Ovary Development in Two Genetic Strains of the Caribbean Fruit Fly (Diptera: Tephritidae)

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Introduction

Olfactory reception and behavioral responses to semiochemicals are influenced by an insect's physiological state, including its sexual maturity (Kendra et al. 2005). To better understand these interactions and develop improved attractants for tephritid fruit flies, reliable methods are needed for assessing maturity status of field-caught adults. Previously, we developed a six-stage system to classify ovary maturation in a laboratory strain of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Fig. 1). In this study, we applied that system to a wild population of *A. suspensa* to document differences in female maturation between the two genetic strains.

Fig. 1

Materials and Methods

Fig. 2

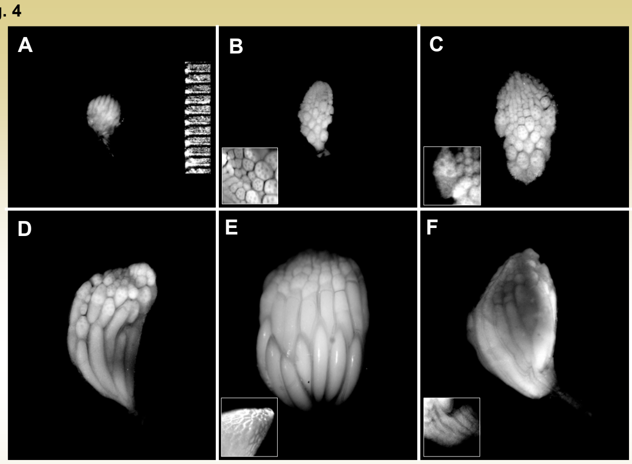
Insects. The laboratory-strain *A suspensa* were obtained from an established colony maintained by the USDA-ARS, Miami, FL using methods previously described (Kendra et al. 2006). Rearing conditions consisted of 25 ± 2°C, 70% RH, and a photoperiod of 12:12 hr (L:D). Adults of known age were held in screen cages and provisioned with water (agar blocks) and food (sugar and yeast hydrolysate, 4:1 mixture) *ad libitum* (Fig. 2).

The feral strain was obtained from infested guava (*Psidium guajava* L.) field collected in Homestead, FL (Fig.3). Fruit was placed in trays of vermiculite until the larvae exited, and puparia were subsequently sifted from the vermiculite. Puparia were held in screen cages for adult eclosion, and known-aged adults were maintained under laboratory conditions (as above) until collected for dissection.

Fig. 3

Morphological Studies. Females were dissected under a stereomicroscope (Leica MZ16, Bannockburn, IL), their ovaries removed and photographed digitally to record length of ovary, width of ovary, and length of terminal follicle using ImagePro MC 5.1 (Media Cybernetics, Bethesda, MD). A fourth character, ovarian index, was calculated by multiplying ovary length by ovary width (Kendra et al. 2005). Sexual maturity status was classified using a six-stage system previously published (Fig. 4; Kendra et al. 2006), and number of mature oocytes per ovary (egg load) was counted. Ovary measurements were recorded from females 1-28 days post-eclosion (n = 5 per day).

Fig. 4



Stages of ovarian development in adult *Anastrepha suspensa.* Stages 1 (A) and 2 (B) represent follicles in early and late previtellogenic development, respectively; Inset B shows clustered trophocytes (nurse cells). Stage 3 (C) marks initiation of vitellogenesis, accumulation of yolk in terminal follicles; Inset shows enlarged follicle containing a yolk-filled oocyte (dark lower portion) capped with trophocytes. Stage 4 (D) indicates late vitellogenesis, at which point yolk occupies more than half the follicle. Stage 5 (E) denotes ovaries with mature oocytes, characterized by an intact chorion (eggshell) with a reflective surface and a reticulated pattern visible at high magnification (inset). Stage 6 (F) indicates onset of oviposition, confirmed by presence of residual follicular bodies (corpora lutea) at base of the ovary (enlarged in inset). [All images at same magnification, scale unit (A) = 0.1 mm.]

Kendra, P. E., W. S. Montgomery, D. M. Mateo, H. Puche, N. D. Epsky, and R. R. Heath. 2005. Effect of age on EAG response and attraction of female *Anastrepha suspensa* (Diptera: Tephritidae) to ammonia and carbon dioxide. Environ. Entomol. 34: 584-590.

Kendra, P. E., W. S. Montgomery, N. E. Epsky, and R. R. Heath. 2006. Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae). Florida Entomol. 89: 144-151.

The authors gratefully acknowledge technical assistance from Carina Allen, Ingris Filpo, Micah Gill, Luis Mazuera, and Jeffrey Tefel. Mention of a proprietary product does not constitute an endorsement by the USDA.

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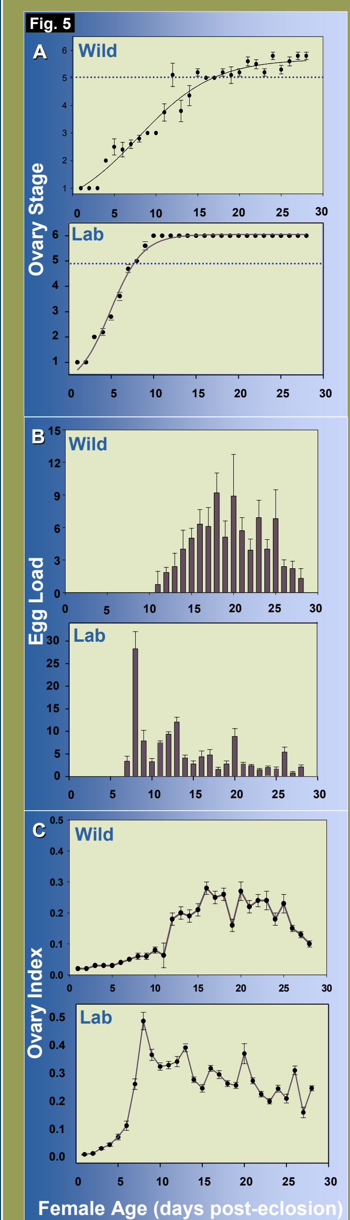
Results and Discussion

Table 1 presents the mean values for ovarian characters and egg load recorded at each developmental stage from the two strains of *A. suspensa*. Stages 1-4 represent sexually immature females, stages 5-6 represent reproductively mature (gravid) females.

Table 1

Stage	Age (d)		Ovary Length (mm)		Ovary Width (mm)		Ovarian Index (mm^2)		Follicle Length (mm)		Egg Load	
	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild	Lab	Wild
1	1-2	1-6	0.29	0.43	0.27	0.29	0.08	0.12	---	---	0.00	0.00
2	3-5	4-14	0.58	0.64	0.36	0.36	0.21	0.23	0.11	0.19	0.00	0.00
3	4-6	5-19	0.9	0.85	0.49	0.46	0.45	0.42	0.26	0.25	0.00	0.00
4	6-7	11-13	1.38	1.42	0.71	0.80	0.97	1.13	0.52	0.78	0.00	0.00
5	7-9	11-28	1.88	1.61	1.29	0.86	2.45	1.39	1.06	0.97	18.2	7.1
6	9-28	12-28	1.56	1.46	1.05	0.64	1.66	0.97	0.96	0.76	4.2	2.0

Fig. 5



Ovary Developmental Stage.

Comparing female chronological age to stage of ovarian maturation, there were significant differences between the two genetic strains (Fig. 5A). The transition from immature females to mature females (stage 5 ovaries) is indicated by the dotted lines on the graphs. With the laboratory strain, this maturity threshold was reached by day 8 and development was synchronous within the female population. In the wild strain, sexual maturation was much less synchronous, and it took an average of 16-17 days for females to attain maturity.

(For both fly strains, the relationship between ovary stage and female chronological age was best fit by a sigmoidal model.)

Egg Load.

There were differences in temporal patterns of egg production between the two strains (Fig. 5B). In the laboratory strain, mature eggs were first detected on day 7, with a peak number of eggs observed by day 8. In the wild strain, there was much more variation. The first mature eggs were observed on day 11, with a gradual increase in number over time to reach a maximum level at about day 18-20. With the synchronous development of females in the laboratory population, there were distinct fluctuations in egg load over time, with peaks observed on days 8, 13, 20, and 26. This cyclic pattern suggests that eggs are laid in batches by female *A. suspensa*.

Ovarian Index.

Of the four morphometric characters evaluated in this study, ovarian index (Fig. 5C) was the best indicator of female sexual maturity. All four characters were positively correlated with egg load, but the highest correlation was obtained with ovarian index. Ovarian index combined the contributions of ovary length and width, thereby maximizing the differences in size between immature and mature ovaries. In addition, the first peak value observed in ovary index corresponds approximately with the threshold age for sexual maturation.

Summary. Female *Anastrepha suspensa* are sexually immature at eclosion and ovarian maturation is dependent upon multiple variables, both biotic and abiotic. Therefore, chronological age is not equivalent to physiological age. This study, conducted under controlled environmental conditions and adult diet, demonstrated that genetic strain differences also influence maturation rate. Classification of sexual maturity based on an ovary staging system is a useful tool for assessing physiological age of field-trapped flies.