Difference in the Timing of Larval Jumping Behavior of
Mass-Reared and Newly-Colonized Strains of the Melon Fly,
Dacus cucurbitae COQUILLET (Diptera: Tephritidae)¹

Hiroshi SUENAGA,² Hidemi KAMIWADA,³ Akira TANAKA²
and Naoya CHISHAKI²

*Melon Fly Control Project Office, Oshima Branch, Kagoshima
Prefectural Government, Naze, Kagoshima 894, Japan*

(Received March 2, 1991; Accepted December 24, 1991)

Mean periods from egg seeding to larval jumping (EJ-period) of the melon fly, *Dacus cucurbitae*, were compared between mass-reared (MS: F₅₀) and newly-colonized strains (NS: F₁₃) at three egg densities. The EJ-period of MS was significantly longer than that of NS at each density: 7.6, 10.1 and 13.1 d in MS, and 6.5, 6.7 and 8.2 d in NS, respectively, at densities of 50, 300 and 3,000 eggs/450 ml of medium. The proportion of MS and NS larvae which had matured by the 6th day after egg seeding amounted to 75.0% and 77.8%, respectively, and those matured by the 8th day attained 98.8% and 99.5%, respectively, at an egg density of 300/450 ml. The difference between the percentages of the two strains was insignificant, indicating that the period from egg seeding to larval maturation (EM-period) of MS was almost equal to that of NS. These results suggest that the prolonged EJ-period of MS was caused not by a lengthened EM-period but by delayed larval jumping behavior after maturation.

Key words: *Dacus cucurbitae*, jumping behavior, mass-reared strain, newly-colonized strain, selection

INTRODUCTION

Domestication, or artificial selection in introduction of wild insects to mass rearing is one of the most crucial problems in insect quality control. Since the BOLLER (1972) review on insect quality, much interest has been focused on this problem (CHAMBERS, 1977; MACKAUER, 1976), and many studies dealing with quality changes have been made.

Most of these studies dealt with the characteristics of adult flies such as flight ability (BUSH et al., 1976; NAKAMORI and SIMIZU, 1983), mating behavior (WONG and

¹ The main points of this work were presented at the annual meeting of the Japanese Society of Applied Entomology and Zoology (April 5, 1987, Ibaraki).

² Present address: Kagoshima Prefectural Agricultural Experiment Station, Kagoshima 891-01, Japan

³ Present address: Ohsumi Branch, Kagoshima Prefectural Agricultural Experiment Station, Kimotsuki-gun, Kagoshima 893-16, Japan

NAKAHARA, 1978; SOEMORI et al., 1980; SUZUKI and KOYAMA, 1980; KUBA and KOYAMA, 1982), oviposition and longevity (RÖSSLER, 1975; SOEMORI and NAKAMORI, 1981) and response to pheromone (FLETCHER et al., 1968). However, few studies have been made on the larval or pupal traits.

In general, one behavioral or quantitative trait is genetically correlated with other traits (BOLLER, 1972; HARTL and CLARK, 1989). Even in the fruit fly, some larval or pupal traits may be associated with those of adult flies, which are important in the sterile insect release method.

In this study, timing of the larval jumping behavior (mature larvae jumping out of the medium in search of pupation sites) in the mass-reared strain was compared with that of the newly-colonized strain. We also discussed what caused the difference in the jumping behavior of the two strains.

MATERIALS AND METHODS

Origin and maintenance of strains. Mass-reared strain (MS) was developed from a source colony collected on the Amami Islands (Kagoshima Pref.) in June, 1981. It had been reared artificially for ca. six years (about 55 generations) in a mass-production facility of the Melon Fly Control Project Office (Kagoshima Pref.). Fifty thousand eggs were seeded in 5 l of medium in a container (65×31×5 cm) and maintained in a room at $27\pm1^{\circ}\text{C}$ and $80\pm5\%$ R.H. under natural-light conditions. Mature larvae began to jump out of the medium on the fifth day after egg seeding. In order to induce larval jumping behavior, matured larvae in the medium were exposed to low temperature and sprayed with water on the sixth and seventh day. For the first three generations, larvae collected from the fifth to seventh day were used as the parent stock, afterwards only those collected on the seventh day were used as it. Flies were selected for short preoviposition period for the first four generations; that is, eggs oviposited by younger females were reared for the next generation. The other rearing techniques are described in NAKAMORI and KAKINOHANA (1980). Larvae used in the following experiments were from the 45th to 55th generations.

Newly-colonized strain (NS) was obtained from about 2,800 wild melon flies collected as larvae from infested cultivated bitter gourd, *Momordica charantia* var. *pevel* CRANTZ, in the Amami Islands in July, 1985. The flies were selected for short preoviposition period for the first nine generations in the same way as MS. Flies and larvae were reared in the same way as MS except that all larvae collected in the interval from the fifth to seventh day after egg seeding were used as parent stock and that the larvae were stimulated by low temperature only. Larvae of the 5th to 13th generations after colonization were used in the following experiments.

Change in the timing of larval jumping of MS with generations. The larvae which jumped out of the medium on the fifth day after egg seeding were counted at every rearing from the 7th to the 40th generation. On the fifth day, larvae were allowed to jump out of the medium spontaneously.

The generations were discrete until the fifth generation and overlapped thereafter. The number of generation of larvae produced weekly (F) was calculated as follows:

$$F = \{\sum(f \times A) / \sum A\} + 1$$

where f is the generation of females from which eggs were collected and A is the egg

amount (ml). The percentage of larval recovery of each generation was calculated by averaging those of weekly-created generations with the same integers.

The effect of larval density on the timing of jumping in MS and NS. In the first experiment, one egg was seeded and reared individually in 8 ml of medium in a Petri dish (3.5 cm dia. $\times$ 1 cm depth). About one hundred individuals were used in each experiment and strain. This experiment was replicated seven times from July, 1986 to September, 1987. The generations of MS and NS at the beginning of these experiments were the 45th and the 5th, respectively, and those at the end were about the 55th and the 12th, respectively.

In the second experiment, groups of 50, 300, and 3,000 eggs were each seeded in 450 ml of the medium in plastic containers (17 $\times$ 11 $\times$ 4 cm) dedicated to each strain. There were five replications of this experiment for each egg density and strain. The MS and NS used in this experiment were of about the 50th and 13th generations, respectively.

Each rearing container and each of the 15 Petri dishes was placed into a larger plastic case (27 $\times$ 21 $\times$ 10 cm) where it was maintained at $27 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ R.H. under 12L–12D. On the fifth day after egg seeding, 300 ml of water was poured into the plastic case. The larvae which jumped out of the medium into the water were counted and removed every day starting from the sixth day. Larval jumping was not stimulated artificially. The larval rearing method hereafter was the same as in this experiment. Larvae were not sexed in any of the experiments.

Larval maturation periods of MS and NS. The larvae with medium in the midguts were regarded as immature and those without medium as mature. India ink (2.5 ml) was added to 450 ml of the medium for easy recognition of the medium in the larval midgut. The India ink had no effect on the larval development (SUENAGA, unpublished).

On the sixth and the eighth day after the egg seeding, the larvae of each strain in the five rearing containers with a density of 300 eggs per 450 ml of medium were separated by screening in water before the onset of larval jumping (before dawn), and percentages of mature larvae were calculated. The MS and NS larvae used in this experiment were of the 48th and 12th generations, respectively.

RESULTS

Change in the timing of larval jumping of MS with generations

Figure 1 shows the change in the percentage of larvae which jumped out of the medium on the fifth day after egg seeding. The percentage increased and reached a peak at generation 13, after which it gradually decreased. After generation 32, only a few larvae jumped out on the fifth day. These results indicate that the MS larvae came to require a longer period before jumping with an increase in generation number.

The effect of larval density on the timing of jumping of MS and NS

In the first experiment in which larvae were reared individually, the total number of larvae collected in seven replications was 546 in MS and 551 in NS. Larval jumping in MS and NS showed peaks on the seventh and sixth day, respectively. The mean periods required from egg seeding to jumping (EJ-period) were 6.92 d in MS and 6.64 d in NS; thus the timing of jumping behavior was different between the two strains

Fig. 1. Change in the percentage of larvae collected on the 5th day after egg seeding in mass production. Larvae collected (%) = (number of larvae collected) / (number of eggs seeded) $\times$ 100.

Fig. 2. Larval jumping behavior of MS (■) and NS (●) under conditions of individual rearing in Petri dishes. Figure drawn on basis of total number of larvae obtained in seven experiments.

(Fig. 2, $p < 0.01$, KOLMOGOROV-SMIRNOV two-sample test). However, all the larvae of both strains had jumped out in the interval from the sixth to the ninth day.

In the second experiment, the total number of larvae collected per container at densities of 50, 300 and 3,000 eggs was, respectively 42, 251 and 2,872 in the MS, and 41, 249 and 2,322 in the NS.

The jumping behavior in NS showed a peak on the sixth day at egg densities of 50 and 300 (Fig. 3-A). At both densities, about 95% of the larvae had jumped out by the eighth day. The EJ-period was 6.5 and 6.7 d at the densities of 50 and 300, respectively. At the egg density of 3,000, the larval jumping reached a peak on the seventh day and then gradually decreased to zero on the 15th day. The EJ-period was 8.2 d. The jumping behavior lasted five, seven and nine days at egg densities of 50, 300 and 3,000, respectively.

On the other hand, the larval jumping behavior in MS at the egg density of 50 reached a peak on the seventh day (Fig. 3-B). At the egg density of 300, the larval jumping showed no clear peak and continued until the 15th day. When the larvae were reared at the density of 3,000, only 3.5% of the larvae had jumped out by the ninth day, after which larval jumping increased, reaching a peak on the 14th day. After the 19th day, no larvae jumped out. The EJ-period and the jumping period, from the

Fig. 3. Effect of egg densities on the timing of larval jumping behavior of newly-colonized (A) and mass-reared (B) strains. Solid, broken, and chain lines indicate the densities of 50, 300 and 3,000 eggs/450 ml of medium, respectively.

Table 1. Percentages of mature larvae of MS and NS on 6th and 8th day after egg seeding^a

Strain	6th day ^b		8th day ^b			Total no. of mature larvae
	No. of larvae in the medium	Total no. of mature larvae	No. of larvae jumping out from 6th to 7th day	No. of larvae in the medium		
				mature	immature	
MS (F ₄₇)	992	744 (75.0) ^c	222	805	12	1,027 (98.8)
NS (F ₁₂)	911	709 (77.8)	678	166	4	844 (99.5)
Result of χ^2 -test for the two percentages		n.s.				n.s.

^a Larvae were reared at the density of 300 eggs/450 ml of medium. Five repetitions were made for each strain in each investigation.

^b Trays were examined before the day's onset of larval jumping (Thus no larvae jumped out from the medium before the investigation on the 6th day).

^c Values in parentheses show percentages of mature larvae.

first to the last jumping behavior, increased with larval density: i.e., 7.6, 10.1 and 13.1 d in the EJ-period, and 7, 10 and 13 d in the jumping period at egg densities of 50, 300 and 3,000, respectively. The EJ-period of MS was significantly longer than that of NS at every egg density ($p < 0.001$, KOLMOGOROV-SMIRNOV two-sample test).

Larval maturation periods of MS and NS

The percentages of mature larvae of both strains on the sixth and the eighth days after egg seeding are shown in Table 1. By the sixth day, when the larvae started jumping, 75.0% of MS and 77.8% of NS larvae had matured. No significant difference was observed between the two strains ($p > 0.10$, χ^2 -test). On the eighth day, 817

larvae of MS remained in the medium and almost all of them (805 larvae) were mature. In NS, only 170 larvae remained in the medium and 166 of them were mature. The portion of the larvae which had matured by the eighth day amounted to 98.8% in MS and 99.5% in NS. These two values also did not differ significantly ($p > 0.10$, χ^2 -test). The NS larvae jumped out of the medium immediately after maturity, while mature MS larvae remained in the medium.

DISCUSSION

In our practical mass-rearing procedure, the larvae began to jump on the fifth day after egg seeding, and most of them had jumped out of the medium by the seventh day when stimulated by low temperature and water spray. Among them, the larvae collected on the seventh day were used as the parent stocks after the fifth generation in MS. This method of using the larvae as parent stock produced two selections: the selection of the long EJ-period and the selection of the tendency to jump out under low temperature and water spray. Thus, the number of MS larvae collected on the fifth day after egg seeding decreased starting from generation 13 (Fig. 1). The increase in recovery during the 7th to 13th generations might be due to an improvement in the rearing techniques, such as temperature control and water spray, or to the adaptation of individuals to the rearing conditions (Fig. 1) (BOLLER, 1972).

MS larvae required more days before jumping and continued jumping for a longer period than NS at every larval density (Fig. 3). MCKENZIE and HOSKINS (1954) reported that the period from oviposition to midpupation and the pupating period from first to last pupation of the housefly, *Musca domestica*, were lengthened by selection of late pupae.

The EJ-period can be divided into two parts: the time from egg seeding to larval maturation (pre-maturation period) and the time from larval maturation to jumping (post-maturation period). As shown in Table 1, the pre-maturation period did not differ between the two strains, but the post-maturation period was longer in MS than in NS. Therefore, the longer EJ-period of MS was caused by its longer post-maturation period as compared to that of the NS. This result suggests that the two selections mentioned above mainly affected the post-maturation period of MS.

Under the individual rearing, the EJ-period of MS (6.92 d) was slightly longer than that of NS (6.64 d) (Fig. 2.). However, the EJ-period of MS increased more markedly with increasing larval density than did that of NS (Fig. 3). This suggests that metabolic heat produced by larvae, and/or physical contact among larvae, will influence the manifestation of the post-maturation period of MS.

Selection for a given trait is likely to affect other traits of the selected population owing to pleiotropic effect or linkage disequilibrium of genes (HARTL and CLARK, 1989). In the housefly, *Musca domestica* (Diptera), genetic correlation exists between the larval period and the DDT-resistance of adult flies (MCKENZIE and HOSKINS, 1954). NAKAMORI (1988) reported that the selection for pre-oviposition period of *Dacus cucurbitae* affected other life history traits and flight ability. The timing of larval jumping of *Dacus cucurbitae* may also relate to other traits such as mating behavior and flight ability. We must therefore pay attention to the selection for larval traits to avoid unexpected changes of insect quality (BOLLER, 1972).

ACKNOWLEDGEMENTS

We express our sincere appreciation to Dr. K. KAWASAKI of the Okinawa Agricultural Experiment Station, Dr. J. YUKAWA of Kagoshima University, Mr. O. SETOGUCHI of the Oshima Branch of Kagoshima Agricultural Experiment Station and Mr. H. KUBA of the Fruit-fly Eradication Project Office, Okinawa Prefectural Government for their critical reading of this manuscript. We also thank Mr. Y. HIGASHIZONO, Sankei Chemical Co., Inc. for his helpful assistance.

REFERENCES

- BOLLER, E. (1972) Behavioral aspects of mass-rearing of insects. *Entomophaga* **17**: 9-25.
- BUSH, G. L., R. W. NECK and G. B. KITTO (1976) Screwworm eradication: Inadvertent selection for noncompetitive ecotypes during mass rearing. *Science* **193**: 491-493.
- CHAMBERS, D. L. (1977) Quality control in mass rearing. *Annu. Rev. Entomol.* **22**: 289-308.
- FLETCHER, L. W., H. V. CLABORN, J. P. TURNER and E. LOPEZ (1968) Difference in response of two strains of screw-worm flies to the male pheromone. *J. Econ. Entomol.* **61**: 1386-1388.
- HARTL, D. L. and A. G. CLARK (1989) *Principles of Population Genetics*. 2nd ed., Sinauer Associates, Sunderland, Massachusetts, 682 pp.
- KUBA, H. and J. KOYAMA (1982) Mating behavior of the melon fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae): Comparative studies of one wild and two laboratory strains. *Appl. Entomol. Zool.* **17**: 559-568.
- MACKAUER, M. (1976) Genetic problems in the production of biological control agents. *Annu. Rev. Entomol.* **21**: 369-385.
- MCKENZIE, R. E. and W. M. HOSKINS (1954) Correlation between the length of the larval period of *Musca domestica* L. and resistance of adult flies to insecticides. *J. Econ. Entomol.* **47**: 984-992.
- NAKAMORI, H. (1988) Behavioral and ecological studies on sexual competitiveness in the melon fly, *Dacus cucurbitae* COQUILLET, mass-production. *Bull. Okinawa Agric. Exp. Stn. Suppl.* **2**: 1-64 (in Japanese with English summary).
- NAKAMORI, H. and H. KAKINOHANA (1980) Mass-production of the melon fly, *Dacus cucurbitae* COQUILLET, in Okinawa, Japan. *Rev. Plant Protec. Res.* **13**: 37-53.
- NAKAMORI, H. and K. SIMIZU (1983) Comparison of flight ability between wild and mass-reared melon fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae), using a flight mill. *Appl. Entomol. Zool.* **18**: 371-381.
- RÖSSLER, Y. (1975) Reproductive differences between laboratory-reared and field-collected populations of the mediterranean fruitfly, *Ceratitis capitata*. *Ann. Entomol. Soc. Am.* **68**: 987-991.
- SOEMORI, H. and H. NAKAMORI (1981) Production of successive generations of a new strain of the melon fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae) and reproductive characteristics in mass rearing. *Jpn. J. Appl. Entomol. Zool.* **25**: 229-235 (in Japanese with English summary).
- SOEMORI, H., S. TSUKAGUCHI and H. NAKAMORI (1980) Comparison of mating ability and mating competitiveness between mass-reared and wild strains of the melon fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae). *Jpn. J. Appl. Entomol. Zool.* **24**: 246-250 (in Japanese with English summary).
- SUZUKI, Y. and J. KOYAMA (1980) Temporal aspects of mating behavior of the melon fly, *Dacus cucurbitae* COQUILLET (Diptera: Tephritidae): A comparison between laboratory and wild strains. *Appl. Entomol. Zool.* **15**: 215-224.
- WONG, T. T. Y. and L. M. NAKAHARA (1978) Sexual development and mating response of laboratory-reared and native mediterranean fruit flies. *Ann. Entomol. Soc. Am.* **71**: 592-596.