

PHYSIOLOGY OF MATING BEHAVIOR IN MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE): CHEMORECEPTION AND MALE ACCESSORY GLAND FLUIDS IN FEMALE POST-MATING BEHAVIOR

E. B. JANG

USDA-ARS, Pacific Basin Agricultural Research Center, P.O. Box 4459, Hilo, Hawaii 96720, USA

ABSTRACT

Studies on behavior of tephritid fruit flies have historically focused on the interaction of external stimuli such as temperature, semiochemicals, seasonality, etc., or the interactions of flies between and among species for a number of observed behaviors such as mating, pheromone calling and oviposition. While descriptive behaviors represent much of what we know about these pest species, less is known about the underlying physiological mechanisms which function in priming or modulation of the observed behaviors. In the Mediterranean fruit fly, virgin females are preferentially attracted to the volatile male pheromone over host fruit odors. This behavior switches as a result of mating. Factors from the male accessory gland have been shown to facilitate the switch suggesting a male role in modulation of female olfactory-driven behaviors. Other physiological factors are likely to further influence the degree to which female behaviors are influenced.

Key Words: medfly, *Ceratitidis capitata*, mating behavior, pheromone, host odors, accessory glands

RESUMEN

Los estudios en cuanto al comportamiento de las moscas tefritidas de la fruta se han enfocado históricamente en la interacción del estímulo externo tal como temperatura, semioquímicos, estacionalidad, etc., o en las interacciones de las moscas, entre y dentro de las especies, para un número de comportamientos observados tales como apareamiento, llamado por feromonas y oviposición. Mientras comportamientos descriptivos representan la mayor parte de lo que sabemos de estas especies de plagas, menos se conoce de los mecanismos fisiológicos subyacentes cuya función es de preparar o modular los comportamientos observados. En la mosca del Mediterráneo, las hembras vírgenes son preferentemente atraídas a la volátil feromona masculina por encima de los olores de fruta de su hospedero. Este comportamiento cambia como resultado del apareamiento. Factores provenientes de la glándula accesoria masculina han demostrado facilitar este cambio sugiriendo el papel del macho en la modulación de los comportamientos olfato conductores femeninos. Otros factores fisiológicos son probables que influyan mas adelante en el grado en que se influye sobre el comportamiento femenino.

Much of our current knowledge on mating behavior of the Mediterranean fruit fly, *Ceratitidis capitata* (medfly) has historically focused on the role of external stimuli such as temperature, pheromones, time of day, seasonality, etc., or the interactions of flies with their physical surroundings (host trees, canopy size, lek sites, etc). Descriptive behaviors represent a sizable portion of what we know about this pest. Less is known about the underlying physiological mechanisms which function in priming or modulation of pre and post mating behaviors. For us to fully understand mating behavior in medfly we must have knowledge of the multiple and often complex internal factors which are involved, and the path / mechanisms by which external stimuli results in observed behaviors. The physiological basis of behavior is a vastly understudied research area which could provide important information on how peripheral receptors receive environmental

cues, the transduction and coding of information centrally (from these receptors to the brain) and how behavior is regulated biochemically. The integration of physiology to help explain behavior is central to the goal of understanding the mechanisms of mating behavior and improving the sterile insect technique (SIT) in this species. In this report I would like to review some of the work in our laboratory looking at the link between mating in female medfly and chemoreception.

Chemoreception and Physiological State in Mediterranean Fruit Fly

We have been studying the mechanisms of chemoreception and its link to behavior in the medfly in such areas as olfaction, feeding, mating and oviposition. Our approach is based on the hypothesis that tephritid behavior is influenced by olfactory, gustatory, visual and tactile informa-

tion inputs as primers of behavior and physiology which internally modulates behavior. Behaviorally, tephritids are often classified within a continuum from monophagus to polyphagus, and from "highly chemoreceptive" to "mostly visual" (Jang & Light 1996). However what is known about the underlying receptor systems which accompany these difference in behavior? Do highly chemoreceptive species have different systems than the non chemoreceptive species for detecting semiochemicals? Do differences exist peripherally (the receptor) or more centrally (the brain)?

In earlier studies on female medfly, we focused our research on the influence of plant volatiles on chemoreception and male pheromone on attraction of females. Both sexes exhibited sensitivity to a range of C6-C8 aldehydes and alcohols but did not show sex specific selectivity (Light et al. 1988). Cosse et al. (1995) identified difference in antennal sensitivity to mango volatiles. We also identified over 50 compounds produced by calling male medflies and compared electroantennogram responses of males and females to the identified chemicals (Jang et al. 1989). Although we found some differences in sensitivity and selectivity between male and female antennae, female sensitive compounds were not attractive when assayed individually in the wind tunnel. A blend of the five most prevalent identified chemicals were found to be attractive to females (Jang et al. 1994). While conducting assays of female attraction to male pheromone and/or host fruit volatiles we noted that virgin females behaved differently than mated females which triggered our interest in the effects of physiological state on behavior.

Physiological state is an important concept which, when applied to behavior, helps to explain some of the differences observed in response of medfly to standard stimuli. Physiological state can be the result of multiple "effectors" which result in generalized states which we commonly

identify as age, mating status, nutritional history, or more specific molecules such as accessory gland fluids, sex peptides, hormones, etc. which may be involved either directly or indirectly in a specific behavior. These specific molecules represent an understudied area of research due to their secondary recognition as real modulators of behavior. Over the last several years we have seen increasing recognition of the influence of physiological state on behavior which has heightened our awareness of its importance to behavior.

Male Accessory Gland Fluid and Female Post Mating Behavior

In many adult tephritids, semiochemicals serve important roles in life history (Jang & Light 1996; Light & Jang 1996). Specific behaviors such as feeding, attraction to pheromone, and oviposition are all probably influenced by semiochemicals such as food odors, pheromones, and host fruit odors. Our current research in Hawaii has focused on the physiological factors which are responsible for olfactory-driven female behavior. Virgin female medflies are attracted to and prefer male pheromone over host fruit odors. However, mating triggers profound physiological (and behavioral) changes resulting in a switch in preference by females to host-fruit odors (Jang 1995). Other physiological and behavioral effects such as inhibition of remating (Nakagawa et al. 1971, Delrio & Cavalloro 1979) have also been reported. When females were injected (abdominally) with extracts of accessory gland fluids from males, they switched their behaviors like their naturally mated counterparts (Table 1). Namely, a switch from preference to male produced pheromone to host fruit (guava) odors. Females injected with saline only did not switch their behavior and continued to be attracted to the pheromone over the host fruit odors. In addition to changes in the

TABLE 1. RESPONSE OF FEMALE MEDITERRANEAN FRUIT FLIES TO MALE-PRODUCED PHEROMONE AND GUAVA HOST ODOR.

	AGF injected	Mated	Unmated	Saline injected
a. Time on Sphere				
Pheromone	4203 ± 786**	2061 ± 312**	8143 ± 1287**	9039 ± 1208**
Guava	14022 ± 2220	18174 ± 1520	3832 ± 1520	3766 ± 645
b. Landings				
Pheromone	6.8 ± 1.9**	4.6 ± 0.7**	12.8 ± 2.4**	12.6 ± 2.1**
Guava	16.2 ± 1.7	17.2 ± 1.2	5.8 ± 1	5.6 ± 1.2
c. Eggs				
Pheromone	0 ± 0*	0 ± 0**	0 ± 0	0 ± 0
Guava	43 ± 13	163 ± 18	6.2 ± 4.6	8.1 ± 5.6

**Significance at $P < 0.01$ level.

*Significance at $P < 0.05$ level.

AGF = Accessory gland fluid.

TABLE 2. RESPONSE OF VIRGIN FEMALE MEDITERRANEAN FRUIT FLIES TO MALE-PRODUCED PHEROMONE AND GUAVA HOST ODOR.

	LRN	LRS	Wild
a. Time on Sphere			
Pheromone	11026 ± 3275**	2779 ± 574	1706 ± 555*
Guava	3859 ± 2116	2148 ± 704	259 ± 152
b. Landings			
Pheromone	11.94 ± 0.93**	6.83 ± 1.47	3.2 ± 0.66**
Guava	5.56 ± 0.78	4.17 ± 1.01	0.6 ± 24
c. Eggs			
Pheromone	0 ± 0**	0 ± 0	0 ± 0
Guava	20.88 ± 3.5	0 ± 0	0 ± 0

**Significance at $P < 0.01$ level.
 *Significance at $P < 0.05$ level.
 LRN = laboratory reared normal.
 LRS = laboratory reared sterile.

olfactory-stimulated switch in attraction from pheromone to host fruit odors, females which were naturally mated or injected with accessory gland fluid, laid significantly more eggs than non-mated or saline injected controls. Females did not exhibit the switch in behavior immediately after mating suggesting that secondary mechanisms may be involved in the regulation of the behavioral switch.

Sterile Males and Female Postmating Behavior

If factors from male accessory glands are important in control of female driven olfactory behavior, what, if any, is the effect of irradiation on the ability of sterile males to switch the behavior of wild-type females? To test this question we set up a series of laboratory flight tunnel assays in which laboratory normal, irradiated and wild-type females were mated to conspecific males and observed for their response to pheromone or host-

fruit (guava) odor. Both laboratory-reared normal and wild virgin females preferred male odor over guava odor (Table 2) (Jang et al. 1998). Laboratory reared normal and wild females mated to normal males switched their behavior and preferred the guava odor over male pheromone. Both laboratory-reared and wild females also switched their behavior when mated to sterile males (Table 3). In both of these tests, sterile females did not exhibit a significant switch in behavior. These studies concluded that irradiated males were equally adept at altering female behavior as non-irradiated males (Jang et al. 1998).

Field Cage Studies on Female Post Mating Behavior

Laboratory-reared and wild female medflies were assayed in outdoor field cages to assess the impact of the mating-induced behavioral switch on mating behavior and oviposition activity. Laboratory and wild type virgin females mated more

Table 3. Response of laboratory normal, laboratory sterile and wild females mated to sterile males.

	LRN	LRS	Wild
a. Time on Sphere			
Pheromone	4556 ± 531**	260 ± 162**	270 ± 140**
Guava	11368 ± 825	1856 ± 445	2821 ± 612
b. Landings			
Pheromone	6.07 ± 0.67**	0.5 ± 0.27**	0.67 ± 0.14**
Guava	14.2 ± 0.88	3.25 ± 0.59	3.83 ± 0.46
c. Eggs			
Pheromone	89 ± 14**	0 ± 0	0.17 ± 0.17
Guava	189 ± 23	0 ± 0	8.33 ± 4

**Significance at $P < 0.01$ level.
 LRN = laboratory reared normal.
 LRS = laboratory reared sterile.

with males on leaves and branches than females which had previously mated with either laboratory normal or irradiated males (Fig. 1) (Jang et al. in press). More of the mated females could be found alighting and ovipositing in artificial spheres emitting guava odor (or authentic apples) hung in host guava trees (Figs. 2 and 3). Females mated with either normal or sterile males exhibited the behavioral switch which we had seen in earlier laboratory studies (increased landings, time on sphere and oviposition/eggs laid). Some quantitative differences were observed between responses of laboratory reared and wild females. Both wild and laboratory reared mated females laid significantly more eggs in artificial spheres emitting guava odor than virgin females (Fig. 4).

Effects of Anti-JH Compounds and Chemosterilants on Behavior

In medfly, anti-juvenile hormone compounds such as precocene and the chemosterilant benzyl 1,3- benzodioxole have been shown to effect synthesis and release of JH from the corpora allata (Chang et al. 1994). This interference in JH production has been shown to affect sex attractancy of male medflies (Chang & Hsu 1982) as well as ovarian development in females (Hsu et al. 1989). Preliminary studies with females treated with these compounds suggest that JH may also be involved in modulation of olfactory behavior.

CONCLUSION

Chemoreception, transduction, age, mating status and nutritional state all play important roles in regulating behavior. Semiochemicals

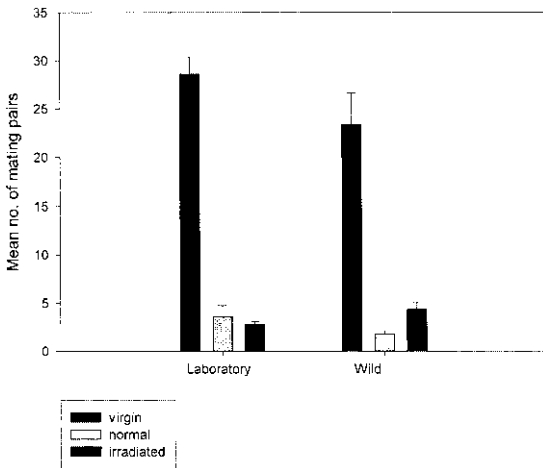


Fig. 1. Mating behavior of virgin and mated laboratory and wild Mediterranean fruit flies in a field cage.

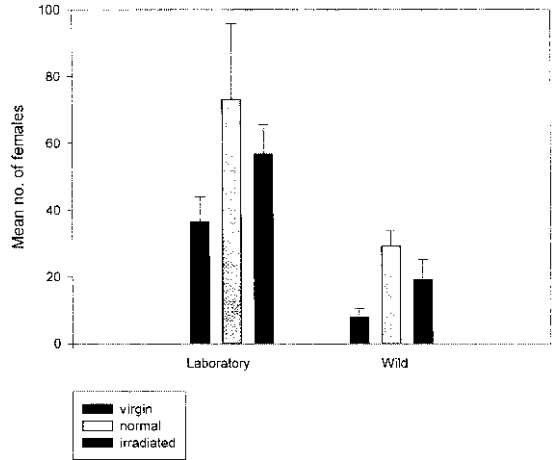


Fig. 2. Cumulative fly counts on spheres of laboratory and wild virgin and mated female Mediterranean fruit flies.

serve as behavioral primers while hormonal activities and cellular homeostasis are further “downstream” in the regulatory process but no less important. In our example we have shown that mating and transfer of accessory gland fluid had a direct impact on female medfly olfactory behavior which probably works through biochemical intermediates and possibly hormones. The specific mechanisms which drive female behavior await further research and promise to be complex but exciting to uncover. Irradiation used in sterilizing mass-reared males for sterile insect release control programs does not appear to affect the ac-

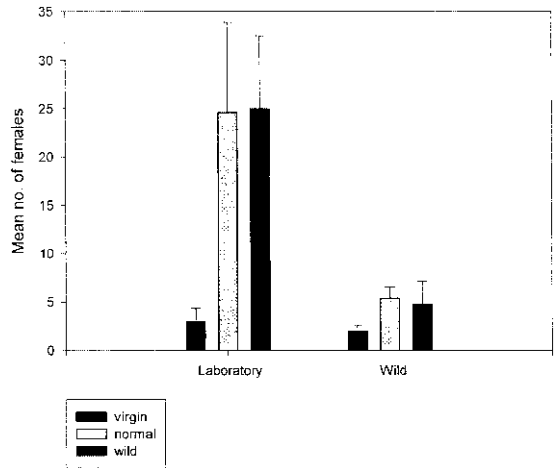


Fig. 3. Cumulative ovipositions on spheres by virgin and mated laboratory and wild female Mediterranean fruit flies.

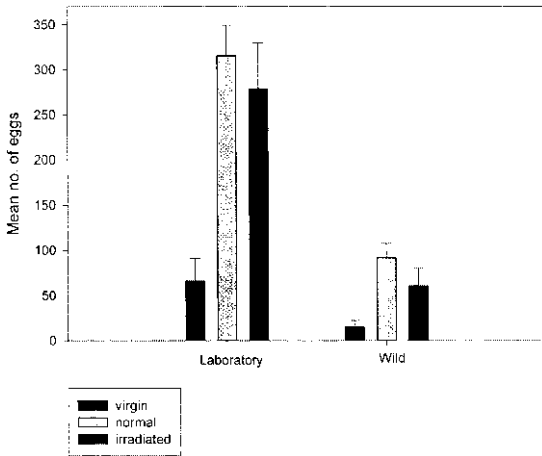


Fig. 4. Number of eggs deposited/h by virgin and mated laboratory and wild female Mediterranean fruit flies.

cessory gland fluid which switches behavior. Improvements in our knowledge of these processes and their control will be the key to development of strategies which target behavioral processes against these pests.

REFERENCES CITED

- CHANG, F., AND C. L. HSU. 1982. Effects of precocene II on sex attractancy in the Mediterranean fruit fly, *Ceratitis capitata*. *Ann. Entomol. Soc. Am.* 75: 38-42.
- CHANG, F., E. B. JANG, C. L. HSU, M. MA, AND L. JURD. 1994. Benzodioxole-1,3-benzodioxole derivatives and their effects on the reproductive physiology of insects. *Arch. Insect Biochem. Physiol.* 27: 39-51.
- COSSE, A. A., J. L. TODD, J. G. MILLAR, L. A. MARTINEZ, AND T. C. BAKER. 1995. Electroantennographic and coupled gas chromatographic-electroantennographic response of the Mediterranean fruit fly, *Ceratitis capitata* to male produced volatiles and mango odor. *J. Chem. Ecol.* 21: 1823-1835.
- DELRIO, G., AND R. CAVALLORO. 1979. Influenza dell'accoppiamento sulla la recettivita sessuale e sull'ovideposizione in femmine de *Ceratitis capitata*. *Entomol. Bari* 15: 127-143.
- HSU, C. L., F. CHANG, H. F. MOWER, L. J. GROVES, AND L. JURD. 1989. Effect of orally administered 5 ethoxy-6-[4-methoxyphenyl] methyl-1,3-benzodioxole on reproduction of the Mediterranean fruit fly. *J. Econ. Entomol.* 82: 1046-1050.
- JANG, E. B., D. M. LIGHT, R. A. FLATH, J. T. NAGATA, AND T. R. MON. 1989. Electroantennogram responses of the Mediterranean fruit fly, *Ceratitis capitata* to identified volatile constituents from calling males. *Entomol. Exp. Appl.* 50: 7-19.
- JANG, E. B. 1991. Olfactory stimulated behavior of fruit flies to the odor of ripening papaya. *Proc. 26th Annual Papaya Industry Association Conference (1990)* pp. 21-24.
- JANG, E. B., D. M. LIGHT, R. G. BINDER, R. A. FLATH, AND L. A. CARVALHO. 1994. Attraction of female Mediterranean fruit flies to the five major components of male-produced pheromone in a laboratory flight tunnel. *J. Chem Ecol.* 20: 9-20.
- JANG, E. B. 1995. Effects of mating and accessory gland injections on olfactory-mediated behavior in female Mediterranean fruit fly, *Ceratitis capitata*. *J. Insect Physiol.* 41: 705-710.
- JANG, E. B., AND D. M. LIGHT. 1996. Olfactory semiochemicals of tephritids, pp. 73-90. *In* B. McPheron and G. Steck [eds.], *Economic Fruit Flies: A world assessment of their biology and management. Proceeding of the Fourth International Symposium on Fruit Flies of Economic Importance*, Sand Key Florida. 586 pp. St. Lucie Press. Delray Beach FL.
- JANG, E. B., D. O. MCINNIS, D. R. LANCE, AND L. A. CARVALHO. 1998. Mating induced changes in olfactory-mediated behavior of laboratory-reared normal, sterile and wild female Mediterranean fruit flies (Diptera: Tephritidae) mated to conspecific males. *Ann. Entomol. Soc. Am.* 91: 139-144.
- JANG, E. B., D. O. MCINNIS, R. KURASHIMA, AND L. A. CARVALHO. 1999. Behavioral switch of female Mediterranean fruit fly, *Ceratitis capitata*: Mating and oviposition activity in outdoor field cages in Hawaii. *Agric. and Forest Entomol.* 1: 179-184.
- LIGHT, D. M., E. B. JANG, AND J. C. DICKENS. 1988. Electroantennogram responses of the Mediterranean fruit fly, *Ceratitis capitata* to a spectrum of plant volatiles. *J. Chem. Ecol.* 14: 159-180.
- LIGHT, D. M., AND E. B. JANG. 1996. Plant volatiles evoke and modulate tephritid behavior, pp. 123-133. *In* B. McPheron and G. Steck [eds.], *Economic Fruit Flies: A world assessment of their biology and management. Proceeding of the Fourth International Symposium on Fruit Flies of Economic Importance*, Sand Key, Florida. St. Lucie Press. Delray Beach, FL. 586 pp.
- NAKAGAWA, S., G. J. FARIAS, D. SUDA, R. T. CUNNINGHAM, AND D. L. CHAMBERS. 1971. Reproduction of the Mediterranean fruit fly: frequency of mating in the laboratory. *J. Econ. Entomol.* 64: 949-950.