratory-reared insects which have been sterilized with gamma radiation and allowed to compete for mates with pest males. Eradication can be achieved at a relatively low cost compared to insecticide treatments on a long term basis without the ecological problems associated with insecticide use. However, the effectiveness of the SIRM relies to a great extent on the ability of the laboratory-reared males to mate successfully with wild females. Thus, proper mating behavior and efficient function of sterilized, mass-reared males in the field are of major concern.

Unfortunately, two important aspects of behavioral quality, (a) searching ability for mating sites or sexual partners and (b) mating behavior, are affected in mass-reared insects (Boller 1972). Examples include the alteration of female responsiveness to male pheromone in screw-worm flies, Cochliomyia hominivorax (Fletcher et al. 1968),the production of a less attractive sex pheromone in boll weevils, Anthonomus grandis males (Gast 1968), and the unsynchronized mating period of laboratory and wild populations in codling moths, Carpocapsa pomonella (Boller 1972). Among tephritid species, specific examples of laboratory effects are reported in two species. Laboratory males of C. capitata have a lower insemination rate than recently established laboratory males (Rossler 1975). Moreover, both sexes of the laboratory strain reach sexual maturity much earlier than the wild strain (Wong and Nakahara 1978). Another example occurs in D. dorsalis in which sexual isolation existed between the laboratory and wild strains, as the members of each strain preferred to mate with individuals of their own strain (Wong et al. 1982).

In addition to effects derived from laboratory rearing, sterilization with gamma radiation also poses some problems related to mating. Sterilization of A. suspensa pupae decreases the frequency of the signaling behavior of males from 95% to 3% (Sharp and Webb 1977). In C. capitata, the ability of sterilized males to compete successfully with non-sterilized males in mating is reduced by about 50%, and the peak of mating activity of both sexes is delayed as a result of sterilization causing unsynchronized sexual activity (Holbrook and Fujimoto 1970). Therefore,it is not surprising that the deviation in sexual behavior of sterilized, laboratory-reared flies from that of wild ones has been suggested as a primary cause for the failure of several SIRM programs (Ito and Kawamoto 1979).

Successful eradication of D. dorsalis using an effective SIRM program requires a thorough knowledge of its complex sexual behavior in order to develop a laboratory strain which can either mate in the field as readily as, or prevent normal matings of wild males. In addition, basic research on the sexual behavior of pest species has recently been recognized as an important component of insect control. At the present time, however, sexual communication of this species remains poorly understood (Arakaki et al. 1984, Keiser et al. 1973, Kobayashi et al. 1978, Roan et al. 1954). Therefore, detailed experimental data regarding the sexual behavior of D. dorsalis will certainly be of value in the effort to control this pest.

RESEARCH GOALS

At dusk, when mating in this species takes place, I have observed the sexual behavior of both sexes. The predominant male behavior is wing fanning, and females, which are passive most of the time, appear to be attracted to these wing-fanning males. However, no study has actually examined the significance of acoustic, olfactory or visual signals produced by the wing fanning of males and how these signals are used in sexual communication. I have therefore designed my research to examine the sexual behavior of _D. dorsalis_ in two main phases:

1. **Attraction phase** which brings males and females from a distance (average 50 cm) to the same place and at the same time for mating, and

2. **Courtship behavior at close-range** (average 6 cm) which is displayed by the male and used by the female to either accept or reject the courting individual.

The overall objective of my research is to understand the significance and methods by which sexual communication is performed by both sexes in both phases of mating behavior. This work will generate new information likely to be useful in developing effective control strategies. For example, if sound and pheromones are as important in attracting and/or mating with females, a control method utilizing these data can possibly be developed. Synthetic sources of odor and sound may be potentially used to confuse wild females which are attempting to locate males, and as a result matings will be prevented.

In a control program using SIRM, knowledge of the normal production of sexual signals may be particularly useful as a baseline for monitoring the quality of mass-reared insects. The results of this research will identify important behavioral traits that should be examined when screening mass-reared, sterilized flies before release. The behavioral bioassays that have been developed in my research may also be used in screening for flies that are capable of producing proper signals. Knowledge obtained from my research about sexual communication of _D_. _dorsalis_ will have potential for both direct and indirect application to developing effective control programs for this pest species as well as other related species. In a broader sense, information regarding sexual communication of _D_. _dorsalis_ will be of value to basic science, especially in the area of animal communication.

CHAPTER II

GENERAL MATERIALS AND METHODS

METHODS OF OBTAINING FLIES FOR EXPERIMENTS

1. Rearing Methods

All flies used in this study were originally obtained as larvae from infested papaya, collected in April, 1985 from the Puna District on the Island of Hawaii. The colony was continually maintained on papaya at 20-26^{0}C, 65$\pm$20 %R.H., and under semi-natural lighting conditions within the laboratory at the University of Hawaii, Honolulu, Hawaii.

Adult flies were separated by sex within 2 days after eclosion and held in groups in 1-gallon plastic holding containers with white organdy mesh covering on the top. Water and food containing a mixture of sugar and/or honey and protein hydrolysate in a proportion of 2:1 were provided inside and on top of the cages. Flies were kept separately by sex until testing (approximately 3-4 weeks). Stock flies of both sexes were maintained in large screen cages of 60 x 120 x 60 cm in a low density of about 120 flies per cage to allow sexual selection to operate within the artificial environment. If flies were held in a more crowded condition, sexual selection might have been even more relaxed. Certain traits necessary for the males to be successful in mating such as ability to attract females may no longer be necessary and may be lost from the laboratory colony. As a result, the behavior of the laboratory flies reared in a more crowded condition may be significantly different from the wild ones.

All flies were held in cages placed in a room next to windows exposing them to natural light cycles. Between 0730 and 1530 hours, additional overhead fluorescent lamps were provided. Therefore, in the period prior to and during dusk, when _D_. _dorsalis_ is sexually active, flies were exposed totally to natural light passing through the windows. Environmental conditions for all experiments were similar to those described for stock rearing.

One of my concerns in rearing this species for the various mating experiments was the difference in colored markings on the scutellum and thorax of the flies. Markings on these structures in most wild _D_. _dorsalis_ flies are yellow, but those of another morph are white which appears to make up about 2-4% of the natural population in Hawaii (Hart and Steiner, 1972). My original field collected population consisted of about 3.5% of the white morph. Flies reared from this population were therefore monitored throughout the course of study for any change in the proportion of these two morphs as compared to that which exists in the natural population. Flies used in the study were those of the third to eighth generation removed from the wild. No change in the proportion of the white morph was detected from generation to generation under the laboratory conditions (Table 1). Therefore, the population of flies used in this research was similar, at least in the proportion of the two morphs, to the wild one.

2. Marking Technique

For most of the experiments conducted in this research, flies were individually color-coded, for identification purposes, on the dorsal surface of the thorax with dots of non-toxic, colored enamel paint using a toothpick as an applicator. Flies were marked after immobilization by chilling in a freezer for a few minutes. Various color combinations of small dot-markings allowed marking experimental flies individually.

PRESENTATION OF THE RESEARCH

This research consists of three independent studies. Each of them is by itself a complete research paper focussing on a particular aspect of sexual communication in *D. dorsalis*. In **CHAPTER III: SEXUAL BEHAVIOR IN *Dacus dorsalis***, the sexual behavior of the species is described, and the relationship of sexual activities which include male wing-fanning, and female responses to matings with respect to age of the individuals performing the behaviors is presented. In addition, the question whether mated females would still respond to signaling males and accept subsequent matings is examined. In **CHAPTER IV: SIGNIFICANCE OF MALE**

Table 1. Percentages of white morph appeared in generations of the laboratory colony of Dacus dorsalis used in this study

Generation	n	% White morph		
		Males	Females	Total
F1	129	2.1	1.6	3.7
F2	165	1.15	1.04	2.19
F3	250	0.78	0.97	1.75
F4	308	3.2	1.3	4.5
F5	2876	0.17	0.18	0.35
F6	3982	1.05	0.88	1.93
F7	3662	0.52	0.46	0.98
F8	2579	1.24	1.59	2.83

WING-FANNING FOR ATTRACTION OF AND MATING WITH FEMALES OF *Dacus dorsalis*, there are two series of experiments. In the first series, the attraction of females to male wing-fanning at a range of approximately 50 cm is demonstrated, and then the various channels of sexual signals that the species may use to attract females are examined. In the second series, the types of signals that the species may use in sexual display at a close range, approximately 6 cm are studied. In **CHAPTER V: CONSTITUTION OF A SEXUALLY SUCCESSFUL MALE IN *Dacus dorsalis***, the behavioral components of successfully mating males are examined which include their ability to attract females, the amount of time they spend producing signals and their ability to win fights with other males. An overall discussion and summary of the research based on the information gained from the entire series of studies is then provided in **CHAPTER VI: SUMMARY AND CONCLUSIONS**.

CHAPTER III

SEXUAL BEHAVIOR OF *Dacus dorsalis*

Patterns or components of sexual behavior of species in the subfamily Dacinae of the Tephritidae have primarily been studied in the genus *Dacus* including the Queensland fruit fly, *Dacus tryoni*, melon fly, *Dacus cucurbitae* and oriental fruit fly, *Dacus dorsalis* (Arakaki et al. 1984, Fletcher 1968, Kobayashi et al. 1978, Kuba and Koyama 1982, Monro 1953, Myers 1952, Roan et al. 1954, Suzuki and Koyama 1980, 1981, Tychsen 1977). The recent publications on detailed descriptions of courtship patterns of these species (Arakaki et al. 1984, Suzuki and Koyama 1980, Tychsen 1977) may be an indication of the increasing interest and significance being placed on their courtship behavior.

Through the works mentioned above, it appears that these three *Dacus* species share certain patterns of sexual behavior. First of all, sexual activities are restricted to the dusk period, during which mature males exhibit courtship behavior. Secondly, males "call" by rapidly vibrating their wings. The calls produce a high-pitch buzzing sound and a so called "stridulation". Possibly, sex pheromones are also released from the males' rectal pheromone glands during calling (Giannakakis 1976, Kobayashi et al. 1978, Kuba and Koyama 1985, Sugimoto 1979). Males of *D. cucurbitae*, and *D. dorsalis* frequently wipe or stroke the posterior region of their abdomen with their rear legs (Arakaki et al. 1984, Kuba and Koyama 1982).

During wing vibration of a D. dorsalis male, the male remains stationary and the receptive female approaches him (Arakaki et al. 1984). These particular sexual activities, male wing-fanning and female response, seem to be the important preliminaries to copulation, and take place only after sexual maturity has been reached.

One of the major factors that may potentially complicate the use of SIRM is the probability that females may mate more than once with males that have differential sperm viability. However, females of some species such as Dacus oleae become unreceptive after the first mating (Tzanakakis et al. 1968). A suggested mechanism causing an unreceptivity is that a male substance is transferred through the semen to the female during copulation. About 40% of Ceratitis capitata females do not remate at all, and those receiving sufficient sperm do not remate for several weeks (Nakagawa et al. 1971).

Even though some species of Tephritidae become unreceptive after mating, several regain receptivity. For example, some females of D. tryoni will mate again several weeks after the first mating (Tychsen 1972). Remating in D. oleae varies depending on various factors such as the origin of the flies, their age, previous sexual activity, diet, and environmental conditions (Economopoulos 1972, Tzanakakis et al. 1968, Zouros and Krimbas 1970). For example, wild females may mate up to six or more times whereas laboratory-reared females may mate only four or five times during their lives. In laboratory adapted C. capitata, about 45% of mated females remate once or twice, and 15% remate more than twice over a seven-week period (Nakagawa et al. 1971). Such receptivity to remating is related to low numbers of sperm transfered (Cunningham et al. 1971). However, receptivity may return before the sperm supply is expended as in Drosophila, family Drosophilidae (Dobzhansky and Pavlovsky 1967). In some groups, females remain receptive throughout their lives after mating, such as in Scatophaga, family Scatophagidae (Parker 1970).

The objectives of this study were:

1. To describe the pattern of sexual behavior of both males and females.

2. To determine the relationship between the onset of particular sexual activities including the age of males when they first exhibit wing fanning, the age at which females first respond to sexual signals, and mating.

3. To determine whether mated females would still be sexually responsive to males, and whether they would be likely to accept subsequent matings during the period of 1-18 days after the first mating.

MATERIALS AND METHODS

Study 1. Description of Sexual Behavior

Three pairs of sexually mature virgin males and females were introduced into a 30 x 30 x 60 cm glass cage during 0930 to 1030 h to acclimate them until the late afternoon experiment. Observations of sexual behavior were made at 20-26^{0}C, 65±20 %R.H., and under natural lighting conditions from 1730 to 1930 h. Detailed activities including movements and interactions performed by both sexes were recorded. Observations ended when all activities ceased at about 35 min after sunset.

Study 2. Sexual Activities with Respect to Age

In this study, the chronological age at the first exhibition of some specific sexual activities was recorded in both sexes from day 1 to 28. These activities including male wing-fanning, female response (visiting wing-fanning males), and matings, were examined separately in addition to the relationships between them.

a. Male wing-fanning

Fifty individually marked males were held together in a glass cage from the first day after eclosion. Each day, males observed to fan their wings were removed from the cage during the observation period (1730 to 1930 h), and replaced with new virgin unmarked males of the same age. This way a constant density of flies in the cage was maintained to minimize any possible influence of density dependent factors. The total number of marked, original males of each age engaging in wing-fanning behavior was recorded. The experiment was continued until about 90% of the 50 males had been seen to fan their wings.

b. Female responsiveness

Fifty individually marked, 1 day old females were released into a 60 x 120 x 60 cm screened cage containing a guava plant. Sexually mature virgin males were confined in several mini-screened cages hung on the plant. These mini cages (3-4 cm in diameter and 5-6 cm in height), screened with white nylon veil, contained 1 or 2 males per cage. Each day, females which were seen to visit males were removed from the cage during the observation period (1730 h to 1930 h), and replaced with new virgin unmarked females of the same age. The total number of the marked, original females at each age visiting males was recorded. The experiment was continued until about 80-90% of the 50 females had visited males.

c. Matings

Fifty individually marked, 1-day old males and 50 1-day old females were confined together in a screened cage of 60 x 60 x 60 cm containing a guava plant. Each day, flies in copula were removed at about 15-30 min after darkness had fallen, and replaced with new virgin unmarked flies of the same age. The total number of marked males of each age achieving copulation was recorded. In addition, the locations where matings occurred were recorded. The experiment was terminated when about 90% of the 50 males and 50 females had mated.

Study 3. Responsiveness and Receptivity of Mated Females

Upon sexual maturity (ages between 25-30 days), 10 virgin males and 10 virgin females from the same age group were placed together in each of 20 cages to allow matings for 3 days. Each day at about 15-30 min after darkness had fallen, pairs in copula were carefully removed from the cage using small glass vials and held until they had separated. The mated males and new virgin females were then placed back into the same cage, but the mated females were held for further experiments. An additional 20 groups of 10 virgin males and 10 virgin females were set up two to three more times, each time after the termination of the previous 20 groups. By this method, a sufficient supply of mated females was obtained for further tests.

The following experiments were then carried out on mated and virgin females of the same ages to determine the effects of having mated once on their sexual responsiveness and receptivity to further matings. The experiments were not carried beyond 18 days because the effect of aging of flies may bias the results.

a. Responsiveness of mated females

Female responsiveness is defined as an exhibition of female approaching response towards signaling males.

The experiment on female responsiveness to males was carried out by releasing a group of 20 virgin and 20 marked mated females from the same age group into a large screen cage of 60 x 120 x 60 cm. One to two sexually mature males were confined in each of several mini-screened cages hung on a plant placed inside the large screened cage. Females visiting the males were removed from the cage during the observation period (1730 to 1930 h). The experiment was carried out at 2, 4, 6, 8, 10, 12, 14, 16, and 18 days after the first mating of mated females. The number of virgin and marked mated females visiting males was recorded.

b. Receptivity of mated females

Female receptivity is defined as a female readiness for matings. Two experiments were conducted to determine if a first mating would inhibit further mating of females.

Experiment 1. Mated and virgin females together

Ten groups of 10 virgin females and 10 marked mated females were placed with 10 males of the same age to test for female receptivity to matings. Each day, pairs in copula were removed from the cages and the number of matings was recorded. Mated females were discarded and the same number of females of the different type, virgin or mated, from the females that had been removed was randomly removed from the cage and discarded. Mated males were placed back into the cage and an equal number of males as those that had mated were then randomly removed and discarded to maintain the constant proportion of sexes. The experiment was continued for 3 consecutive days. New groups of flies were set up in the same way for successive three day periods; i.e. 1-3, 4-6, 7-9, 10-12, 13-15, and 16-18 days, after the first mating of mated females. A total of four replicates was conducted at each time period.

Experiment 2. Mated and virgin females separately

Virgin and mated females were tested separately for their mating receptivity. Three groups of 15 males and 15 virgin or mated females were placed together and allowed to mate for 3 consecutive days. Each day, pairs in copula were removed from the cages and the number of matings was recorded. Mated females were discarded, unmated males of an equal number to the mated males were randomly removed from each cage and discarded, but mated males were placed back into the cages. By this method, a constant proportion of sexes and a high number of experienced mating males that were potentially able to copulate were maintained. The experiments were carried out at several periods, 1-3, 4-6, 7-9, 10-12, 13-15, and 16-18 days, after the first mating of mated females.

RESULTS

Study 1. Description of Sexual Behavior

There are a number of components comprising a pattern of sexual behavior in D. dorsalis. The following are three major components:

a. Signal emission

As light intensity decreased during the 1-h period around dusk, sexually mature males increased their locomotor activities including rapid walking and short-distance flight (about 4-8 cm). As a result, encounters between individuals became frequent. Females were mostly stationary, but showed some movements such as walking away, or aggression with encountered individuals. Then, males assumed "signaling" activities consisting of rapid wing fanning and rhythmic abdominal stroking with the rear legs. During this period, an odor which was hypothesized to be a sex pheromone could be smelled. The males fanned their wings up and down rapidly while holding them over their backs. This wing fanning produced an audible buzzing sound emitted in a series of pulses for varying duration from 5 sec to 2 min. At the same time, the males frequently stroked the posterior region of the abdomen by using both of the hind legs alternately. This abdominal stroking rhythmically coincided with a "chik" sound which could be heard intervening in the buzzing sound produced by the wing fanning. Based on my observations of the male's signaling activities, such "chik" sound seemed to be produced by touching the legs to the wings at the moment when the male was stroking his abdomen. The odor mentioned above was also easily detectable during the active signaling by males. During signaling, a male responded quickly to any movement nearby. Between bursts of wing fanning, a male frequently changed direction and then resumed wing fanning. The signaling male usually remained in one place, or walked toward other individuals. Activities of these sorts usually occurred several times prior to the first mating attempt by males. The signaling by males appeared to excite females which became active, walking about, and quivering their abdomens vertically. The onset of dusk alone did not seem to excite the sexual activity of females. Females confined in a cage without males were inactive, and showed no walking and quivering.

b. Courtship behavior

In general, females approached signaling males rather than vice versa. A female either walked towards a male or flew about until she landed near him. The male would then turn toward the female, usually in a face-to-face position, and walk closer to the female, while still fanning his wings rapidly in a long burst. During a brief frontal orientation after the female approached to about 1 cm from the male, he fanned his wings as previously described. When the female stopped, the male flew (while on the underside of a leaf) or jumped (while on the vertical surface of a leaf) from the frontal position over the head of the female and onto her back. The male usually "flapped" his wings as he tried to mount the female and settle on her back. However, this wing flapping seemed to function merely as a way to balance himself while attempting to achieve intromission rather than serving as an acoustic signal like the rapid wing fanning performed previously.

c. Copulation

The female in a non-receptive state repeatedly resisted a male's attempts to mount. She would bend her abdomen and ovipositor downwards preventing the male from successfully mounting her. Simultaneously, the female tried to dislodge the male from her back using her hind legs and wings. Both of them struggled and eventually the female dislodged the male, or the attempting male gave up. However, if the female was receptive, she would turn the distal end of her abdomen upward and extend her ovipositor. The male would bend his abdomen downwards attempting to insert his aedeagus into the female's ovipositor, and copulation could be achieved within a few seconds. The male held the female by the abdomen with his forelegs, but held his middle and hind legs onto the substrate. The copulating pair sometimes flew a short distance or walked away from the area of intense activities to settle on the upper part of the cage. Pairs remained in copula beyond dusk and into darkness when the observation ceased. Other sexual activities e.g. male wing-fanning and female responses subsided abruptly with the onset of darkness.

Males were seen to attempt to copulate with individuals of both sexes, suggesting that they could not discriminate between sexes. Homosexual attempts in copulation were observed and several of them continued for an extended period.

Study 2. Sexual Activities with Respect to Age

a & b. Male wing-fanning and female responsiveness

The cumulative frequency (%) of male wing-fanning and female approaching are plotted in Fig. 1. Males first exhibited wing-fanning behavior at an earlier age than females first became responsive to the males' signals. The first time that wing fanning in males was observed was at the age of 7 days, but females first approached males at 9 days. More than 50% of the males were observed wing fanning at 13 days. More than 50% of the females were observed responding to male signals at 18 days. At the age of 28 days, 94% of the males were observed wing fanning and 80% of the females were observed to respond to signaling males.

c. Matings

The first mating pair was observed at the age of 8 days (Fig. 2) which was close to the age of the first wing-

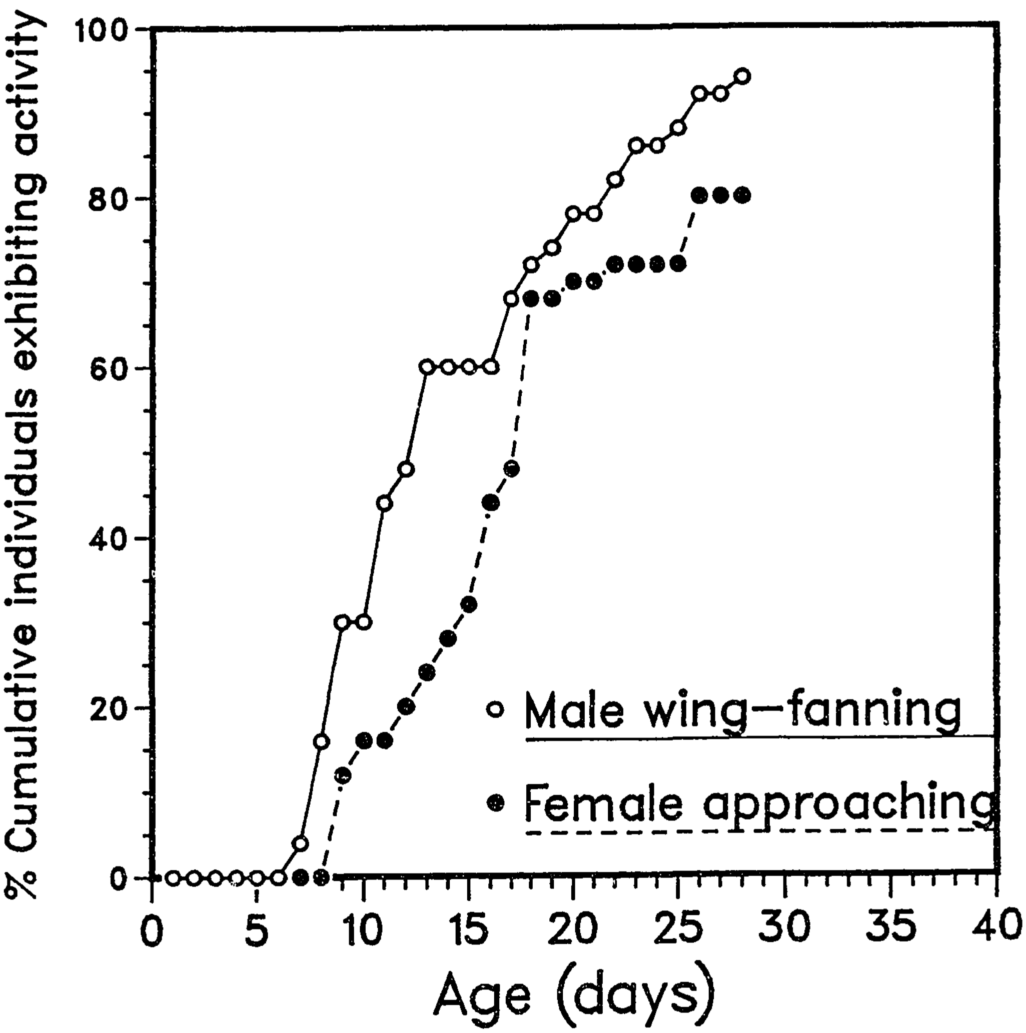

Fig. 1. Cumulative percentage of individuals exhibiting the activities of male wing-fanning or female approach, at various ages after adult eclosion (n =47 males, 40 females).

fanning male and first approaching female. At the age of 15 days, more than 50% of the matings had been observed. At the age of 26 days, 94% of the matings had been observed and no further mating was seen through day 28.

In this mating experiment, the majority of signaling males and matings were seen on the underside of the leaves. Out of 250 mating pairs observed, 54.4% were on the bottom surface of the leaves, 38.4% were on the cage walls, and the remaining 7.2% were on the ceiling or bottom of the cage and on the plant pot.

During the dark phase within about 2 hours after sundown, several brief checks (using a flashlight) did not detect any sexual activity or new copulating pairs. Most of the flies were immobile, but slight movements or short-range walking were observed if the flies were disturbed by a strong flashlight for longer than 30 sec.

Study 3. Responsiveness and Receptivity of Mated Females

a. Responsiveness of mated females

In all tests during the period of 2-18 days after mating, mated females were significantly less responsive to signaling males than virgin females (Table 2). At 2 days after first mating, only 16.67% of responding females were mated females compared to 83.33% virgins of the same age. The lack od significance of the heterogeneity chi-square

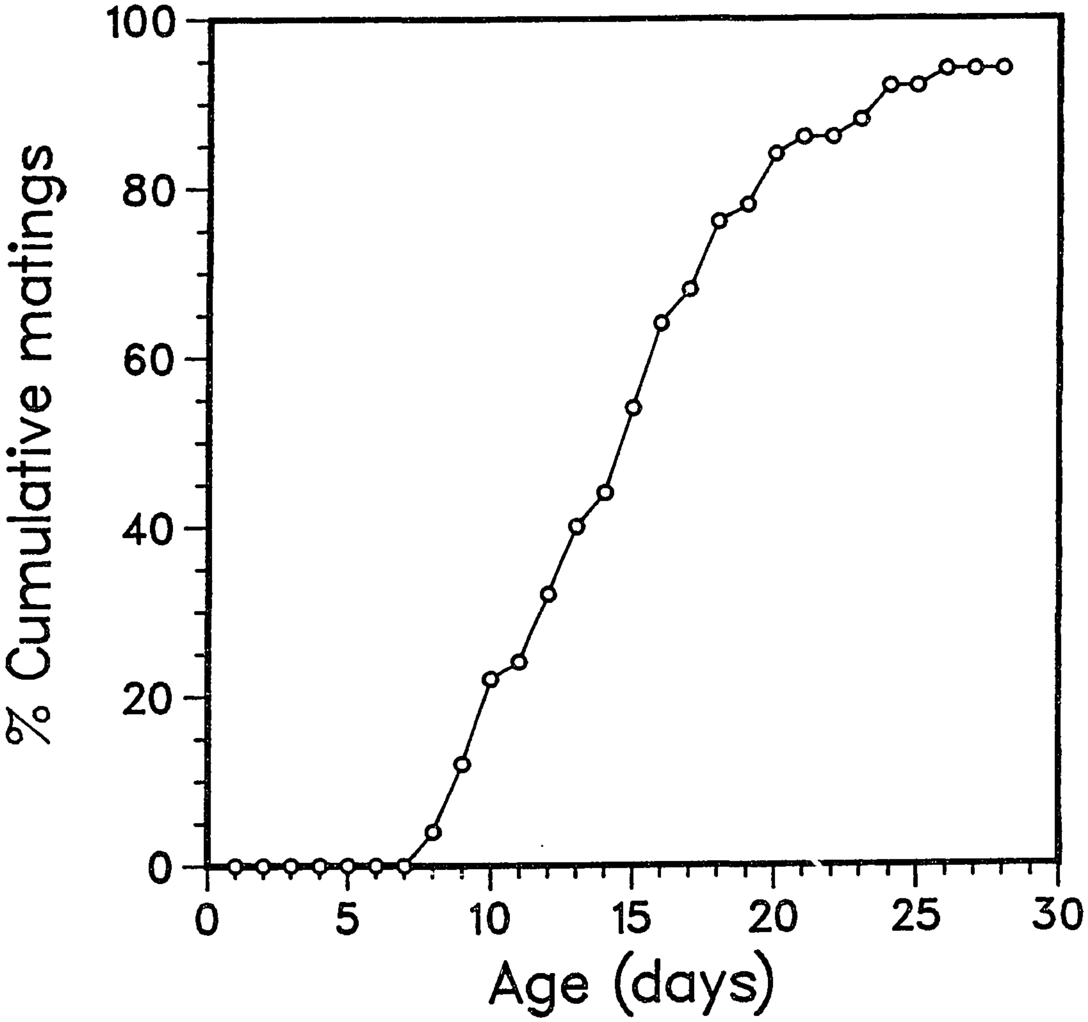

Fig. 2. Cumulative percentage matings at various ages after adult eclosion (n =47).

Table 2. The effect of the first mating on likelihood of approach by <u>Dacus</u> <u>dorsalis</u> females to males during the period of 2-18 days after mating

Days after 1st mating	Number females tested	Females which approached males[a]		chi-square[b]	P[c] ≤
		Virgin female	Mated female		
2	40	15 (75)	3 (15)	8.0	0.01
4	40	11 (55)	3 (15)	4.57	0.05
6	40	10 (50)	2 (10)	5.33	0.05
8	40	14 (70)	5 (25)	4.26	0.05
10	40	14 (70)	3 (15)	7.12	0.01
12	40	6 (30)	0 (0)	6.0	0.05
14	40	8 (40)	0 (0)	8.0	0.01
16	40	9 (45)	2 (10)	4.45	0.05
18	40	12 (60)	2 (10)	7.14	0.01

[a] Each trial consists of 20 virgin and 20 mated females that were free to approach the wing-fanning males. Values in the parentheses are percentages.

[b] chi-square values are pooled.

[c] The chi-square test is based on the actual numerical frequencies. The chi-square test for heterogeneity among days is non-significant (chi-square =2.42, df =8, P ≥0.975).

test among days suggests that approaches by mated females did not increase with increasing time since the first mating.

b. Receptivity of mated females

In **Experiment 1** when both virgin and mated females were caged with males, mated females were significantly less receptive to further matings than virgin females (Table 3). This highly significant level ($P \leq 0.001$) of decreased receptivity to further matings continued in mated females throughout the period of 1-18 days after the first mating. The lack of significance of the heterogeneity chi-square test among days suggests that matings by mated and virgin females did not increase with increasing time during the entire period of the experiment after the first mating.

In **Experiment 2** when virgin or mated females were caged alone with males, mated females were still significantly less receptive to further matings than virgin females (Table 4). This highly significant level of decreased receptivity to further matings was continued in mated females throughout the period of 1-18 days after the first mating. The lack of significance of the heterogeneity chi-square test suggests that matings by females, whether mated or virgin, did not increase with increasing time since the first mating.

Table 3. The effect of the first mating on subsequent mating of *Dacus dorsalis* females during the period of 1-18 days after mating, and in the presence of virgin females

Days after 1st mating	Number females tested	Females mated[a] Virgin female	Mated female	chi-square[b]	$P^c \leq$
1-3	190	102 (53.7)	22 (11.6)	51.61	0.001
4-6	190	105 (55.3)	9 (4.7)	80.84	0.001
7-9	168	111 (66.1)	15 (8.9)	73.14	0.001
10-12	124	85 (68.6)	14 (11.3)	50.92	0.001
13-15	119	59 (49.6)	15 (12.6)	26.16	0.001
16-18	61	41 (67.2)	10 (16.4)	18.84	0.001

[a] Each trial consists of an equal number of virgin and mated females placed in groups with a half as many males as the total females. Values in the parentheses are percentages.

[b] chi-square values are pooled.

[c] The chi-square test is based on the actual numerical frequencies. The chi-square test for heterogeneity among days is non-significant (chi-square =4.36, df =5, $P \geq 0.5$).

Table 4. The effect of the first mating on subsequent mating of _Dacus_ _dorsalis_ females during the period of 1-18 days after mating, and in the absence of virgin females

Days after 1st mating	Number females tested	Females mated[a]		chi-square[b]	P[c] ≤
		Virgin female	Mated female		
1-3	90	27 (60.0)	8 (17.8)	10.31	0.005
4-6	90	30 (68.2)	6 (13.6)	16.0	0.001
7-9	90	28 (62.2)	3 (6.7)	20.16	0.001
10-12	90	36 (80.0)	11 (24.4)	13.30	0.001
13-15	90	25 (58.1)	3 (7.0)	17.29	0.001
16-18	90	38 (84.4)	12 (26.67)	3.52	0.001

[a] Each trial consists of an equal number of males and females. Virgin and mated females were tested separately. Values in the parentheses are percentages.

[b] chi-square values are pooled.

[c] The chi-square test is based on the actual numerical frequencies. The chi-square test for heterogeneity among days is non-significant (chi-square =3.8, df =5, P ≥0.9).

DISCUSSION

D. dorsalis is restricted in its mating period to about half an hour each day during the decrease in light intensity at dusk. This narrow daily period of mating in D. dorsalis has been known since the work by Roan et al. (1954), and confirmed several times (Arakaki et al. 1984, Keiser et al. 1973, Kobayashi et al. 1978). In **Study 1** in which the components of sexual behavior were examined, the results again confirm that this species exhibits sexual behavior and mates only at dusk. In addition, several brief checks made after sundown did not detect any more copulating pairs or any sexual activities suggesting that darkness suppresses their sexual behavior and mobility. However, copulation of D. dorsalis in total darkness, reported by Keiser et al. (1973), may be a consequence of the limited space in the small cages that they used leading to encounters and matings of a small number of flies. Other species of sub-tropical and tropical tephritids that normally mate only at dusk like D. dorsalis include D. cucurbitae, D. cacuminatus, D. tryoni, D. oleae, Pterandrus rosa, and the Mexican fruit fly, Anastrepha ludens (Back and Pemberton 1917, Causse et al. 1966, McPhail 1933, Myburgh 1962, Myers 1952). The restricted period of sexual activity may result from an interaction between the declining light intensity and an endogenous daily rhythm of sexual responsiveness in both sexes as occurs in D. tryoni (Tychsen 1975, Tychsen and Fletcher 1971).

In D. dorsalis, the wing fanning by males and the touching of legs with the wings while stroking their abdomen during dusk appeared to generate sound. The "buzzing" and the "chik" sound, heard rhythmically intervening with the buzzing sound, were generated by the wing fanning and the touching of legs with the wings respectively. These sounds may serve as an auditory stimulus to females. Sound produced in some tephritid species such as D. cucurbitae, and D. tryoni has been reported to result from "stridulation" of the wings on the abdominal cilia (Fletcher 1968, Monro 1953, Suzuki and Koyama 1980). Even though the male of D. dorsalis possess some structures hypothesized to be the stridulatory organs, Keiser et al. (1973) failed to prove that stridulation exists in this species. In addition to the production of sound, wing fanning may be associated with the release of a sex pheromone in this species as an odor was released during the signaling of males. This is also reported in D. cucurbitae (Kuba et al. 1984). The exact role of wing fanning and the types of signals emitted by males will be investigated further in subsequent chapters.

An increase in the number of wing-fanning males and responding females to the males' signal and mating are closely correlated with the chronological ages of D.

dorsalis adults (Fig. 1 and 2). Since the onset of mating assumes sexual maturation, the results of **Study 2** (Fig. 1) showed that the onset of all sexual activities of D. dorsalis investigated in this study was coincident with the attainment of sexual maturity. Even though the onset of sexual responsiveness in D. tryoni does not depend on ovarian maturation (Fletcher and Giannakakis 1973), there is a positive correlation between the percentage of female mating and the stage of ovarian development in D. cucurbitae (Suzuki and Koyama 1980). Furthermore, the greatest response of A. suspensa females occurs as the ovary reaches mature size and decreases in both younger and older females. (Nation 1972). It is therefore reasonable to hypothesize that the female D. dorsalis response of approaching signaling males is correlated with female ovarian development.

Although it may be expected that female responsiveness and receptivity to males would not be totally lost after a single mating or regained after a refractory period, the results of this study indicate a tremendous effect of mating on both the responsiveness and receptivity of females (Table 2, 3, and 4). Mated females neither approached signaling males nor accepted subsequent matings as readily as virgin females. The inhibition of responding and remating did not decline, even at 18 days after the first mating. It should be noted that even though the males may be monopolized by caging with only one type of female, the results were not different from when they were caged with both types of females, virgin and mated. The results of this study correspond well with the findings of Kobayashi et al. (1978) that previously mated females of *D. dorsalis* do not respond to either live males or rectal glands. Surprisingly, these results contradict the report of Christensen and Foote (1960) that females of this species mate and remate frequently at 4-5 day intervals. However, there is insufficient detail of their study to determine why our results are different. It is possible that the inhibition of responsiveness and receptivity of mated females over a period of time during my study may be due to a lack of opportunity to utilize the sperm from the first mating. In my study, females were not provided with substrate for oviposition after the first mating. On the other hand, oviposition may not be the actual inhibiting factor because mating occurs repeatedly in both laying and non-laying females of another species of tephritids, *Euleia fratria* (Tauber and Toschi 1965).

In other tephritids, mated females become sexually unresponsive and unreceptive for at least a few days like *D. dorsalis*. For example, most mated *C. capitata* are not attracted to male pheromone for at least 10 days after mating (Feron 1962). The male pheromone of *D. tryoni* does not elicit any response in females that mated two days previously (Fletcher and Giannakakis 1973). The refractory period in *D. tryoni* and *D. oleae* females that had mated may last for several weeks after mating (Fay and Meats 1983, Tzanakakis et al. 1968).

The evolutionary reason for a refractory period to remating may be associated with the time and energy costs of mating. First of all, copulation may be disadvantageous to the female if she has already obtained a sufficient sperm supply. For example, females of C. capitata do not remate for several weeks after receiving an adequate amount of sperm (Nakagawa et al. 1971). The expenditure of energy may act as a selective pressure favoring unreceptivity if the female has a full supply of viable sperm to fertilize her eggs. Secondly, it would be an advantage for the male to develop any mechanism to reduce chances of further mating of the same female by a different male. Possibly in this species, a male substance or chemical stimulus associated with sperm or the accessory fluid may induce female unreceptivity as suggested in D. oleae (Tzanakakis et al. 1968).

Sexual receptivity in some species may be regained when sperm in the spermatheca is utilized. For example, the proportion of remated females in C. capitata gradually increases to about 30% at four weeks, and 50% at seven weeks after the initial mating (Nakagawa et al. 1971). Such female receptivity to repeated mating is negatively correlated with the sperm content in the spermatheca. However, the return of sexual receptivity of mated D.

dorsalis females did not occur during the period of my 18 day experiment. The cause could be a lack of opportunity for females to oviposit and deplete their sperm supply.

At present, there is experimental evidence from this study which indicates a reduction in responsiveness and receptivity in mated D. dorsalis females as compared to those of virgin ones, at least during the 18 days after the first mating. The mechanism reducing female responsiveness and receptivity is unknown. This aspect of sexual behavior in this species needs further investigation. If the inhibition of remating in females can be overcome and sperm can compete successfully with those from a previous mating, there may be selection favoring males that court mated females (Parker 1970, 1974). In SIRM programs, the reduced receptivity of females to further mating is advantageous because females would be more likely to be inseminated by released, sterilized males due to their higher proportion in the wild. If there is differential sperm viability, a return of receptivity of mated females may affect the use of this method. Wild females may be able to produce viable offspring if they were subsequently inseminated by wild males whose sperm can compete successfully with those from the previous, sterilized males. On the other hand, remating of wild females with sterilized males whose sperm are as viable as those from the previous wild males is advantageous to SIRM programs.

CHAPTER IV

SIGNIFICANCE OF MALE WING-FANNING FOR ATTRACTION OF AND MATING WITH FEMALES OF Dacus dorsalis

Sexual signals are advertisements produced by individuals for the purpose of gaining mates. There are large differences in sexual signal complexity produced by different species. Some species do not appear to signal at all while others utilize various modes of signal emission including visual, acoustic, and/or olfactory signals. For example, Tephritis dilacerata males attempt to copulate with any individual similar to females in size and shape, apparently without producing any kind of signal (Berube 1978). In gall-forming tephritids, signaling consists of simple wing waving (Zwolfer 1974). More complex sexual signals occur in species in which sexual communication may involve two steps, male-female assembly and courtship. In such cases, the step of assembly which is the bringing together of males and females from a distance to the same place for mating, relies a great deal on attraction signals.

Attraction signals utilized in sexual communication are defined as those which are emitted over a reasonably long range to attract individuals of another sex. The attraction phase is the first important step to occur prior to courtship, which is defined as being a close-range interaction between two individuals leading to copulation. In the attraction phase, signaling individuals do not aim the signals at any one particular individual. As soon as a signal receiver arrives close enough to the signaling individual, courtship begins. Therefore, sexual signals in Dacus dorsalis would be examined in two main phases, attraction and close-range courtship.

Attraction signals may include visual, acoustic, and olfactory modes. Visual signals such as a variety of markings, colors, or movement positions have never been reported to be utilized as attraction signals in any tephritid species. Among various types of signals, acoustic and olfactory signals are most commonly observed in many tephritids. Males of Rioxa pornia (island fruit fly) produce and disperse pheromones for attracting females (Pritchard 1967). Several other tephritid species utilize similar forms of attraction signals. Attraction signals in Anastrepha suspensa (Caribbean fruit fly) consist of stereotyped "calling songs" generated by bursts of rapid wing fanning, and a sex pheromone (Bateman et al. 1976, Nation 1972, Perdomo 1974, Webb et al. 1976, 1983a). Those in Ceratitis capitata (Mediteranean fruit fly) consist mainly of a sex pheromone (Bateman et al. 1976, Feron 1962, Ohinata et al. 1977), accompanied with distinct sound of wing fanning (Rolli 1976). In Dacus cucurbitae (melon fly), males vibrate their wings and emit a sex pheromone (Kuba et al. 1984). Pheromones of Dacus tryoni (Queensland fruit fly) can attract females over a distance of at least 0.5 meters (Bellas and Fletcher 1979).

All of the mate-attracting signals in various tephritids presented above are produced by males. In some other species such as Dacus oleae (olive fruit fly), both sexes produce similar pheromones (Baker et al. 1980, Mazomenos and Pomonis 1982). A sex pheromone which is a mixture of several components emitted by females attracts males (Haniotakis 1974, Haniotakis et al. 1977, Mazomenos and Haniotakis 1981, Mazomenos et al. 1985). Mate-attracting signals may, therefore, be produced by either sex depending on the species.

In the second phase, close-range courtship, communication signals including visual, acoustic, and olfactory stimuli are important in varying degrees to successful matings of several tephritid species. Distinctive color or markings on the body or wings and elaborate movements or postures in some species suggest their significance as visual signals. For example, the shiny gold body, patterned wing-markings, and elaborate wing or body movement in A. suspensa are suggested to be significant visual signals (Dodson 1978). Descriptions of male performances and female responses associated with mating in leaf-mining tephritids Euleia fratria and Tephritis stigmatica seem to imply the significance of visual stimuli in their communication during courtship (Tauber and Toschi 1965a, 1965b).

Acoustic signals have been characterized and reported for their important functions during courtship in several tephritid species. A particular song serves to persuade a female of Toxotrypana curvicauda (papaya fruit fly) to remain in place (Sivinski and Webb 1985c). In A. suspensa, sound produced by the wings stimulates sexual activity and response in both sexes (Sivinski and Webb 1985, Webb et al. 1984). Other tephritids such as D. tryoni and C. capitata also produce species-specific acoustic signals during courtship (Monro 1953, Myers 1952, Webb et al. 1983b). Males of C. capitata produce acoustic stimuli consisting of three different types of sound, "calling, courtship, and copula" sound, prior to successful mating (Feron 1962, Webb et al. 1983b). However, the actual roles of these sounds are not known.

The presence of a male-produced sex pheromone has been reported in numerous tephritids including A. suspensa, C. capitata, D. cucurbitae, D. tryoni, Rhagoletis pomonella (apple maggot fly), and R. pornia (Feron 1959, 1962, Fletcher 1968, Kobayashi et al. 1978, Nation 1972, Pritchard 1967, Prokopy 1975). The male-produced pheromones have been shown to evoke copulatory responses at close range in D. cucurbitae, D. tryoni, and three Australian Dacus spp.(Dacus opiliae, Dacus aquilonis, and Dacus tenuifascia) (Baker et al. 1982, Bellas and Fletcher 1979, Fitt 1981).

Males of D. dorsalis and the other tephritids discussed above share a common behavior, wing fanning. Wing fanning occurs strictly during the time period associated with sexual activity (**CHAPTER III**). However, the nature of the stimulation provided by this wing fanning in D. dorsalis is still not clear. Two major types of stimuli, acoustic and olfactory, have frequently been discussed as possible important signals produced by male wing-fanning in D. dorsalis.

First of all, it is known that the wing fanning of males produces a buzzing sound (Roan et al. 1954 and **CHAPTER III**). D. dorsalis males possess curved cilia on the 3rd abdominal tergite which were believed to function as stridulatory organs during courtship in several dacine species (Drew et al. 1982, Monro 1953, Munro 1984). However, stridulation does not exist in D. dorsalis (Keiser et al. 1973).

Secondly, the presence of attractive male pheromones in D. dorsalis has been suggested because live males and the gland complex in the male rectal sac are highly attractive to virgin females (Schultz and Boush 1971, Kobayashi et al. 1978). Furthermore, a substance emitted as "smoke" by the males during their wing-fanning activity is somewhat attractive to females in laboratory tests (Ohinata et al. 1982). Even though the behavior and responses discussed above may give suggestions as to which stimuli are important in mating success, there is no experimental proof to indicate which stimuli elicit sexual responses from females.

A sexual signal produced by an animal causes a specific reaction in a conspecific individual of the opposite sex upon its reception of such a signal. There are several types of sensory receptors utilized in signal reception by individuals of various species. In one Dipteran species, Drosophila melanogaster, the acoustic receptor is the feathery arista situated on the 3rd antennal segment of the female. When the arista is stimulated by the sound wave, it stimulates the Johnston's organ located in the 2nd antennal segment (Bennet-Clark 1971, Manning 1967). There is no information available on the location of acoustic receptors in tephritids, however, species of Diptera may possess a similar type of sound receptor.

In addition to the sound receptor, the 3rd antennal segment of D. melanogaster also bears olfactory receptors (Kellogg et al. 1962). Among tephritids, the same third antennal segment in D. tryoni and D. oleae is covered with large numbers of chemosensilla (Giannakakis and Fletcher 1985, Hallberg et al. 1984). Studies of D. oleae indicate that sensilla on the antennae can respond to sex pheromone and other volatiles (Bateman and Morton 1981, Van der Pers et al. 1984). Many tephritid species including Anastrepha ludens (Mexican fruit fly), A. suspensa, C. capitata, D. cucurbitae, and D. dorsalis possess sensilla on the third antennal segment (Dickens et al. 1988, Robacker and Hart 1987). They are believed to function as olfactory receptors because of their similar morphological characteristics with other proven chemoreceptors in insects. The sensilla found on the antennae of both male and female of _A_. _ludens_, and _A_. _suspensa_, respond similarly to the tested chemicals (Robacker and Hart 1987, Robacker et al. 1986). One type of these antennal sensilla in _C_. _capitata_ also responds to a male attractant called trimedlure (DIckens et al. 1988).

Based on the above specific studies of sensory receptors and behavioral evidence, it was believed that interference with the functioning of sensory receptors in receiving signals would provide some evidence regarding sensory modalities used by the species. Thus, modification of various sensory receptors in females was the primary approach used in this study. In addition, male wing-fanning has never been experimentally demonstrated to be a significant component of sexual communication . Therefore, my experiments were designed to evaluate the significance of wing fanning during the sexual activity.

Two series of experiments were conducted. Each series focused on several aspects of one particular phase during sexual communicaiton. The **First Series (Study 1-**4) focused on the attraction phase over an intermediate range (average 50 cm; range 15-105 cm). The **Second Series (Study 5-6**) emphasized courtship at close-range (average 6 cm; range 2-11 cm).

The objectives of this study were as follows:

First Series. Attraction Phase

1. To determine the attraction between sexes, i.e. either the males attracted females or vice versa.

2. To examine the distribution of attractiveness in relation to time during dusk.

3. To examine the attractiveness of males whose wing fanning was limited or prevented.

4. To determine the possible roles of acoustic and olfactory signals in sexual attraction.

Second Series. Close-Range Courtship

1. To examine the matings of males whose wing fanning was limited or prevented during close-range encounters with females.

2. To investigate the sensory systems that females use in accepting or rejecting copulation with courting males.

MATERIALS AND METHODS

Two series of experiments were conducted. The **First Series (Study 1-4)** was on the attraction phase, while the **Second Series (Study 5-6)** was on the close-range courtship.

First Series (Study 1-4). Attraction Phase

The goal of the first series of experiments included in **Study 1-4** was to examine the bringing together of a male and female into close proximity of one another which is the first step in the mating behavior of this species.

General procedures. Experiments in **Study 1 to 4** were conducted to examine the attraction and assembly of flies over an intermediate range. The maximum distance, limited by the size of an experimental cage (60 x 120 x 60 cm), was much less than distances available in the wild. Such limited distance was likely to be an "intermediate range" rather than "long range" available in the wild. The average distance was about 50 cm ranging between 15-105 cm depending on where the free-flying individuals were when the experiments were started.

Individuals were released into the cage during morning hours to acclimatize them for the afternoon experiments. Individuals being tested for their attractiveness were confined in several small, screened (white nylon veil), cylindrical cages (3-4 cm in diameter, 5-6 cm in height), 1 or 2 individuals per cage (called "mini cages" from now on). Two individuals per cage were confined in 2/3 of the mini cages to increase the chance of obtaining data: Two males confined together in a mini cage were noticed to fan wings more frequently than males confined singly. These mini cages were hung on potted guava plants placed inside the large cage. Individuals that landed on the mini cages and remained there for at least 1 min were recorded as being attracted by the tested flies in the mini cages.

Mini cages were placed on the plant about two hours before sunset. Observations were started about one hour later and continued until darkness fell when all activities ceased. The times presented throughout this chapter was relative to the different time of the year when the work was conducted. Generally, the experiments were conducted during the two-hour period around dusk. The number of times that individuals landed on (**Study 2**), the number of individuals (**Study 1, 3**), or each type of females that landed on (**Study 4**) mini cages within a 45 min observation period (2 hr for **Study 2**) was recorded. The results were analyzed by using chi-square tests, and chi-square tests for heterogeneity between trials conducted on different days were also performed.

Study 1. Attraction between Sexes

In this study, two experiments were conducted to determine the attractiveness of both males and females to the same or the opposite sex.

Mature virgin males were pre-screened to select those that had been seen wing fanning. These males or mature virgin females were then confined in each of several mini cages, 1 or 2 individuals per cage, as explained in the **General procedures**. These mini cages were hung on two different plants, one for each sex. Each plant was placed on each side of a large screened cage. Each day the confined flies were alternated between the two plants to minimize any effect of plant preference that could occur. Forty individuals of the sex being tested, males in the first experiment and females in the second experiment, were released into this large cage. The number of times individuals landed on each mini cage on the plants was recorded at 2 min intervals. Flies used in the study were replaced for each observation period. A total of 10 groups of males and 11 groups of females was tested.

Study 2. Distribution of Attractiveness in Relation to the Time of Day

This study was carried out to examine the distribution of attractiveness during a 2 hour period around dusk, 1730-1930 hours.

Mature males pre-screened as described in **Study 1** were confined in each of several mini cages, 1 or 2 individuals per cage as before. These mini cages of males were hung on a plant and placed in a large screened cage. Twenty females, the sex found in **Study 2** (Table 5) to be attracted to the wing-fanning males, were released into this large cage. The number of times individuals landed on the mini cages was recorded at 5 min intervals. Six replicates for a total of 120 individuals were tested.

Study 3. Effect of Wing Modification on the Attractiveness of Males

This study was conducted to determine the necessity of male wing-fanning in attracting females. The normal wing fanning behavior of males was either limited or prevented to determine its significance.

Males were pre-screened as previously described. They were then anaesthesized by chilling at 5 ^{o}C in the freezer compartment of a refrigerator for 1-2 min before any modification was made to their wings. The modifications, made under a dissecting microscope, were to clip off more than 50% of the wing area (Fig. 3), or to put a drop of wax at the base of each wing. The purpose of clipping was to remove the portion of the wing that may rub against the abdominal cilia, but to allow males to still fan their wings normally. Acoustic or olfactory signals produced may have been altered. The waxing procedure was intended to inhibit the male wing fanning completely. Non-modified males used in a control treatment were simply anaesthetized by chilling. In the experiment, one or two modified and non-modified mature males were confined separately in each of several mini cages like those used in **Study 1**. These cages of males were then hung on two separate plants, one containing only modified males, and the other containing non-modified males. The two plants were placed in a large screened cage into which 40 mature virgin females were released. The number of times females visited each type of

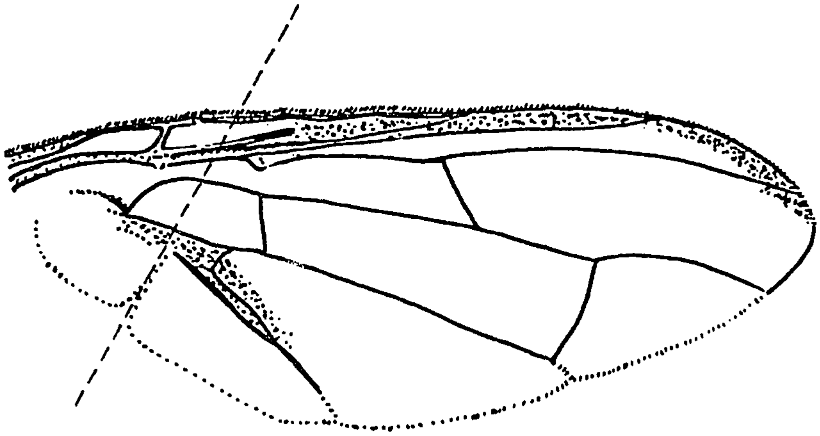

Fig. 3. Wing of <u>Dacus</u> <u>dorsalis</u> male showing the position at which the portion of >50% of wing was removed.

male was recorded at 2 min intervals during a 1-h period around dusk. Each day, all new, virgin individuals were replaced for each test. The plants containing modified males were alternated each day to minimize any effect of plant preference that could occur. A total of 130 males possessing each type of modified wings and 260 non-modified (control) males was tested. The virgin females were replaced for each day of observation.

Study 4. Importance of Female Sensory Receptors for the Response to Males

The goal of this study was to determine the purpose and significance of the male wing-fanning over an intermediate range. The two major sensory receptors, acoustic as well as olfactory, that females may use for receiving male signals produced by wing fanning were modified.

Mature virgin females were anaesthetized by chilling at 5 °C in the freezer compartment of a refrigerator for 1-2 min before any modification to their sensory receptors was made. Modifications of female sensory receptors were done in three different ways under a dissecting microscope. Each method of modification was intended to prevent the female reception of a specific type of signal from males. The modifications were removal of the aristae (acoustic receptor) or entire antennae (both acoustic and olfactory receptors) by using a pair of microscissors, and painting of the antennae (olfactory receptor) with black enamel leaving the aristae free to receive acoustic signals. The modified females were then tested to determine whether they were able to locate and approach males as readily as non-modified females. Attraction over an intermediate range was tested by confining one or two pre-screened, mature males as described previously in each of several mini cages. These cages containing males were then hung on a plant and placed in a large screened cage. Forty females, 20 modified and 20 non-modified, were then released into this large cage. Females landing on male cages were captured during the test and identified later to obtain the number of each type of female attracted to males. New, virgin individuals were replaced for each day of observation. The total number of modified and non-modified (control) females tested is shown in Table 7.

Second Series (Study 5-6). Close-Range Courtship

The series of experiments included in **Study 5-6** were conducted to examine in detail the significance of sexual signals utilized at close range (average 6 cm; range 2-11 cm). Matings as measured by actual copulations of males were used as a criterion indicating the significance of signals produced.

General procedures. Mature males used in all experiments were pre-screened to select those that had exhibited wing-fanning behavior. Individual flies were anaesthetized by chilling at 5 °C in the freezer compartment of a refrigerator for 1-2 min before any modification was made. Matings were conducted by placing tested individuals in a paper, cylindrical cage (8 cm in diameter, 8 cm in height). Each day after sundown, matings were recorded, allowing only one mating in each cage. The test in each cage was terminated when mating was observed or after 7 days, whichever came first. The results were analyzed by using a chi-square test.

Study 5. Role of Male Wing-Fanning in Mating

Wing fanning performed by males at close range was examined in two separate experiments to determine its importance in mating success. The normal wing-fanning behavior of males was limited or prevented by the methods of modification described below. Modified and non-modified (control) males were then tested to determine their mating success.

The same procedures of wing modifications were used in both experiments. Normal wings of immobilized males were altered under a dissecting microscope in three different ways. Portions of their wings at either one of two positions, less than 50% (the tip; Fig. 4) or more than 50% of their wings, were clipped off, or a drop of wax was put at the base of each wing. Males could still fan their wings normally when part of a wing was removed. The removal of less than 50% of the wing area by the wing tip changed or

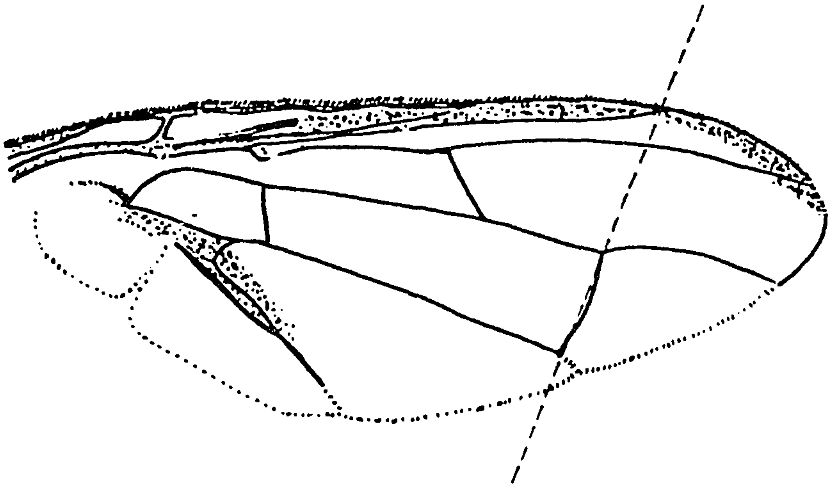

Fig. 4. Wing of Dacus dorsalis male showing the position at which the portion of <50% of wing was removed.

reduced the production of stimuli only slightly. The removal of more than 50% could affect the acoustic or olfactory stimulus that may be produced more drastically. The waxing procedure inhibited the wing fanning completely: the waxed-wing males were not seen to fan their wings. These modified males were then used in behavioral tests. Non-modified males used as controls were simply anaesthetized by chilling.

Experiment 1. Matings were conducted by placing two males, one each with modified and non-modified wings, together with one female. A total of 45 to 50 groups of males from each type of modification was tested (Table 8).

Experiment 2. Matings were conducted by placing two males, either non-modified and modified males, or two males modified in two different ways, together with one female. A total of 14 to 42 groups of males from each type of modification was tested (Table 9).

Study 6. Mechanism of Female Response

Two experiments were carried out to determine the significance of the mechanism by which females accepted or rejected copulation with the courting males. Each type of the three major sensory receptors, visual, acoustic, or olfactory, that females may use for receiving male signals was modified. Modified and non-modified females were then further tested to evaluate any effect of modifications on mating.

The same procedures of sensory receptor modifications were used in both experiments. Normal sensory receptors of anaesthetized females were altered under a dissecting microscope in four different ways. Each method of modification was intended to prevent the female reception of a certain type of signal from males. They were removal of the aristae (acoustic receptor) or entire antennae (both aristae and olfactory receptors) by clipping with microscissors, and painting the eyes (visual receptor) or antennae (olfactory receptor) with black enamel. Painting of the antennae left the aristae free to receive acoustic signals. These females were tested in two separate experiments. Non-modified females used as controls were simply anaesthetized by chilling.

Experiment 1. Matings were conducted by placing two females, one each with non-modified (control) and modified sensory receptors, together with one male. A total of 76 to 105 groups of females from each type of modification was tested (Table 10).

Experiment 2. Matings were conducted by placing two females, either non-modified and modified females, or two females modified in different ways, together with one male.

A total of 32 to 98 groups of females from each type of modification was tested (Table 11).

RESULTS

First Series (Study 1-5). Attraction Phase

Study 1. Attraction between Sexes

Males were equally attracted to females and to wing-fanning males (Table 5). These free-flying males landed on the mini cages hung on both plants at approximately equal frequency. They then started their wing fanning and attempted copulations at about the same time of day as those males confined in mini cages did. Females confined in mini cages seemed to be excited and walked around in an agitated manner, especially when males were fanning wings nearby. Free-flying females showed no attraction to other females but were significantly attracted to males confined on the plant (Table 5). Attracted females flew to cluster on the male's mini cages, and probed through the screen with their ovipositors in a movement that was similar to the extension of the ovipositor by receptive females.

Study 2. Distribution of Attractiveness in Relation to the Time of Day

Most females observed during the afternoon hours usually remained inactive under leaves and on cage walls

Table 5. Attraction between sexes of <u>Dacus dorsalis</u>

Sex tested	Total flies tested	Approached[a] Males	Approached[a] Females	Did not approach	chi-square[b]	P[c] ≤
Male	400	145 (36.3)	130 (32.5)	125	0.82	NS
Female	440	262 (59.6)	56 (12.7)	122	133.45	0.001

[a] Each trial consists of 40 tested flies, males or females, that were free to approach males or females confined on two separate plants. Values in the parentheses are percentages.

[b] chi-square values are pooled.

[c] The chi-square test is based on the actual numerical frequencies. The chi-square test for heterogeneity among trials is non-significant in either the male or female (Male: chi-square =8.98, df =9, P ≥0.5; Female: chi-square =10.41, df =10, P ≥0.5).

until about an hour before sundown. As the light intensity started to decrease at 1800 h, males started to wing fan, the females began to approach these signaling males and to exhibit probing responses. The number of landings was low at 1730 to 1755 h, and gradually increased (Fig. 5). Attractiveness, measured by the number of female landings, increased between 1820 and 1855 h, and peaked at 1855 h, reaching a level of 32 landings per 5 min when most males in the mini cages fanned their wings actively and stroked their abdomen at the same time. Such responses declined sharply at the time of sunset, approximately 1900 h. Within about 10 min after sunset, this number declined to 9 landings as most females began to leave the signaling males to settle on leaves or cage walls. However, a very small number of females, fewer than one female in each replicate, remained on the mini cages at 1925 h when it was completely dark.

Study 3. Effect of Wing Modification on the Attractiveness of Males

Males with a large portion of their wings removed were significantly less attractive to females than non-modified males (Table 6). These males seemed to fan their wings actively; even though the quality of signals may be altered. Similarly, males with waxed wing-bases were not as attractive to females as non-modified males. These males usually walked around or were inactive. It seemed that the prevention of wing fanning by waxing their wing bases also

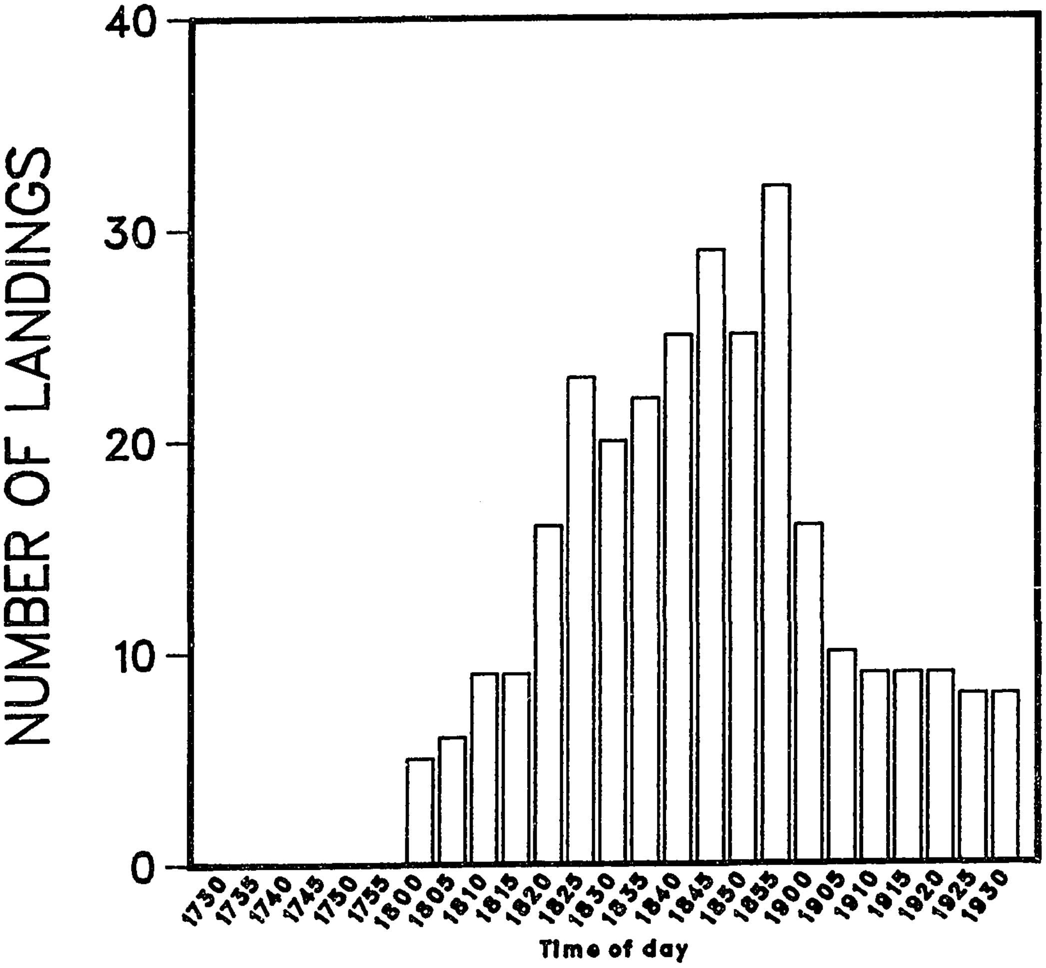

Fig. 5. Distribution of females attracted to wing-fanning males at 5 min intervals between 1730 and 1930 h.

Table 6. Attraction of *Dacus dorsalis* females to males possessing non-modified or modified wings

Wing modification	Total males tested	Total females tested	Females approaching male type[a] Non-modified male	Females approaching male type[a] Modified male	chi-square[b]	P[c] ≤
Clipped >50%	130	400	215 (53.8)	37 (9.25)	125.73	0.001
Waxed	130	400	226 (56.5)	37 (9.3)	135.92	0.001

a Each trial consists of 40 females that were free to approach non-modified or modified males confined on two separate plants. Values in the parentheses are percentages.

b chi-square values are pooled.

c The chi-square test is based on the actual numerical frequencies. The chi-square test for heterogeneity among trials is non-significant in either the males with clipped or waxed wings (Clipped: chi-square =2.45, df =9, P ≥0.995; Waxed: chi-square =2.62, df =9, P ≥0.995).

inhibited abdominal stroking with their rear legs. Males possessing clipped wings still stroked their abdomen while fanning wings.

Study 4. Importance of Female Sensory Receptors for the Response to Males

The responses to males by females with any of their sensory receptors modified were significantly reduced (Table 7). Significantly fewer females with modified sensory receptors visited males than did non-modified females. The low magnitude of effect due to aristae removal alone suggests its weaker effect than that of the other treatments.

Second Series (Study 5-6). Close-Range Courtship

Study 5. Role of Male Wing-Fanning in Mating

Experiment 1. Males with the tips of their wings (less than 50%) removed mated as frequently as non-modified males (Table 8). Males with more than 50% of their wings removed were significantly less successful in mating than non-modified males. Similarly, males with the wing bases waxed were also significantly less successful in mating than non-modified males. The effect of removing the majority of the wing and of waxing appeared to be similar in magnitude.

Table 7. Attraction of *Dacus dorsalis* females, possessing non-modified or modified sensory receptors, to males

Sensory receptor modification	No. trials	Total females tested	Females approaching normal males[a]		chi-square[b]	P[c] ≤
			Non-modified	Modified		
Aristae removed	13	520	176 (33.8)	95 (18.3)	24.21	0.001
Antennae painted	13	520	167 (32.1)	54 (10.4)	57.78	0.001
Aristae & Antennae removed	11	440	139 (31.6)	27 (6.1)	75.57	0.001

[a] Each trial consists of 40 females, 20 individuals each possessing non-modified or modified sensory receptors, that were free to approach males. Values in the parentheses are percentages.

[b] chi-square values are pooled.

[c] The chi-square test is based on the actual numerical frequencies. The chi-square for heterogeneity among trials was non-significant in all treatments (Aristae removed: chi-square =6.73, df =12, P ≥0.9; Antennae painted: chi-square =5.87, df =12, P ≥0.975, Aristae & Antennae removed: chi-square =4.69, df =10, P ≥0.975).

Table 8. Effects of wing modifications in *Dacus dorsalis* males on mating success

Wing modification	Total males mated[a]	Males mated[b] Non-modified	Modified	chi-square	P ≤
Clipped <50%	55	31 (51.7)	24 (40.0)	0.89	NS
Clipped >50%	54	46 (76.7)	8 (13.3)	26.74	0.001
Waxed	45	38 (63.3)	7 (11.7)	21.36	0.001

[a] 60 pairs of males tested for each modification.

[b] Each sample consists of two males possessing non-modified or modified wings. Values in the parentheses are percentages.

Experiment 2. The results (Table 9) support those obtained from **Experiment 1.** Males missing the tip portion of their wings mated as successfully as non-modified males. Clipping off more than 50% of the wings and waxing the wing bases had a similar effect in reducing male mating success. First, males with more than 50% of the wing area removed were significantly less successful in mating than non-modified males. Similarly, males with the wing bases waxed were also significantly less successful in mating than non-modified males. Second, males with more than 50% of the wing area removed mated significantly less frequently than males with the wing tip removed. Third, males with either more than 50% of the wing area removed or the wing bases waxed mated equally well. The number of males tested was similar in all treatments. However, the low number of matings by males (n=14) with more than 50% of their wings clipped off or with their wing bases waxed is due to the failure of mating by either one. As noted earlier, waxing the wing bases inhibited both wing fanning and abdominal stroking with the rear legs, but clipping the wings did not.

Study 6. Mechanism of Female Response

Experiment 1. Females with their eyes painted or sound receptors (aristae) removed mated as well as the non-modified females did (Table 10). However, when pheromone reception (antennae painted) was prevented, modified females

Table 9. Matings of *Dacus dorsalis* males possessing non-modified or modified wings when placed with other types under all possible combinations

Wing modification		Total males mated[a]	Males mated[b]		chi-square	P ≤
Male-I	Male-II		Male-I	Male-II		
None	Clipped <50%	36	19 (42.2)	17 (37.8)	0.1	NS
None	Clipped >50%	42	37 (82.2)	5 (11.1)	24.38	0.001
None	Waxed	35	31 (68.9)	4 (8.9)	20.83	0.001
Waxed	Clipped <50%	33	4 (8.9)	29 (64.4)	18.94	0.001
Waxed	Clipped >50%	14	5 (11.1)	9 (20.0)	1.14	NS
Clipped >50%	Clipped <50%	37	5 (11.1)	32 (71.1)	19.7	0.001

[a] 46 pairs of males tested for each combination. Values in the parentheses are percentages.

[b] Each sample consists of two types of males designated as male-I and male-II, possessing non-modified or modified wings as indicated, placed with a virgin female.

Table 10. Effects of Sensory receptor modifications in *Dacus dorsalis* females on matings

Sensory receptor modification	Total females tested	Total males tested	Females mated[a] Non-modified	Modified	chi-square	$P \leq$
Eyes painted	100	93	46 (46.0)	47 (47.0)	0.01	NS
Aristae removed	110	105	57 (51.8)	48 (43.6)	0.77	NS
Antennae painted	105	99	68 (64.8)	31 (29.5)	3.83	0.001
Antennae & Aristae removed	85	76	54 (63.5)	22 (25.9)	13.47	0.001

[a] Each sample consists of two females, one with non-modified and another with modified sensory receptors, placed with a virgin male. Values in the parentheses are percentages.

Table 11. Matings of *Dacus dorsalis* females possessing non-modified or modified sensory receptors when placed in a choice situation with another type under all possible combinations

Sensory receptor modification[a]		Total females	% Females mated[b]		chi-	
Female-I	Female-II	mated	Female-I	Female-II	square	P ≤
a. None	Eyes	36	55.6	44.4	0.44	NS
b. None	Aristae	98	57.1	42.9	2.0	NS
c. None	Antennae	76	69.7	30.3	11.84	0.001
d. None	Antennae & Aristae	54	70.4	29.6	8.96	0.01
e. Eyes	Aristae	35	45.7	54.3	0.26	NS
f. Eyes	Antennae	38	73.7	26.3	8.5	0.01
g. Eyes	Antennae & Aristae	32	87.5	12.5	18.0	0.001
h. Aristae	Antennae	92	65.2	34.8	8.5	0.01
i. Aristae	Antennae & Aristae	46	73.9	26.1	10.52	0.01
j. Antennae	Antennae & Aristae	40	82.5	17.5	16.9	0.001

[a] Modifications include non-modified, eyes painted, aristae removed, antennae painted and antennae & aristae removed.

[b] Each sample consists of two types of females designated as female-I and female-II, possessing non-modified or modified sensory receptors as indicated, placed with a male.

mated significantly less frequently than non-modified females. A significant reduction in mating was also found in females whose entire antennae were removed. In the latter cases, both sound and pheromone receptors in the females were missing.

Experiment 2. The results (Table 11) support those obtained from **Experiment 1.** Females without the visual or sound receptors mated as well as non-modified females, and as each other (lines a, b, and e). Based on lines b, c, and h, olfactory signals appeared more important than acoustic signals in determining whether a female would mate or not. Pheromone and pheromone-plus-acoustic receptor modification had equivalent effects (lines c, d, f, g, h, i). Acoustic signals appear important only where olfactory-plus-acoustic signal elimination significantly reduces mating of females by a greater magnitude than the effect of olfactory signal elimination alone.

DISCUSSION

First Series (Study 1-4). Attraction Phase

Mature males of D. dorsalis were equally attracted (low magnitude) to either females or wing-fanning males, however, females were strongly attracted to males, but not to females (Table 5). These findings were similar to results for D. dorsalis, and D. cucurbitae in other bioassays which were conducted in much smaller cages (25 x 25 x 25 cm) and using a higher density of flies (Kobayashi et al. 1978). In other species such as *C. capitata*, it has been suggested that the pheromone calling of males serves to attract other males to the mating arena in addition to attracting females (Arita and Kaneshiro 1983). Similar results were found in *A. suspensa* and *D. tryoni* under field conditions (Fletcher 1968, Perdomo 1974).

In this species, assembly of both males and females at the same place and time for mating is restricted to only a short period during dusk. Since signaling males of *D. dorsalis* do not strongly attract other males (Table 5), males may orient towards their preferred site based on other favorable environmental conditions. Preliminary data showed that two or more males confined in the vicinity of each other fanned wings more frequently and actively than those confined singly. Movements and interactions among males appeared to stimulate each other to fan wings producing signals simultaneously in order to attract females.

The approach of females to males increased gradually between 1800 and 1925 h, peaked at 1855 h, and decreased suddenly at 1900 h (Fig. 5). These results suggest that there was a temporal distribution of either signal emission or female responsiveness, or both. This distribution resulted in varying degrees of male attractiveness with time. Male attractiveness peaked at 1855 h lasting for a few minutes. This pattern suggests a strong dependence of a mechanism triggering sexual activity on factors that were changing during the time of dusk. A circadian clock and light intensity are known to control the timing of sexual behavior in _D_. _tryoni_ (Smith 1979).

Males which were unable to fan their wings normally were less attractive to females (Table 6). Such a reduction in male attractiveness is likely to be the result of changes in the signals produced by the modified wings. Removal of more than 50% of the wing may have affected the quality and amount of both acoustic and olfactory signals produced by wing fanning. Even though _D_. _dorsalis_ males whose wings had been reduced to stubs were reported to still produce the "high-pitched" sound normally, male attractiveness was never tested (Keiser et al. 1973). Removal of wings could have also affected the production of an olfactory signal because small portions of wing left intact may not be efficient in the dispersion of the pheromone. On the other hand, waxing of the wing bases may have totally prevented the production and dispersion of acoustic and olfactory signals respectively. The significant reduction in attractiveness of _D_. _dorsalis_ males whose wing fanning had been altered certainly demonstrated that the normal wing fanning played an important role in the production of signals. These signals could be acoustic, olfactory, or visual, or a combination of them.

Females with experimentally impaired receptors for sound, pheromone, or both sound and pheromone were significantly less attracted to males than females with unmodified receptors (Table 7). These results strongly indicate that both acoustic and olfactory stimuli are necessary to attract females. This study is the first to experimentally demonstrate the role of an acoustic stimulus in attracting D. dorsalis females. Furthermore. this study clearly demonstrates the attraction significance of sound and pheromone from the perspective of female behavior towards signaling males. One study was conducted in field cages in an attempt to test the significance of male-produced stimuli that were artificially administered in A. suspensa (Webb et al. 1983a). Traps baited with recorded calling sound or sex pheromone extract alone were found to catch significantly more females than the empty control traps, but caught significantly less females than traps with caged mature males. Their study has provided evidence in which sound and pheromone play a role in attracting females of another tephritid species.

Other studies reported evidence of the presence of attracting pheromones in this species, but did not differentiate the significance of pheromonal from acoustic signals on attraction of females. For example, females of D. dorsalis were attracted by both live males and rectal glands removed from males (Kobayashi et al. 1978, Schultz and Boush 1971). These glands were suggested to be used for both production and storage of the pheromone because their attractiveness increased progressively during the day and peaked during the period associated with sexual activity. The substance suggested to be a sex pheromone and released as "smoke" by males of _D_. _dorsalis_ was, however, found to be only slightly attractive to females (Ohinata et al. 1982).

The influence of acoustic signals on the attraction of females to signaling males was also demonstrated in this study (Table 7). Certain sound components may be important in inducing female visitation, and utilized to facilitate female orientation towards males. The pheromone dispersed by the same wing-fanning behavior of males may be used primarily for attracting females. This was suggested by the stronger approaching response of females without the acoustic receptors as compared to that of females without the pheromone receptors. However, most of the females with either type of sensory system eliminated failed to approach males (Table 7).

Second Series (Study 5-6). Close-Range Courtship

At close range (average 6 cm), matings were drastically reduced in males with more than 50% of their wings removed or the wing bases waxed (Table 8 and 9). These results conclusively demonstrate that the normal wing fanning of males during courtship is important for successful mating. Males were better able to mate if they possessed sufficient wing area (only wing tips removed) and fanned their wings normally. Males with more than 50% of their wings removed still fanned their wings normally, although they may not emit proper signals. On the other hand, males with only the tips of their wings removed were as successful as non-modified males.

Keiser et al. (1973) reported that wingless males of D. dorsalis mated as successfully as winged males. The difference in materials and methods between their study and mine may explain the discrepancy in results. First, I used a recently established laboratory colony of the 4th-6th generation, but they used a long-term laboratory colony of about 22 years (about 264th generation). Second, in my study matings were conducted individually as opposed to continued mass matings for 3 days. Females were likely to be repeatedly experiencing attempted copulations by several competing males and forced to mate eventually under a crowded condition of small cages. Third, I scored the actual copulations, but they scored "matings" by measuring the total numbers of eggs and percentages of egg hatch. The number of eggs is an inherent character of the female. Furthermore, the percentages of egg hatch may be influenced by sperm displacement resulting from repeated matings with several males, and thus is not an accurate measurement of mating.

Females without either visual or sound receptors mated as effectively as females with non-modified receptors (Table 10, and 11). D. cucurbitae females also mate successfully when their compound eyes are painted (Keiser et al. 1973). Visual signals may have minor importance in the mating behavior of D. dorsalis, first, because mating takes place at dusk. Furthermore, males of this species possess neither an elaborate visual courtship display nor distinctively patterned wings which could be utilized as visual signals during male-female encounters at close range. The results of my other experiments (unpublished data) showed that males mated normally in spite of extra black markings that were added to their wings or black paint covering the yellow markings on their thorax.

Apparently acoustic signals play little or no role at close range in eliciting copulation (Table 10, and 11) . The male wing-fanning may play a major role in dispersal of pheromones, but the buzzing sound could be produced incidentally. Even though sound may not be significant for mating success in D. dorsalis, it is believed to play a role in the mating of other tephritids. In T. curvicauda, for example, the recorded "approach song" (directed at females prior to mounting) and "precopulatory song" (produced immediately after mounting the female), when played back to virgin females provided results suggesting that sound serve to hold a female in place (Sivinski and Webb 1985a). The "precopulatory song" produced by A. suspensa males during the insertion of the aedeagus modifies the female behavior resulting in female reception and longer mating durations (Sivinski et al. 1984). Surprisingly, females with the aristae removed still responded to the precopulatory song normally, and rejected mounted males as frequently as normal females (Sivinski and Webb 1985b). They concluded that the aristae are not the only sound receptor in A. suspensa, and that sound produced as the male mounted a female could be received by mechanoreceptors present on the body, legs, and/or wings of the female. Their results suggest that an examination of the role of mechanoreceptors in D. dorsalis might prove fruitful. Various types of male sounds have been qualitatively described and correlated with male mating success (Webb et al. 1983b, 1984). In one study, acoustical properties of sound produced by males and thought to be important in sexual success were quantified and compared among males that copulated and those that did not mate (Webb et al. 1984). In contrast to the above analysis, my study has used experiments to directly test whether or not acoustic signals play a role in mating success.

Mating was significantly reduced when female antennae were painted (Table 10, and 11: lines b, c). This clearly demonstrates that a chemical stimulus was crucial for mating by the females. This is the first study in tephritids that has directly demonstrated the significance of an olfactory signal for mating. Previous studies on A. ludens, A. suspensa, C. capitata, D. cucurbitae, D. latifrons (Malaysian fruit fly), D. tryoni, and R. pomonella reported only the presence of pheromone (Feron 1959, 1962, Fletcher 1968, Kobayashi et al. 1978, Little and Cunningham 1987, Nation 1972, Prokopy 1975, Robacker and Hart 1986). In my study a significant reduction in mating success was detected when the entire antennae and aristae were removed from females (Table 10, and 11: line d). Such a reduction in mating could be due entirely to the lack of pheromone reception alone, since sound was not a significant close-range signal (Table 10, and 11: line b). This is similar to the mating inhibition that occurs in C. capitata when both antennae were removed from females (Nakagawa et al. 1973).

Based on lines b, c, e, f, and h of Table 11, pheromones appear more important than acoustic signals in determining whether a female would mate or not. However, on line j the effect of pheromone-plus-acoustic signal elimination significantly reduces mating of females greater than the effect of pheromone elimination alone. The lack of acoustic signals alone was insufficient to significantly reduce female mating as shown by its lower magnitudes in effect than the pheromone elimination (Table 11: lines b, c, e, and f). However, the elimination of acoustic signals may have had some degree of additive effect in one case (line j). Even though antennal removal may be thought as such a drastic operation that it may cause females to behave abnormally, they were physically active, but did not respond to signaling males as readily as normal females. Many of these females with their antennae removed also mated.

D. dorsalis females were noticed to extend their ovipositors and probe through the screen of the mini cages containing males (**Study 2**). This probing response has also been reported from sexually excited females of C. capitata, or D. tryoni females exposed to the content of a "male pheromone gland" (Feron 1962, Fletcher and Giannakakis 1973). It was used as an indicator of sexual response of D. tryoni females to pheromone. This female probing response was also seen in D. dorsalis females when they were near males in the mini cages. This female behavior reinforces the findings that females respond to pheromones, which are significant for mating success.

In summary, visual stimuli did not play a role at close range for successful mating. During the attraction phase, males used acoustic signals as well as pheromones to attract females. Pheromones appeared to be dispersed by the wing fanning, and may be used as a primary stimulus for attracting females. Acoustic stimuli, on the other hand, may be less important than olfactory signals. During the courtship phase, females appeared to mate based solely on an olfactory signal produced in close-range encounters. Females did not discriminate among the courting males on the basis of the presence or absence of an acoustic signal which could be produced incidentally while the males apparently fanned their wings to disperse the pheromone. Sexual communication in this species depends on olfactory and acoustic stimuli emitted by the male wing-fanning, and olfactory signals clearly plays a major role in attracting females to the mating sites as well as satisfying the courtship requirements of the females.

CHAPTER V

CONSTITUTION OF A SEXUALLY SUCCESSFUL MALE IN Dacus dorsalis

Variations of mating success in males may be the result of one or both of the two primary components of sexual selection, intersexual selection and intrasexual selection (Darwin 1871). Intersexual selection is the process in which individuals of one sex, females in most cases, exhibit a preference for mating with individuals of the other sex, males, possessing particular traits. Intrasexual selection occurs through the competition between members of one sex, usually males, for the individuals of the other, females. Darwin (1859, 1871) viewed females as the more discriminating sex in mate choice. Bateman (1948) reasoned that the difference in discrimination is caused by the sexual inequality in reproductive success. In more general terms, the sex which provides the greater parental investment should be the discriminating sex in mate choice (Trivers 1972). In most animals, the female is more likely to be the discriminating sex.

There is a large number of examples in which intermale competition determines male mating success (Partridge et al. 1987). Direct evidence of female preferences for particular male traits is limited (Thornhill and Alcock 1983). There are two reasons for the limited evidence for female preferences. First, variation in female preferences towards males may be masked by intermale competition (Borgia 1981, Cox and LeBoeuf 1977, Halliday 1983, Partridge and Halliday 1984). Second, female choice may be exercised in a very subtle way involving passive attraction to sexual signals emitted by males (Parker 1982). Nonrandom mating of males has been suggested as evidence for female choice, but this may also result from intrasexual selection. Stronger evidence for female choice, however, exists in the case in which females exhibit consistent preferences among males that do not interact physically. Another is an indication of a mating advantage of males possessing certain characteristics towards which females respond positively (Searcy 1982). For example, females of cockroach, Nauphoeta cinerea prefer dominant males and their odor (Breed et al. 1980).

Sexual selection results in the evolution of traits such as elaborate or secondary sex characteristics and sophisticated mating rituals (Darwin 1871). Some of these traits have evolved as a result of mate competition occurring prior to copulation. The forms of competition may include the ability to search or fight for possession of partners, territories, or dominance status that is related to mate acquisition (Thornhill and Alcock 1983). The nature of mate competition varies between species. For example, in matings which take place on resources controlled by males, as in Tephritis dilacerata, males compete to locate resources quickly, defend them against other males, and seize encountered females (Parker 1970, 1974a). If matings take place away from resources, competition may be by direct male-male aggressive encounters. Competing males may fight for favorable signaling stations, or may produce more attractive signals. Examples include aggression by individual males on territories where the attracting signals are produced as in *Anastrepha suspensa*, *Ceratitis capitata*, and *Dacus tryoni* (Prokopy and Roitberg 1984).

Several behavioral characteristics may influence the variance in males mating success. First of all, success in fights of some species is known to determine male mating success (Aspey 1977, Dow and von Schilcher 1975, Jacobs 1960). In tephritids, the ability to establish a calling station is believed to indicate successful fights against other males (Burk 1979). Secondly, males with greater mating success may produce more intense or more continuous signals than those with lower mating success (Burk 1979, Crankshaw 1979, Forrest 1980, Hedrick 1986). In several tephritid species, males possessing such superior behavior in producing signals are evidently successful. For example, successful males of *A. suspensa* have a higher calling propensity, repeatedly produce calling song pulses, and win more fights than non-successful ones (Burk 1983, Burk and Webb 1983). Those of *C. capitata* and *Toxotrypana curvicauda* (papaya fruit fly) also produce louder and more attractive sounds to females (Churchill-Stanland et al. 1986, Sivinski and Webb 1985). Therefore, based on these studies, it seems likely that male characteristics which are correlated with sexually successful males may involve signal production, and ability to win fights against other males.

The goal of this study was to determine particular behavioral components that make certain males of *D. dorsalis* so sexually successful. "Male sexual success" was measures by the number of females a male could attract and mate with, i.e. the greater the number of females each individual male attracted and mated with, the more successful he was. "Highly sexually successful males" were defined as those that could attract and mate with three or more females, and "intermediate" category males were defined as those that mated once or twice.

The objectives in this study were:

1. To determine what percent of a group of males was successful in both attracting and copulating with females.

2. To determine if attractiveness and mating ability were inherent in the same males by examining if attractive males could mate with many females, and successfully mating males could also attract many females.

3. To look for differences between males in intermale aggression and the amount of time spent in signaling.

MATERIALS AND METHODS

Study 1. Variation in Male Success

As defined earlier, a "sexually successful male" was one with high capability for attracting and mating with females. In this study, attractiveness (defined below) and mating ability (defined below) of males were measured in two separate tests.

Experiment 1. Attractiveness

Fifteen mature virgin males were placed singly in fifteen mini, screened (white nylon veil), cylindrical cages (3-4 cm in diameter, 5-6 cm in height) hung on a potted guava plant. This plant was placed in a large screened cage (60 x 120 x 60 cm) into which 40 mature virgin females were released. Females landing on male cages were captured and their numbers were recorded during an hour around dusk. Males tested were then grouped, according to the number of females attracted to them, into 3 categories: "Low" did not attract any females, "Intermediate" attracted one or two females, and "High" attracted three females or more. A total of 9 groups of males was tested.

Experiment 2. Mating ability

Twelve pairs of mature virgin males and females were placed in each of 34 plastic gallon cages (20 cm in diameter, 20 cm in height). Males were individually marked before being tested. Matings were recorded for 6 to 11 consecutive days. Matings in the first four groups of five cages each were observed for 6, 7, 8, and 9 days, and those of the last two groups of seven cages each were observed for 10 and 11 days. Each day, mated males were placed back into the cage but all females were replaced with new virgin females. The number of times males copulated was used to categorize their mating ability into 3 groups: "Low" never mated, "Intermediate" mated 1-2 times, and "High" mated 3-6 times. The distribution of individual male mating success was obtained from a total of 408 males in 34 groups.

Study 2. Attractiveness and Mating Ability of Males

In this study, several groups of males were tested in two separate experiments, each consisting of two steps. The first step was to test for differential success in either attractiveness or mating ability. The second step was to further test for differential success in the aspect which had not been tested in the first step.

Experiment 1. Attractiveness

Fifteen mature virgin males were individually marked and placed singly in mini-screened cages (as in **Study 1**). These fifteen cages of males were hung on a guava plant at random. Randomization of the male cages on the plant was done by using a randomization program written by A. Moore. Each of the 15 males as well as 15 different leaves was numbered. Each day, mini cages of numbered males were hung on numbered leaves according to the randomization program. This plant was placed in a large cage into which 40 females were released. Females landing on each male mini-cage were captured during the hour of the test (between 50 min before and 10 min after dusk) and used in **Experiment 2** conducted on the following day. The number of females attracted to males was used to placed the males into 2 categories: "non-attractive" males did not attract any females, and "attractive" males attracted one female or more (the number of females attracted per male: mean = 1.07, SD = 2.16).

Another test was conducted by placing two males obtained from the previous test, an attractive and a non-attractive male, with one female in a cage (8 cm in diameter, 8 cm in height). Matings were recorded each day after dusk. The test in each cage was terminated when a mating was observed or after 5 days, whichever came first. A total of 46 males of each type, attractive and non-attractive, was tested.

Experiment 2. Mating ability

Twelve pairs of mature virgin males and females were placed in each of 25, 1-gallon cages. Males were individually marked before being tested. Matings were recorded approximately 15 min after dusk. After mating, mated males were placed back into the cage and all females were replaced with new virgin females. This test was continued for several consecutive days until at least one male in each of four categories: 0, 1, 2, and 3-time maters was obtained. These males were then used in the next experiment to test for their ability to attract females.

One male from each category was then confined singly in a mini cage. The four cages, each holding a male from a different mating success category, were placed at random on one plant. Randomization of the male cages on the plant was done by using a method similar to that described in **Study 2: Experiment 1,** except that only four cages and leaves were used. This plant was placed in a large cage into which 40 females were released. The females landing on male cages were captured and counted during the test hour between 50 min before and 10 min after dusk. A total of 21 groups of males was tested incorporating 42 non-successful and 42 successful mating males. Non-successful males included males that never mated and those which mated only one time, successful males were defined as males that mated two or three times.

Study 3. Behavioral Components of Sexually Successful Males

The overall activity of males during the hour before dusk, consisting of wing fanning, numerous male-male aggressive encounters and copulation attempts was observed. Special attention was placed on wing fanning and aggression. A pre-test was conducted to find males which were successful in mating. Successfully mating males obtained from the **Pre-test** were further investigated for their differential attractiveness in **Experiment 1**, and for any difference in intermale aggression and the duration of wing fanning in **Experiment 2**.

Pre-test. Twelve pairs of mature virgin males and females were placed in each of 21 plastic gallon cages. Males were individually marked before being tested. Each day after matings were recorded, mated males were placed back into the cage and all females were replaced with new virgin females. This test was continued until at least one male in each of four categories: 0, 1, 2, and 3-time maters was obtained. These males were then used in the following two experiments.

Experiment 1. Attractiveness

One male from each category was then confined singly in mini cages and hung on a plant at random as described in **Study 2: Experiment 2**. This plant was placed in a large cage into which 40 females were released. The total landings by females were recorded during an hour around dusk. A total of 21 groups of males was tested. Spearman correlation coefficients were computed for the number of times the males had mated and the number of times that females landed.

Experiment 2. Behavioral components

One male from each category was placed in a glass cage 30 x 30 x 30 cm. The components of male behavior which were examined were (a) wing fanning, and (b) intermale aggression.

(a) The duration of wing fanning was examined to determine whether males of different mating success differed. The total time that each male devoted to wing fanning was measured by using four stopwatches. Spearman correlation coefficients were computed for the number of times the males had mated and the duration of wing fanning.

(b) The other component of male behavior that was examined was intermale aggression. The majority of encounters occurred when a second male approached the wing-fanning male. At this moment, the male stopped fanning his wings and then engaged in a fight or attempted to copulate. Two males fought with one another by butting heads, clashing the extended mouthparts, or pushing and bumping each other at an angle by the head or thorax. A male was considered to be a "winner" if he caused another male to leave the fighting area, or chased another male. He was declared a "loser" if he left the fighting area, or was chased by another male. When both males left the fighting area simultaneously, the bout was declared to be a tie. Observations of aggressive encounters were recorded on tape and transcribed later to determine the frequency with which certain males won fights.

RESULTS

Study 1. Frequency of Sexually Successful Males

Males which attracted (**Experiment 1**) and mated with (**Experiment 2**) many females comprise only a small portion of all males, i.e. 16 and 18% respectively (Table 12). About 2/3 of successfully mating males could attract females. The majority of males did not attract or mate with females at all.

Study 2. Attractiveness and Mating Ability of Males

When the mating ability of males was tested, "attractive" males were significantly more successful in mating with females than "non-attractive" males (Table 13, part A). When male attractiveness was tested, "successful" mating males attracted significantly more females than "non-successful" mating males (Table 13, part B). Evidently, highly attractive males were also more successful in mating than "non-attractive" males and vice versa. Highly sexually successful males seemed to possess both attractiveness and mating ability.

Study 3. Behavioral Components of Sexually Successful Males

The results on the differential attraction of males based on the total landings by females (**Experiment 1**) are presented in Table 14. As the male mating scores increased,

Table 12. Variation in male sexual success (both attraction and mating)

Category	No.males tested[a]	% Males in each category		
		Low	Intermediate	High
Attractiveness	135	62.22	22.22	15.55
Mating	408	42.89	38.73	18.38

[a] The period during which the mating test was conducted varied from 5-11 days, and no indication of variation among days was found.

Table 13. Success of *Dacus dorsalis* males in attracting and mating with females

Initial score	Number males tested	% Mating (case A) or % Female landings (case B)	chi-square[a]	P[b] ≤
(A). Test of mating success of males initially scored by attractiveness:				
Non-attractive	46	26.09	10.52	0.01
Attractive	46	73.91		
(B). Test of attractiveness of males initially scored for mating success:				
0-1 copulations	42	5.56	583.11	0.001
2-3 copulations	42	94.45		

a chi-square values are pooled.

b The chi-square test is based on the actual numerical frequencies of response, however, the data are presented in percentages for ease of understanding. The chi-square test for heterogeneity is non-significant in both cases (case A: chi-square =2.52, df =7. P ≥0.975; case B: chi-square =7.34, df =20, P ≥0.995).

Table 14. Attraction of females in 30 min observation period in relation to mating success

Male category	Number males tested	Number of times females landed	
		Mean	SD
0-time mater	21	0.52	0.98
1-time mater	21	1.43	1.80
2-time mater	21	3.71	3.65
3-time mater	21	29.48	13.76

the mean number of female landings increased. The significantly positive correlation between the number of times the males had mated and the number of times that females landed, was r_s =0.79 (P =0.0001). Highly successful males, 3-time maters, attracted four times as many female as those attracted by all other less successful males (0-, 1-, and 2-time maters) combined (83.9% vs 16.1%). Moreover, they continued to be the most attractive males for at least 4-5 days. Females usually approached fanning males. However, 3-time maters appeared to be able to attract more females than other less successful males whenever two males fanned their wings at the same time. Sometimes, less successful males were able to attract a few females when they fanned their wings while the 3-time maters were inactive. Such attractiveness of the less successful males often subsided, i.e. they attracted only 1-2 females or none as soon as the 3-time maters resumed fanning their wings.

The amount of time males spent in wing fanning was the first component of male behavior to be measured, since several observations made previously indicated that females were attracted to males which were fanning their wings (**CHAPTER IV**). During a narrow period of sexual activity at dusk, 3-time maters spent twice the amount of time (70.8%) in wing fanning that the other males did (combined total =29.2%) (Table 15). The mean duration of wing fanning in the 30 min observation period became longer as the male mating scores increased. The correlation between the number

Table 15. Duration of wing fanning in 30 min observation period in relation to male mating success

Male category	Number males tested	Time (min) males spent in wing-fanning	
		Mean	SD
0-time mater	19	0.30	0.61
1-time mater	19	1.05	1.21
2-time mater	19	2.54	3.13
3-time mater	19	9.42	2.88

of times the males had mated and their duration of wing fanning was significantly positive (r_s =0.76, n =21, P =0.001).

Aggressive encounters among males was the other behavioral component which was examined in detail. Three-time maters were superior in fighting. First of all, 3-time maters did not frequently engage in intermale aggression unless they were approached by less successful males. Most fights ended when the less successful male flew or ran away. Out of 19 cases in which males of all four categories were involved in fights, 3-time maters had the top rank in 16 cases, tied for top in one case, and lost top rank to the 2-time maters twice. Second, when a 3-time mater fought, the fight ended within a few seconds, and he resumed his wing fanning. Third, in several encounters, one male landed right next to the fanning male who was a 3-time mater and flew away immediately without any fight initiated. In a few cases, the determination of a win/tie/loss was not obvious. For example, sometimes two males approached one another until they were about 2 cm from each other, then one or both flew away. Often, two or three males were seen to push each other simultaneously with their heads and stay in such a position for 5 min or more.

The number of aggressive acts/cage/hr averaged 9.6 (range 2-20). Three-time maters did not frequently engage in aggressive interactions unless challenged by the other less successful males, 0-,1-, or 2-time maters.

DISCUSSION

About 38% of the males produced acoustic and olfactory signals (**CHAPTER IV**) attractive to females through the wing-fanning behavior (Table 12). However, about 57% of all males were successful in mating. In other words, about 2/3 of successfully mating males were able to attract females, but about 1/3 were not. In **Study 1: Experiment 1**, females were completely free to visit males without being harassed or controlled by males. The score of 38% attractive males was therefore purely based on female preferences. However, in **Experiment 2**, both males and females were constrained at close range. Some non-attractive males could mate females that would have been attracted by other signaling males. The production of attractive signals is, therefore, an important first step to mating success in most cases, as it brings males and females together. Nevertheless, some of the unattractive males, 1/3 of successfully mating males, could have mated with females successfully given the opportunity to be near females. Females may have been the recipients of repeated attempts to copulate by these males and might copulate because they were unable to escape. Another possibilty is that certain males produce adequate courtship signals despite being unable to attract females.

In **CHAPTER IV**, the combination of both sound as well as pheromones was shown to be significant in attracting females from a distance. However, at close range females chose males based primarily on male pheromones that appeared to be dispersed by the wing fanning. These pheromones, used in attracting females or to facilitate mating, could be of the same or different compounds. The results of study 2 (Table 13) showed that both attractiveness and mating success were present in the same individual males. These seemed to indicate that sexually successful males were able to produce sounds and pheromones used for both functions.

In addition to producing the necessary sexual signals, D. dorsalis males showed some degrees of aggressive interactions during the period of sexual activity, similar to those in A. suspensa (Dodson 1978). Aggression may be settled in a form of dominance hierarchy, in which a subordinate male usually withdraws from a fight with the dominant male. In A. suspensa, fights among males for possession of leaf territories determines access to females (Burk 1983).

Differences in mating success among males could be the result of intermale competition as well as of female choice operating under the situation in which the **Pre-test** experiment was set up: individuals of both sexes were together and may have interacted. Intermale aggression was common during the period of sexual activity, but did not occur at a substantial rate. Nevertheless, the outcome of aggressive interactions among D. dorsalis males (**Study 3**) appeared to be correlated with which male would mate. The 3-time maters had the top rank in 16 out of 19 cases. Thus, intrasexual selection may be a component of sexual selection operating in this species.

Time wasted as a result of participating in a prolonged dispute with other less succesful males reduced the amount of time that a male could perform the behavior necessary to gain mates, i.e., wing fanning. By winning disputes quickly, the 3-time maters may have had more available time to attract and mate with females. In many instances, less successful males were relatively inactive i.e. did not either fan their wings or fight, while the 3-time maters were active throughout the daily period of sexual activity. The ability to produce continuous signals over a long time may be important in determining mating success.

In this species, the communication signals included pheromones as primary, and sound as secondary components (**CHAPTER IV**). The proper chemical structures of pheromones and their concentration, or proper sound characteristics and intensity may be important components in these signals, as well as the duration of their emission. For example, A. suspensa females preferred males that produced loud precopulatory songs (Burk and Webb 1983, Webb et al 1984). The results in which the 3-time maters with a long duration of signal production had higher attractiveness and mating success than other less successful males may be analogous to female preferences for long calling bout length of male field crickets, Gryllus integer (Hedrick, 1986). In addition to the production of signals through the intense and long duration of wing fanning, males which signal frequently may often, by chance, signal alone and thus be easily located by the females.

Sometimes, larger male size has been reported to be related to higher mating success (Burk 1983, Burk and Webb 1983, Partridge et al. 1987). In fact, the possibility for larger males to be more sexually successful than smaller males may be due to the larger males' superior ability in producing signals rather than female preferences for large male size. For example, successfully mating males which repeated their calling song pulses more rapidly as in _A_. _suspensa_ (Burk 1983, Burk and Webb 1983a), or produced more courtship songs and sang more loudly as in _D_. _melanogaster_ (Partridge et al. 1987) were also larger than non-successful ones. On the other hand, no correlation between male size and mating was found in _A_. _suspensa_ (Webb et al. 1984).

On several occasions, 3-time maters were the most attractive males among four categorized males, and continued having top rank for the 3 or 4 days over which they were observed. In _C_. _capitata_, however, the most dominant male did not remain dominant for the 3 days that they were observed (Arita and Kaneshiro 1985). It would be worthwhile to further examine how long the dominant status in _D_. _dorsalis_ can be maintained.

Strong evidence for female preferances for male characteristics is scarce (Thornhill and Alcock 1983). Nevertheless, this study is one of a few successful attempts to detect female discrimination, in addition to its significance as the only study of this sort in Tephritidae. In the test of attraction, visitation of males by females was completely under female control involving an active approach by females to sexually signaling males. Males were singly confined in each mini cage and hence could not interact. Moreover, females were shown (Table 13) to exert consistent preferences for certain males by visiting 3-time maters significantly more frequently than other males ("attraction preferences"), or mating with attractive males significantly more than non-attractive males ("mating preferences"). The above experimental evidence indicates that female preferences are an important component of sexual selection in this species.

Several reasons led me to believe that female choice may be a significant component of sexual selection in this species. First, there was a substantial rate of males attempting to copulate with each other, even by the 3-time maters. Evidently, males were unable to differentiate between the sexes. Second, a low incidence of male rivalry initiated by the 3-time maters could simply allow them more time for signaling. Other males, besides the 3-time maters, usually remained relatively inactive or engaged in prolonged fights. Third, the present evidence indicated that mated females became less responsive to signaling males, and less receptive to subsequent matings (**CHAPTER III**). If most of the females do not become receptive after mating, i.e. mate only once, they may be very selective in mate choice in order to ensure a high level of egg fertility. Fourth, perhaps the most important evidence, female choice was experimentally demonstrated to be exercised in this species by the females making active choice in visiting the three-time maters more frequently than other less successful males.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Public awareness over the excessive use of chemical insecticides has led many entomologists concerned with insect pest control to turn more toward integrated pest management based on a solid foundation of biological and ecological information. As a result, research entomologists currently place great emphasis on the use of the Sterile Insect Release Method (SIRM) for control and eradication of fruit fly species in the family Tephritidae. The SIRM involves the release of sterilized, laboratory-reared flies into a natural population to compete for mating with wild females. The effectiveness of the SIRM, therefore, relies on the ability of the laboratory-reared males to find or attract females, and to perform courtship displays properly to achieve successful matings with most of the females in the natural population. Therefore, the quality of sexual behavior in the laboratory-reared strains is of major concern in any SIRM program.

The lack of information on sexual communication of the Oriental fruit fly, D. dorsalis, and the realization that an understanding of its sexual behavior is a significant element in any control efforts, especially those using SIRM, led to the development of this research. Various aspects in the sexual behavior of the species in order to increase our overall understanding of its complex mating system was investigated.

In CHAPTER III, experiments were conducted to describe the overall pattern of sexual behavior in this species. It was found that there were three major components in the sexual behavior: 1) signal emission and attraction phase, 2) courtship behavior, and 3) copulation. The predominant male behavior during both signal emission and courtship behavior was wing fanning. Sexually receptive females were attracted towards these signaling males. During close-range courtship, males simply fanned their wings rapidly and continuously before attempting copulations, while females remained relatively inactive.

The above sexual activities including male wing-fanning, attraction of females to the males' signal, and mating were found to be closely correlated with the ages of the adults. The onset of these sexual activities coincided with the attainment of sexual maturation. However, if females had already mated, they neither approached signaling males nor accepted subsequent matings as readily as virgin females. Moreover, such inhibition of responding and remating did not decline, even at 18 days after the first mating.

The wing fanning by males accompanied by the touching of the wings with the legs while stroking their abdomens appeared to generate two types of audible sound. The "buzzing" sound was generated by the wing fanning, and the "chik" sound appeared to be produced by the touching of the wings with the legs which was heard rhythmically intervening with the buzzing sound. In addition, an odor suspected to be a sex pheromone was being released during the wing fanning of males. Therefore, the role of male wing-fanning and the signals emitted through this behavior was further investigated.

In CHAPTER IV, sexual behavior in this species was investigated in two main phases: 1) the attraction phase which brings males and females from a distance to the same place and at the same time for mating, and 2) close-range courtship behavior which is displayed by the male and used by the female to either accept or reject the courting individual.

The attraction signals produced by male wing-fanning was significantly more attractive to females than other males. On the other hand, males were equally attracted to both males and females. Movements and interactions among males in the vicinity of each other appeared to stimulate them to actively fan their wings, increasing signal production.

Experiments were conducted to demonstrate the significance of male wing-fanning from the perspective of female behavior toward signaling males. This was accomplished by inhibiting normal male wing-fanning or female sensory reception. The wing fanning of males was found to play a significant role in the production of signals necessary for attraction of and mating with females.

During the attraction phase, males utilized both acoustic and olfactory signals to attract females to their mating sites. Pheromones appeared to be dispersed by wing fanning and used as a primary stimulus for attracting females. Even though the acoustic stimulus produced by the same wing-fanning behavior appeared to be important for attracting females, it may be less significant than the olfactory signal. This was suggested by a stronger response of females without the acoustic receptor as compared to that of females without the pheromone receptor.

During the courtship phase, males continued to wing-fan. Olfactory signals were the only important stimulus for successful mating. Neither acoustic nor visual signals appeared to be important during courtship.

In CHAPTER V, experiments were conducted to determine the variation in male sexual success, in terms of both attraction and mating. It was found that attractiveness as well as mating ability was mostly present in the same individual males. About 0.66 of successfully mating males produced acoustic and olfactory signals attractive to females. About 0.33 were not able to attract females but when given the opportunity to be at close range with females they mated successfully. About 43% of the males did not mate at all.

Males showed some degree of aggression during the period of sexual activity. Males which frequently had the top rank in aggressive interactions were successful in mating with several females. This occurrence suggested that intrasexual selection may influence mating success in this species. These dominant males in aggression did not frequently initiate or engage in prolonged fights.

Females were shown to exert consistent preferences by visiting 3-time maters significantly more frequently than less successful males, or mating with attractive males significantly more than with non-attractive males. This study is one of a few successful attempts to detect female preferences for certain males, in addition to its significance as the only study of this sort in Tephritidae. The results provide strong evidence indicating that female preferences affect male mating success in this species.

In addition to the above experimental evidence of female choice, an indirect evidence also suggests its role in this species. First, the fact that males frequently attempted to copulate with each other indicates their low ability to differentiate between the sexes. Second, mated females became less responsive to signaling males, and less receptive to subsequent matings. If females never regain receptivity after mating, i.e. mate only once, they may be very selective in mate choice in order to mate with males that ensure a high level of egg fertility.

In conclusion, this research has generated new information pertaining to sexual communication in D. dorsalis. Such information has several practical benefits in the development of effective control strategies.

For example, the strong implication that pheromones are used for both attracting and mating with females, and thus more important than acoustic stimuli provided a useful direction for developing more effective control programs. Further investigations on the nature of these volatile chemicals such as their characterization, synthesis, and the possibility of developing blocking agents seem worthwhile endeavors. Synthetic sources of either pheromones or blocking agents can be used to confuse wild virgin females which are searching for mates.

The presence of female choice in D. dorsalis can influence the success or failure of a program using SIRM. Females are likely to discriminate against males that lack certain attributes. In order to participate in mating with the wild females, sterilized, laboratory-reared males must possess attributes which make them attractive to females and be able to compete effectively against wild males in mating with the wild females. The basic knowledge of the sexual signals required by choosy females gained from this research can be useful as baseline information for maintaining laboratory strains with high behavioral quality, and monitoring the quality of mass-reared, sterilized stocks.

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