Effects of Maternal Age and Egg Quality on Mass Rearing of Mediterranean Fruit Flies (Diptera: Tephritidae)

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Abstract: Tests were conducted to determine if age of mass reared adult female Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), affected the viability of their offspring. Samples of eggs (0.4 g each) were collected on five consecutive days (4 to 8 d past adult emergence) from individual 660-liter adult colony screen cages and were reared in separate trays containing 0.5 liter of artificial diet. The mean volume of pupae obtained declined steadily with the age of the parents from 297 ± 21 (S.E.) ml per liter of larval diet for day 1 eggs to 158 ± 24 ml/liter for day 5 eggs. Hatch of eggs varied little, indicating that the viability of larvae declined with maternal age. The yield of pupae also varied significantly among replicates (adult cages), ranging from 174 ± 24 to 258 ± 23 ml per liter of diet. In a second test, pupal yield again declined with maternal age, whereas giving females a "break" from oviposition by withholding egging substrates on various schedules had no discernable effect on the relationship between age. In a second test (but not the first), maternal age also had significant effects on a number of quality traits in the progeny, including percentages of hatch, adult emergence, and flight ability. The authors propose that the observed cross-generation effects on insect survival and quality are likely a maternal effect and discuss possible implications of the findings for mass rearing operations.

Keywords: Insecta, Diptera, *Ceratitis capitata*, maternal effect

Mediterranean fruit flies, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), are mass reared for Sterile Insect Technique (SIT) programs at facilities in at least six countries. In Hawaii alone, two rearing facilities currently produce a total of ≈400 million sterile flies per week for SIT programs in southern California. Although these facilities adhere to quality control procedures and strict rearing protocols, the yield and/or quality of mass-produced *C. capitata* fluctuate and sometimes drop to unacceptable levels. For SIT programs on this scale, such lapses in rearing can substantially increase costs and compromise the efficacy of control efforts.

In some instances, past periods of poor production of *C. capitata* in Hawaiian rearing facilities were traced to known causes such as insecticide contamination of diet ingredients, but many were intractable and eventually self-correcting (J.I.N., unpublished data). During a problematic period in 1996, production and experimental data led us to suspect that egg quality was having a pronounced effect on efficiency of the rearing operation at the USDA-APHIS Hawaii Fruit Fly Rearing Facility (HFFRF) in Waimanalo. We initiated studies to determine if age and cohort (batch) of the parents significantly affected the survival and quality of mass-reared *C. capitata*.

Materials and Methods

Source of insects. All *C. capitata* used in these tests were from the Maui-93 strain that was originally collected in commercial coffee in Kapalua, Maui, Hawaii, in April of 1993. The strain had been in culture for ≈40–50 generations when the tests described below were run. Larvae were reared in stacked trays on a wheat-based artificial diet (5 liters per tray) similar to that described by Tanaka et al. (1970). Diet ingredients included (with % dry weight): wheat mill run (69%), sucrose (21%), torula yeast (9%), and preservatives (1%; equal parts sodium benzoate and methyl paraben). Dry ingredients were mixed into highly diluted HCL (roughly 3:5 dry:wet ingredients by weight), resulting in a larval diet with an initial pH of ≈4.0. Larvae that emigrated from the diet were collected in water and then transferred to fine vermculite to pupate. After 2 d, pupae were sifted from the vermiculite and held on screen trays at 19°C until placed in screen cages for emergence. Adult flies were provided with a water source and food (yeast hydrolysate:sucrose, 2:3).

Egg quality versus fly age. For this test, eggs were obtained from stock colony screen cages (660 liters) that were used for mass production at the HFFRF. In accordance with rearing facility protocol, each cage was stocked with 4 L of *C. capitata* pupae (≈240,000 insects) that were within 1–2 d of adult emergence. An egging tube (PVC pipe, 120 cm x 10 cm diam with ≈4000 1-mm diam holes in the sides and top) extended horizontally through each cage (Vargas et al. 1985). At 3 d after pupae were added to the cages, a small amount of water was added to the egging tubes to induce the females to begin ovipositing. Eggs were collected (gently rinsed) from egging tubes daily on days 4 through 8, and the flies were then discarded. Cages were held at 55–70% RH, with a temperature of 24°C for 2 d and at 27°C thereafter.

For each replicate (n = 5), a sample of eggs was taken daily from an individual cage on each of days 4 through 8. A 0.4-g aliquot of eggs (≈12,000) was placed onto diet in a small tray that contained 0.5 liter of standard larval diet (see above). Trays of diet were covered with fine mesh and held under standard larval rearing conditions of 28°C for 3 d and 25°C for 2 d at >80% RH. Covers on the trays were then removed, and the trays were held over vermiculite for 4 d to allow mature larvae to emigrate from the diet and pupate.

Frequency of egg collection. *C. capitata* pupae were obtained from HFFRF production within 1–2 d of adult emergence (see above) and placed in plastic cups within 27-liter screen cages at 95 ml (À5700 pupae) per cage. Oviposition receptacles were placed into cages on various schedules, depending on the treatment. The receptacles consisted of 250-ml polypropylene bottles with holes similar in size and density to those used in the production egging tubes (above). Several ml of water was added each bottle to provide moisture.

Cages were assigned to one of three egging regimes. For the standard treatment (#1), oviposition receptacles were kept within each cage throughout days 3 through 10 after pupae were initially placed into the cages. Eggs were rinsed from the receptacles daily. On odd-numbered days, samples of eggs were reared to pupation in 0.5-liter trays as described above; eggs from even-numbered days were discarded. Treatment #2 was similar to treatment #1, except that the egging receptacles were withheld from the cages on even-numbered days. For treatment #3, flies were provided with oviposition receptacles only on days 3, 7, and 9. This test was replicated three times.

Evaluation. For both tests, an additional 500 eggs from each sample that was taken for rearing was used to estimate percent hatch. The eggs were spread out on piece of moist black filter paper that was held in a covered petri dish at 25 ! 1°C. After 3 d, hatched and unhatched eggs were counted.

Pupae were sifted from the vermiculite daily from 6 to 9 d after eggs had been placed on the diet. The quantity of all pupae recovered from each tray was determined volumetrically. For evaluations of insect quality, sub-samples of pupae were taken from groups of insects

that had emigrated from the diet as larvae at 7 d after eggs were placed on the diet. These pupae were weighed and counted, and 100 pupae were placed into each of five 10-cm high by 9-cm diam black acrylic tubes at 25°C. The insides of the tubes were lightly dusted with unscented talc so that the flies could not crawl up the walls. After all emerging flies had either flown out of the tubes or died, the numbers of flies and unemerged pupae in each tube were counted. From these data, we computed percentage emergence and "flight ability"; that is, the percentage of pupae that produced flies that were capable of flying out of a 10-cm high tube. These are standard procedures that are used to monitor insect quality for sterile fly release programs (IAEA 1998).

Data were analyzed by analysis of variance (ANOVA) using the General Linear Models option in SYSTAT (SPSS 1998). A repeated-measures design was used for the "frequency of egg collection" data. Percentages were subjected to arcsine-square root transformation before ANOVA.

Results

Egg quality versus fly age. The volume of pupae per unit of diet dropped steadily as age of the maternal flies increased (Figure 1). Overall, eggs from the first egging day (i.e., those deposited between 3 and 4 d after emergence) yielded almost twice as many pupae (297 ± 21 [S. E.] ml pupae per liter of diet) as did eggs from the fifth collection day (158 ± 24 ml/liter; $F = 11.96$, $d.f. = 4, 16$; $P < 0.001$). Yield of pupae also varied among replicates from 174 ± 24 to 258 ± 23 ml per liter of diet ($F = 4.47$; $d.f. = 4, 16$; $P = 0.013$). The variation among replicates suggests that egg quality varied significantly from batch to batch of flies, although this trend would have been confounded with trends in overall diet quality that may have occurred during the test period.

Other indices of insect quality and performance varied relatively little with maternal age (Table 1). Mean egg hatch for all collection days was between 86 and 93% and was actually numerically highest for Day 3 (Table 1). The differences in survival that produced significant variation in larval yield among egging days occurred primarily after eclosion.

Frequency of egg collection. Yield of pupae per liter of larval diet declined with the age of the maternal flies, producing an overall decrease of 29% from Day 3 to Day 9 eggs (Table 2; $F = 136$; $d.f. = 2, 8$; $P < 0.001$; data from Day 5 after emergence were not included in the analyses in order to maintain a balanced design). Yield also varied significantly among replicates ($F = 7.89$; $d.f. = 2, 4$; $P = 0.041$) but was similar among eggs from the different egg-collection treatments ($F = 0.91$; $d.f. = 2, 4$; $P = 0.47$). The interaction of collection treatment with collection day also was not significant ($F = 1.75$; $d.f. = 4, 8$; $P = 0.23$), indicating that egg quality declined similarly with time regardless of the frequency of egg collection. It should be noted, though, that when egging bottles were withheld for an extended period, females would deposit an obviously reduced (though unquantified) number of eggs at various locations around the cage.

In this test, percentage of eggs that hatched also declined significantly with maternal age (Table 2; $F = 47.5$; $d.f. = 2, 8$; $P < 0.001$). Nonetheless, survival of larvae from eclosion to pupation fell from an estimated 50% for eggs that were deposited on the third day after adult emergence to 39% for Day 9 eggs (based on estimates of 30,000 eggs/g and 60,000 pupae/liter; J.I.N., unpublished data). Percent hatch for eggs from females that were offered oviposition substrates only on Days 3, 7, and 9 was lower (84.4 ± 1.9) than percent hatch of eggs from females that were offered oviposition substrates continuously (89.6 ± 1.0) or on every second day (89.3 ± 1.4; $F = 9.82$; $d.f. = 2, 4$; $P = 0.029$).

Percentages of pupae that produced adult flies and "flying" adults tended to decline with maternal age; mean pupal weight varied somewhat from day to day but showed no consis-

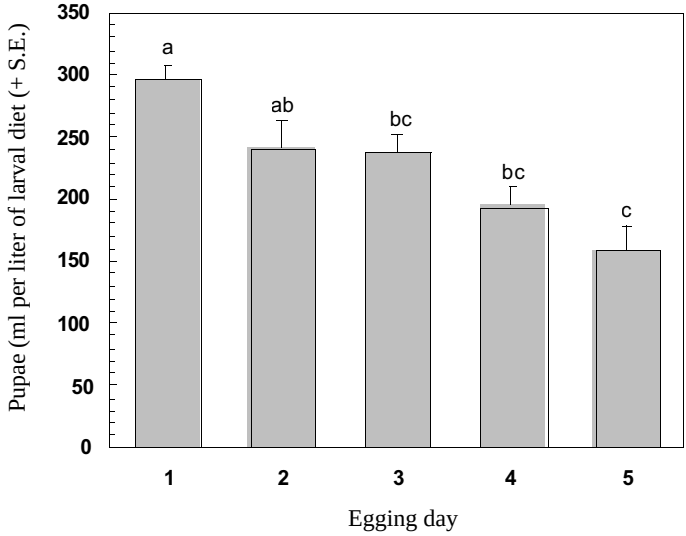

Figure 1. Mean yield (ml + S.E.; $n = 5$) of *C. capitata* pupae produced per liter of larval diet using eggs (0.4 g per 0.5-liter tray of diet) taken on five consecutive days from individual cages of adult flies (eggs collected daily from 4 to 8 d after adult emergence). Bars with the same letters above them represent means that are not significantly different, as determined by Tukey's HSD test (SPSS 1998) ($F = 11.96$; $d.f. = 4, 16$; $P < 0.001$).

Table 1. Quality indices for Mediterranean fruit flies reared from eggs collected from individual cages of adult flies on sequential days of egg production ($n = 5$; for F-tests, $d.f. = 4, 16$).

Collection day	Hatch[a] (%)	Pupal weight (mg)	Emergence[a] (% of pupae)	Flight ability[a] (% of pupae)
1	88.0 ± 1.8	8.6 ± 0.1	96.1 ± 0.4	93.7 ± 0.7
2	89.9 ± 0.5	8.7 ± 0.1	95.3 ± 0.4	93.0 ± 0.4
3	91.7 ± 0.6	8.8 ± 0.0	94.6 ± 0.8	92.5 ± 0.5
4	88.2 ± 1.4	8.8 ± 0.0	94.2 ± 0.8	92.1 ± 0.8
5	86.6 ± 1.9	8.9 ± 0.1	94.0 ± 0.8	91.6 ± 0.3
F	1.80	1.84	2.50	2.92
P	0.18	0.17	0.08	0.054

[a] Actual means are shown, but an arcsin-square root transformation was used prior to ANOVA.

Table 2. Effects of age of flies on the quality of eggs produced by Mediterranean fruit flies that were subjected to various egg collection treatments (see text).

Days after emergence	Treatment (egging days)	Hatch (%)	Pupae (ml/liter)	Pupal Weight (mg)	Emergence (%)	Fliers (%)
3	All days	91.2 ± 1.4	181.3 ± 4.7	8.2 ± 0.0	93.9 ± 0.2	88.9 ± 0.5
	3, 5, 7, 9	93.2 ± 0.5	180.3 ± 2.6	8.2 ± 0.1	93.5 ± 0.5	88.0 ± 0.6
	3, 7, 9	90.1 ± 0.9	185.0 ± 2.6	8.2 ± 0.0	94.0 ± 0.8	89.1 ± 0.9
5	All days	92.7 ± 1.3	170.3 ± 25.3	8.0 ± 0.1	93.2 ± 0.7	87.0 ± 0.7
	3, 5, 7, 9	92.9 ± 1.0	169.3 ± 13.0	8.1 ± 0.1	90.2 ± 0.2	85.9 ± 0.7
	3, 7, 9	—	—	—	—	—
7	All days	89.5 ± 0.7	142.0 ± 11.0	8.2 ± 0.0	91.3 ± 0.6	86.9 ± 0.1
	3, 5, 7, 9	88.8 ± 0.2	146.0 ± 5.0	8.2 ± 0.0	90.1 ± 0.1	87.3 ± 0.5
	3, 7, 9	84.7 ± 1.9	129.7 ± 9.0	8.2 ± 0.0	90.9 ± 1.1	85.5 ± 0.8
9	All days	85.2 ± 1.4	129.3 ± 20.3	8.1 ± 0.0	90.9 ± 0.7	86.0 ± 0.8
	3, 5, 7, 9	82.5 ± 1.8	133.0 ± 20.1	8.1 ± 0.0	91.3 ± 0.6	87.0 ± 1.0
	3, 7, 9	78.3 ± 2.0	124.0 ± 17.0	8.1 ± 0.0	89.5 ± 1.0	85.7 ± 1.1

tent pattern. For all three parameters, effects of maternal age were significant ($F \geq 17$; $d.f. = 2, 8$; $P < 0.002$), as were the interactions between maternal age and replicate ($F. \geq 4.4$; $d.f. = 4, 8$; $P < 0.04$).

Discussion

In our tests, yield of *C. capitata* per unit of larval diet was controlled in large part by a cross-generation influence on egg quality. While our data do not pinpoint the source of this influence, we believe that it is most likely a maternal effect. Maternal effects are developmental influences that extend across life cycle stages in which genetic or environmental differences in the maternal generation are expressed as phenotypic differences in the offspring (Mousseau and Dingle 1991). In various species, these effects manifest themselves through phenotypic variation among progeny in such traits as sex ratio, diapause characteristics, development rate, or survival (Mousseau and Dingle 1991, Rossiter 1994). Maternal effects can produce phenotypic variation among progeny both within and between dams (e.g., Rossiter 1991, Keena et al. 1998). In many cases, the environment of the female parent mediates the effects by (for example) influencing the quality and quantity of provisions in a female's eggs (Mousseau and Dingle 1991, Rossiter 1994).

Maternal effects in insects have been studied primarily in regard to their influence on life histories and population dynamics (Mousseau and Dingle 1991, Rossiter 1994), but they can be of pragmatic interest to mass rearing operations. In particular, the possibility of environmentally based cross-generation effects on yield or quality of insects has led many rearing specialists to a dogmatic assertion that new diet components and rearing procedures should be tested for three to four consecutive generations before being transferred to mass production operations (personal observation; also see Dadd [1985]). This assertion was borne out in a recent series of studies on laboratory colonies of the gypsy moth (*Lymantria dispar* [L.]). A shortage of an available form of iron (amorphous ferric phosphate) in a female's larval diet was shown to result in slow, asynchronous development and poor survival of her offspring (Keena et al. 1995, 1998; ODell et al. 1997). For *C. capitata*, our results demonstrate that cross-generation effects on egg quality are potentially important determinants of the efficiency of sterile fly production and need to be considered when troubleshooting rearing problems, assaying diet ingredients, or testing components of the rearing operation. Furthermore, rearing tests should be performed fully across generations, as percent hatch is not, in itself, an adequate indictor of egg quality.

Our findings also have implications for managing production colonies of *C. capitata*. One of the basic tenets of population ecology is that relatively modest reductions in the age at which an organism reproduces (or "first" reproduces) can substantially increase its reproductive rate and, all other things equal, its fitness (Andrewartha and Birch 1954). In the production system used by the Hawaii Fruit Fly Rearing Facility, a rearing "colony" (i.e., a self-reproducing group of insects that generate eggs for sterile insect production) is not maintained separately from insects that are reared for sterile release programs. Thus, adaptation to the rearing system likely favors insects that put as much effort (nutrients, etc.) as possible into the earliest batches of eggs to ensure that those eggs produce the maximum number of viable offspring. Indeed, females typically deposit nearly a third of their total eggs on the first of the five days that eggs are collected (data from HFFRF production records; also see Vargas et al. 1984). One argument for maintaining a separate "colony" of flies is that the colony could potentially be managed to make the quality and, perhaps, quantity of a female's eggs more consistent throughout her life. In addition, holding colony insects under conditions that are more "relaxed" than those used for sterile insect production could result in higher quality flies and, possible, higher quality eggs for sterile insect production.

Although the findings of the current study have important implications for mass rearing of *C. capitata*, many pertinent questions remain unanswered. At present, we are not sure that the observed cross-generation effect on survival and quality of mass-reared *C. capitata* is a maternal effect, nor do we know the degree to which other strains of *C. capitata* will exhibit similar cross-generation effects. The significant replicate-to-replicate variation that we observed in larval yield may have been caused not only by variation in egg quality but, at least in part, by confounding factors such as possible trends in the quality of diet ingredients. As a result, we can't confidently extrapolate the present findings to possible relationships between egg quality and the yield of pupae in sterile insect production. Most importantly, though, identifying the cause of this cross-generation effect on egg quality could potentially be a critical step in stabilizing and optimizing operations for mass rearing *C. capitata.*

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