Temporal Aspects of Mating Behavior of the Melon Fly,
Dacus cucurbitae COQUILLET (Diptera : Tephritidae) :
A Comparison between Laboratory and Wild Strains

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(Received January 5, 1980)

The laboratory (L) strain of *Dacus cucurbitae* maintained on artificial diet for about 49 generations had a shorter pre-mating period than the wild (W) strain. Ovarian development of the L-strain was faster than that of the W-strain, which suggested a reduction in the pre-mating period of the L-strain. A positive correlation was found between the stage of ovarian development and the female receptivity to mate in the L-strain. The L-strain showed no considerable deviation from the W-strain in daily rhythms of feeding, locomotion, and preening activities. Courtship and copulation attempts were restricted to dusk, irrespective of strain, but the L-strain initiated these sexual activities under higher light intensity than the W-strain.

INTRODUCTION

BOLLER (1972) first stressed the importance of quality control over low production costs in mass-rearing programs, although both are indispensable requisites in the sterile insect release method (SIRM) for eradicating or suppressing pest populations. It is now widely recognized that many unsuccessful applications of SIRM were due to failures in maintaining quality control. IWAHASHI (1979) pointed out, in his thorough discussion on the Kume project, that the success in eradicating the melon fly, *Dacus cucurbitae* COQUILLET from the island was attributable in part to high mating competitiveness of the released males. However, quality control must be given serious attention in the forthcoming project on the eradication of the melon fly in the South-western Is. As ITÔ and KAWAMOTO (1979) suggested, deviation in sexual behavior of released flies from that of wild ones was primarily responsible for the failure in many applications of SIRM so far performed. Thus, monitoring of such deviations is of prime importance. This paper deals with the differences in temporal aspects of sexual behavior between laboratory and wild strains of *D. cucurbitae*.

MATERIALS AND METHODS

Experiments were conducted at the Okinawa Prefectural Agricultural Experiment Station, Naha, Okinawa, Japan, from June to August, 1979.

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Insects. The laboratory (L) strain of *D. cucurbitae* was obtained from a mass-reared culture maintained on artificial diet at the Yaeyama Branch of the Okinawa Prefectural Agricultural Experiment Station, Ishigaki, Okinawa. Rearing techniques have been described in KOYAMA (1979). The culture had been reared through about 49 generations from initial laboratory colonization. The wild (W) strain was obtained as larvae from infested bitter cucumber, *Momordica charantia* var. *pevel* CRANTZ collected at Itoman, Okinawa in June, 1979.

Rearing. Adult flies of both strains were sexed within a day after emergence. Batches of flies were kept in screen cages ($30 \times 30 \times 45$ cm) containing sugar, protein hydrolysate, water, and pieces of squash, *Cucurbita moschata* POIR. Similar cages were used in all of the experiments described herein. The caged flies were maintained in either one of the following rearing rooms:

Room A: Air temperature and photoperiod were maintained at $27.5 \pm 1.5^\circ\text{C}$ and 15L-9D, respectively; fluorescent tubes supplied a light intensity of ca. 3,000 lx at the center of each cage for 13 hr, and ca. 1,500 lx for each of the first and last 1-hr periods of the 15-hr photophase.

Room B: Air temperature was $29.2 \pm 2.3^\circ\text{C}$, which was nearly equivalent to natural conditions; natural daylight entering through the window served as the only light source.

Sexual maturation. The age at the first mating and the relation of ovarian development to receptivity in females were determined with virgin L- and W-strain flies in three different experiments. The experiments and the rearing of flies were performed in room A.

In the 1st experiment, 50 each of L-strain males and females and a similar set of W-strain flies were held in separate cages from the day of emergence. Mating pairs were counted and removed from the cages at about half an hour after the termination of the photophase on successive days with the aid of a pencil torch.

In the 2nd experiment, ovarian development was compared between the two strains in 0-, 5-, 10-, 15-, 20-, and 30-day-old females by dissection of 20 samples for each age. Each female was placed in one of 5 categories, depending upon the stage of the most advanced terminal ovarian follicles. Stages 1, 2, 3, and 4 represented immature ovaries with the follicles being less than 0.26, 0.26–0.50, 0.51–1.00, and more than 1.00 mm in length, respectively, while stage 5 ovaries contained mature eggs.

In the 3rd experiment, 50 male and 50 female 4-day-old L-strain flies were confined in a cage 5 hr before the initiation of the darkphase. Both mated and unmated females were killed 5.5 hr after the confinement to examine their ovaries.

Daily activity rhythm. Three experiments (4th to 6th) were performed in room B with sexually mature virgin flies of the two strains, the 4th to compare general activity throughout daylight hr and the others to compare sexual activity in detail at dusk. In all the experiments, light intensity was measured by an electric photometer (Tokyo Photo-electric Co., Ltd. ANA-300 type) positioned at the center of the experimental cage, facing the window.

For the 4th experiment, batches of 10 males, 10 females, or 10 males and 10 females were prepared for each of the two strains. The batches were reared in room A for 10 days (L-strain) or 15 days (W-strain) after emergence, and transferred to room B 5 days before the experiment. Observations on the activity rhythms of the two strains

were made from 04:30 to 20:00 hr on different days with 16-day-old (L-strain) and 21-day-old (W-strain) flies. Each set was observed twice for 1-min within a 10-min period spaced at 15- or 60-min intervals. The data are presented as the average of the two observations.

For the 5th experiment, a total of 4 batches (2 batches per strain), each containing 10 males and 10 females, were prepared. They were reared in room A for 12 days (L-strain) or 15 days (W-strain) after emergence, and then in room B for 12 days (both strains) till the day of the experiment. The number of males engaged in courtship and the number of pairs in copula were counted at 5-min intervals during 17:00–20:00 hr.

In the 6th experiment, one male and one female, either 10 to 23 days old (L-strain) or 17 to 35 days old (W-strain), were confined in cages at 17:00 hours and their behavior was continuously observed from then until the time when either copulation occurred or light intensity decreased to less than 1 lx. The flies were maintained in room B for at least 3 days before the day of the experiment. The test was replicated 11 times for the L-strain and 20 times for the W-strain. One or two replications were conducted a day. When copulation occurred early enough to allow additional tests, the mated flies were removed from the cage and another pair of flies introduced for observation. The data on such flies are presented separately.

RESULTS

Sexual maturation

The results of the 1st experiment, in which the same number of males and females were confined in a cage from the day of emergence, are shown in Fig. 1. It is clearly demonstrated that the L-strain reached sexual maturity earlier than the W-strain; in the L-strain the first mating and more than 50% accumulated mating occurred

Fig. 1. Accumulated percentage matings in two batches, each containing 50 male and 50 female 0-day-old flies of L- or W-strain at the start of the experiment. One male and one female died on the 2nd day and 16th day, respectively, in W-strain.

Table 1. COMPARISON OF OVARIAN DEVELOPMENT BETWEEN L- AND W-STRAINS
IN 0- TO 30-DAY-OLD FEMALES

Strain	Stage ^a	Days after emergence					
		0	5	10	15	20	30
L	1	20	1	0	0	0	0
	2	0	2	0	0	0	1
	3	0	10	0	1	0	0
	4	0	4	0	0	0	0
	5	0	3	20	19	20	19
W	1	20	4	4	5	0	1
	2	0	4	1	2	2	1
	3	0	4	0	0	1	0
	4	0	1	2	0	0	0
	5	0	1	13	13	17	18

^a Stages 1 to 4 represent immature ovaries with terminal follicles less than 0.26, 0.26-0.50, 0.51-1.00, and more than 1.00 mm in length, respectively; stage 5 ovaries contain mature eggs.

at 3 and 4 days after emergence, respectively, compared with 5 and 12 days after emergence, respectively, in the W-strain.

Data on the dissection of ovaries in virgin females of the L- and W-strains in the 2nd experiment are presented in Table 1. The data did not allow precise comparisons of ovarian development in the two strains, because those females whose ovaries were mature and which discharged all of their eggs were not discriminated from females in stages 1-4. However, the presence of such postpartal females may be negligible, at least in 5-day-old females, since *D. cucurbitae* females are not known to oviposit by the 5th day after emergence. Ovaries of 5-day-old L-strain females were at more advanced stages than those of W-strain females of the same age, and this result indicates faster ovarian development in the L-strain than in the W-strain.

It was suggested from the above results that faster ovarian development in L-strain females has close bearing on their earlier sexual maturation. The 3rd experiment was designed, therefore, to elucidate the relation of ovarian development to receptivity in L-strain females. Table 2 shows the results. Among fifty 4-day-old females tested, 20 mated. The mated females were detected from females at every stage of ovarian

Table 2. FREQUENCY DISTRIBUTION OF MATED AND UNMATED 4-DAY-OLD L-STRAIN
FEMALES AMONG FIVE DIFFERENT STAGES OF OVARIAN DEVELOPMENT

Stage ^a	No. females		Total	% mated
	mated	unmated		
1	1	14	15	6.7
2	3	7	10	30.0
3	8	7	15	53.3
4	6	2	8	75.0
5	2	0	2	100.0
Total	20	30	50	

^a For explanation see Table 1.

development. A positive correlation, however, was found between the stage of ovarian development and the percentage of mating in Kendall's τ -test ($\tau=1.00$, $p<0.01$).

Daily activity rhythm

Records on the daily activity rhythm in the 4th experiment taking 4 types of behavior, i.e., locomotion (walking and flying), preening, feeding, and stridulation are shown in Figs. 2 and 3, for batches containing 10 individuals of either sex. The number of physical contacts observed per min per 10 individuals is also shown in the figures. Two definite peaks were observed in locomotion and preening activity in males and

Fig. 2. Daily activity rhythm of locomotion (B), preening (C), feeding (D), and stridulation (E); and frequency of physical contact per min per 10 individuals at different times of day (F) in batches containing 10 males or 10 females of L-strain. Solid and dotted lines represent male and female activities, respectively. Changes in light intensity (A) is also shown.

Fig. 3. Daily activity rhythm in batches containing 10 males or 10 females of W-strain. For explanation of symbols, see Fig. 2.

females of both the strains at almost the same hours of the day, the first early in the morning and the second at dusk, when light intensity at the center of the cages was less than 2,000 lx. Feeding occurred sporadically throughout the daytime hours, though a small peak followed shortly after the first peak of locomotion activity. This trend was common to every batch, irrespective of sex and strain.

The mating behavior of *D. cucurbitae* usually involves stridulation and repeated physical contacts between the sexes (SUZUKI and KOYAMA, unpublished data). Stridulation was restricted to dusk in only males of both L- and W-strains. In contrast, the rhythm of physical contact in both males and females had two peaks in parallel with locomotion activity, with a higher peak at dusk than early in the morning.

Fig. 4 shows the accumulated percentage mating and the light intensities at the onset of stridulation and copulation in batches containing 10 males and 10 females.

Fig. 4. Accumulated percentage mating in L- and W-strain batches at different times between 17:00 and 21:00 hours. Hollow and solid arrows indicate observation times when stridulation and copulation were first detected, respectively. Numerals above the arrows show corresponding light intensity (lx).

Table 3. COMPARISON OF LIGHT INTENSITY AT INITIATION OF STRIDULATION, MOUNTING, AND COPULATION BETWEEN L- AND W-STRAINS IN THE COMBINATION OF ONE MALE AND ONE FEMALE

Behavior	Strain	No. observed	Light intensity (lx)	
			Median	Range
Stridulation	L ^a	11	1100 ^c	220-2600
	W ^a	11	230	90-1620
	L ^b	12	38	8-140
Mounting	L ^a	10	170 ^c	78-740
	W ^a	10	36	1-198
	L ^b	12	32	5-140
Copulation	L ^a	7	170 ^c	78-245
	W ^a	6	35	1-198
	L ^b	11	30	5-140

^a Test flies were introduced into the experimental cage at 17:00 hr. The experiment was replicated 11 times for L-strain and 20 times for W-strain.

^b Test flies were introduced into the experimental cage after 19:00 hr. The experiment was replicated 13 times.

^c Figures were significantly different from those of W-strain at the 5% level of MANN-WHITNEY's U-test.

Records taken for 04:30–17:00 hours, during which neither stridulation nor copulation were observed, are cut off in the figure. The onset of stridulation, as well as that of copulation, took place earlier and under higher light intensity in the L-strain than in the W-strain.

Similar results were obtained in the 5th experiment, where observations on the two strains were made on the same day at 5-min intervals instead of at 15-min intervals as in the 4th experiment. It is shown in Fig. 5 that L-strain males exhibited stridulation over a wide range of light intensity in comparison with males of the W-strain, and that the occurrence of copulation began under higher light intensity in the L-strain than in the W-strain.

Table 3 summarizes the results of the 6th experiment carried out with single pairs of one male and one female. When the test flies were confined in the experimental cage at 17:00 hours, L-strain males initiated both stridulation and mounting (attempt of males to mount potential mates) under higher light intensities than W-strain males. Copulation by the L-strain occurred within a range between 78 and 245 lx, with a median of 170 lx, which was also significantly higher than the value obtained for the W-strain. The upper threshold of the range was, however, markedly decreased, compared with the light intensity measured at the onset of copulation in L-strain batches containing 10 males and 10 females (Figs. 4 and 5). Tests started after 19:00 hours with L-strain flies showed that they were sexually active throughout the period when light intensity was low enough for initiation of W-strain male courtship and copulation.

Fig. 5. Accumulated percentage mating and percentage males engaged in stridulation (histogram) at different times between 17:00 and 20:00 hr in L-strain (B) and W-strain (C) batches, each containing 10 males and 10 females. Averages of the two replications are presented. Changes in light intensity (A) are also shown.

DISCUSSION

SOEMORI (1980) has reported the earlier sexual maturation of the laboratory strain than the wild strain in *D. cucurbitae*. The results of the 1st experiment (Fig. 1) confirm the difference between the two strains. However, the W-strain used in the present study showed a marked reduction in the pre-mating period in contrast to the wild strain used by Soemori. The reasons for this difference are unknown. Reduced pre-mating periods in flies which have been mass-cultured for years under laboratory conditions have also been noted in other species of Tephritidae including *D. oleae* (GMELIN) (ECONOMOPOULOS et al., 1976) and *Ceratitis capitata* (WIEDEMANN) (RÖSSLER, 1975; WONG and NAKAHARA, 1978). The shorter pre-mating period of mass-cultured flies may be closely related to their earlier onset of oviposition.

FLETCHER (1969) and FLETCHER and GIANNAKAKIS (1973) have demonstrated that *D. tryoni* females do not become responsive to the male pheromone until their ovaries are in the final stages of maturation. Also in L-strain females of *D. cucurbitae*, a positive correlation is found in the percentage mating and the stage of ovarian development (Table 1). Although it is not certain if a similar correlation exists in W-strain females, the faster ovarian development of L-strain over W-strain females may have intimate bearing on the reduction in the pre-mating period of L-strain flies.

That *D. cucurbitae* mates only at dusk has been known since the work by BACK and PEMBERTON (1917). According to SUGIMOTO (1979), mass-reared males of the species initiate stridulation when laboratory light intensity falls below 320 lx, and this activity peaks at around 160 lx, which corresponds to natural light intensity at sundown. By contrast, the results (Figs. 4 and 5, and Table 3) reveal that W-strain males begin their courtship activities under much higher light intensity than that indicated in SUGIMOTO's data, and L-strain males start courtship at still higher levels of light intensity. The results further reveal the lack of perfect synchrony between the two strains in the timing of male courtship and copulation. However, this itself may not predict complete mating failure of L-strain males in competition with W-strain males for mates, since the former are still sexually active at the low light intensity levels required to stimulate mating activity of the latter.

The question remains as to whether any deviate trait found in the present study of L-strain *D. cucurbitae* is due to genetic differentiation or due to environmental influences. Further studies are needed to answer this question.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Mr. OHINATA, Hawaiian Fruit Flies Laboratory, ARS, USDA, for his critical reading of the manuscript. The authors are also thankful to the staffs of the Fruit Flies Laboratory, Okinawa Prefectural Agricultural Experiment Station for their valuable suggestions.

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