

Ovarian Development in a Laboratory Strain of the Caribbean Fruit Fly, *Anastrepha suspensa* (Diptera: Tephritidae)

Paul E. Kendra, Wayne S. Montgomery, Nancy D. Epsky, & Robert R. Heath

USDA-ARS, Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158-1334 USA

ABSTRACT: Female *A. suspensa* are sexually immature at eclosion and the maturation rate is variable, depending upon diet and environmental factors. A classification system for sexual maturity based on ovarian developmental stage, in conjunction with assessment of egg load in the mature stages, will facilitate evaluation of the age structure of a fly population responding to specific lures in the field (Kendra et al. 2006). Although a laboratory strain of *A. suspensa* was used for this study, the proposed classification system should have broad application since it reflects female physiological age determined by examination of multiple ovarian characters. In addition, standardization for insect size may improve resolution of ovary measurements as parameters for assessing maturity status in more variable field populations. The utility of this method for wild populations of *A. suspensa* and other tephritid species is being addressed by our group through ongoing laboratory and field studies.

Key Words: Sexual maturation, oocyte, follicle, vitellogenesis, egg load

INTRODUCTION

Tephritid fruit flies in the genus *Anastrepha* are serious economic pests of fruit crops throughout tropical and subtropical regions of the Americas (Aluja 1994). In the U.S. state of Florida, the Caribbean fruit fly, *A. suspensa* (Loew), is a quarantine pest of citrus (particularly grapefruit, *Citrus paradisi* Macf.) and a production pest of guava (*Psidium guajava* L.) (Greany and Riherd 1993). Since ammonia has been identified as the primary fruit fly attractant emitted from protein food sources (Bateman and Morton 1981), current monitoring programs rely on ammonia-based synthetic lures (Heath et al. 1995, Thomas et al. 2001). However, attraction of *Anastrepha* to these lures has been variable in the field (Epsky et al. 2004).

In an effort to develop improved synthetic lures for pest *Anastrepha*, research was initiated to identify factors responsible for the observed variability in attraction. Using electroantennography (EAG, a technique which measures response of antennal ol-

factory receptors), Kendra et al. (2005a) evaluated dose-response of mature male and female *A. suspensa* to ammonia. EAG recordings from females 1-14 days post-eclosion documented that antennal response to ammonia was not constant, but varied depending upon maturity status of the female (Kendra et al. 2005b). The antennal response of sexually mature and immature females correlated with differences in behavioral response to ammonia measured in flight tunnel tests (Kendra et al. 2005b). These laboratory results support the hypothesis that the variability seen in field captures may be due, in part, to the physiological age structure of the fly population during the monitoring period.

Testing this hypothesis requires a reliable method by which maturity status of field-caught flies can be determined from morphological evidence. At eclosion, female fruit flies are sexually immature, and the ovarian maturation process is dependent upon multiple factors, including temperature, photoperiod, diet, chemical cues, and availability of mates (Fletcher 1989, Wheeler 1996, Papaj 2000, Aluja et al. 2001, Pereira et al. 2006). To differentiate between sexually mature and immature females, previous studies have used measurements of ovary

Author for correspondence.

Paul E. Kendra

e-mail: paul.kendra@ars.usda.gov

length (Nation 1972, Dodson 1982), ovary width (Dodson 1978) or ovarian index (ovary length multiplied by width, Landolt and Davis-Hernandez 1993). Nation (1972) also confirmed sexual maturity by the presence of mature oocytes that are ~1 mm long and opaque. However, there are decreases in both the percent of sexually mature females with mature oocytes once oviposition starts (Dodson 1982) and in the number of eggs oviposited over the fairly long lifespan of *A. suspensa* females (Sivinski 1993), making reliance on single factor determinations unreliable for flies trapped in the field. In this report, we examine several ovarian morphometric characters, document changes in these characters over a four week period following adult eclosion, and assess how reliably each character serves as an indicator of sexual maturity in *A. suspensa*.

MATERIALS AND METHODS

Insects. *A. suspensa* were obtained from a laboratory colony maintained at the USDA-ARS, Subtropical Horticulture Research Station, Miami, FL, USA. Rearing conditions consisted of a photoperiod of 12:12 (L:D), 70% RH, and ambient room temperature ($25 \pm 2^\circ\text{C}$). Adult flies were held in mixed-sex cages (1:1 ratio) and provisioned with water (agar blocks) and food (sugar and yeast hydrolysate, 4:1 mixture) *ad libitum*. Known-aged females were collected and stored in 70% ethanol prior to dissection.

Morphological Studies. Flies were dissected under a stereomicroscope (25x magnification), their reproductive system (Fig. 1) examined, and ovarian development classified by adapting the system of Raghu et al. (2003) described for *Bactrocera cacuminata* (Hering). Measurements were taken of ovary length (from top of germarial region to base of lateral oviduct), ovary

width (at widest point), and length of terminal follicle (the largest, most advanced follicle). All measurements were made with a hand-held micro-scale (to 0.1 mm; Mini-tool, Inc., Los Gatos, CA).

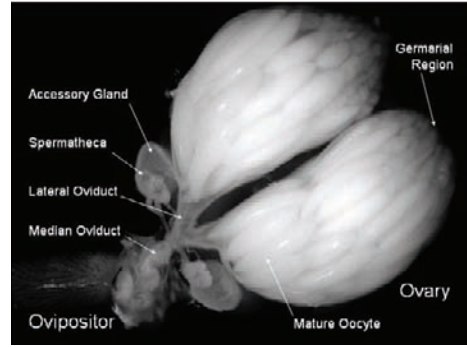


Figure 1. Reproductive system of a mature (Stage 5) female Caribbean fruit fly, *Anastrepha suspensa* (Loew).

In addition, ovary length was multiplied by ovary width to obtain an ovarian index (Landolt and Davis-Hernandez 1993, Kendra et al 2005b). Ovarioles were then teased apart with fine insect pins to count the number of mature oocytes per ovary (egg load). To be considered mature, oocytes had to lack accompanying trophocytes (nurse cells) and possess a fully developed chorion (eggshell), visible at 100x magnification. Finally, the forewing length (from base of costal vein to wing apex where vein R_{4+5} terminates at the margin) was measured as an independent indicator of insect size. Measurements were recorded from females 1-28 days post-eclosion ($n = 10$ females per day).

Statistical Analyses. Regression analysis (SigmaPlot 8.0; SPSS, Inc. 2002) was used to describe the relationship between female chronological age and ovary developmental stage. Differences in ovarian characters among stages were analyzed by one-way analysis of variance (ANOVA) with PROC GLM (SAS Institute 1985) followed by Tukey's test ($P = 0.05$) for mean separation. The Box-Cox procedure, which is a power transformation

that regresses log-transformed standard deviations ($y + 1$) against log-transformed means ($x + 1$), was used to determine the type of transformation necessary to stabilize the variance before analysis (Box et al. 1978). Correlations between egg load and ovarian characters were determined using PROC CORR, and analysis of covariance (ANCOVA) with PROC GLM was used to evaluate effect of female size on comparisons of ovarian characters.

RESULTS AND DISCUSSION

The ovarian maturation process in *A. suspensa* was classified into six distinct stages, depicted in Figure 2. Ovary stages 1 (Fig. 2A) and 2 (Fig. 2B) represented follicles in early and late previtellogenic development, respectively. Stage 3 (Fig. 2C) marked initiation of vitellogenesis, the accumulation of yolk in terminal follicles; this is illustrated better in

the photo inset of Fig. 2C, which shows an enlarged follicle containing a yolk-filled oocyte (dark lower portion) capped with trophocytes. Stage 4 (Fig. 2D) indicated late vitellogenesis, at which point the yolk occupied more than half the follicle. Stage 5 (Fig. 2E) denoted ovaries with mature oocytes, characterized by an intact chorion with a reflective surface and a reticulated pattern (especially pronounced near the micropyle) visible at high magnification (Fig. 2E inset). Stage 6 (Fig. 2F) indicated onset of oviposition, confirmed by the presence of residual follicular bodies (corpora lutea) at the base of the ovary (enlarged in Fig. 2F inset).

The relationship between ovary stage and female age was best fit by a sigmoidal model (Fig. 3A), and comparisons of ovary characters among the six stages are given in Table 1. The ovaries of *A. suspensa* initially increased in length and then in width during a maturation phase which spanned the first 8 days post-eclosion in this laboratory population.

TABLE 1. Ovarian Characters (Mean \pm SD) at each Developmental Stage in Female *A. suspensa*.

Stage	n	Age (d)	Ovary Length (mm)	Ovary Width ¹ (mm)	Ovarian Index ¹ (mm ²)	Follicle Length (mm)	Egg Load ²
1	20	1 - 2	0.29 \pm 0.06 a	0.27 \pm 0.05 a	0.08 \pm 0.03 a	0.10 \pm 0.00 a	0.0 \pm 0.00 a
2	20	3 - 5	0.58 \pm 0.15 b	0.36 \pm 0.09 ab	0.21 \pm 0.08 ab	0.11 \pm 0.03 a	0.0 \pm 0.00 a
3	14	4 - 6	0.90 \pm 0.15 c	0.49 \pm 0.08 b	0.45 \pm 0.12 b	0.26 \pm 0.07 b	0.0 \pm 0.00 a
4	9	6 - 7	1.38 \pm 0.34 d	0.71 \pm 0.12 c	0.97 \pm 0.28 c	0.52 \pm 0.19 c	0.0 \pm 0.00 a
5	21	7 - 9	1.88 \pm 0.22 e	1.29 \pm 0.26 e	2.45 \pm 0.71 e	1.06 \pm 0.12 e	18.2 \pm 13.49 c
6	196	9 - 28	1.56 \pm 0.20 d	1.05 \pm 0.18 d	1.66 \pm 0.43 d	0.96 \pm 0.12 d	4.2 \pm 3.80 b
	F		279.95	205.08	204.32	479.28	64.01
	df		5,274	5,274	5,274	5,274	5,274
	P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same letter are not significantly different (Tukey's mean separation test [$P = 0.05$]).

¹Data were square-root ($x + 0.5$) transformed prior to analysis, non-transformed means are shown.

²Data were log ($x + 1$) transformed prior to analysis, non-transformed means are shown.

Of the characters examined, ovary length provided the best separation of immature stages during this maturation phase, but ovary length alone did not discriminate between stage 4 (immature) and stage 6 (mature) ovaries. Distinguishing between these two stages required inspection for residual follicular bodies and assessment of gross ovary morphology. Ovarian index, which combined the contributions of length and width, effectively maximized the differences between immature and mature ovaries.

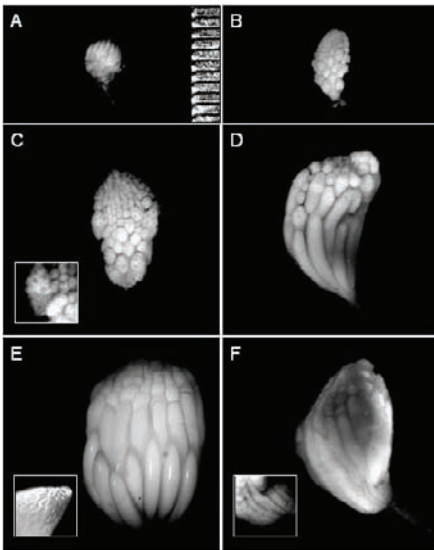


Figure 2. Stages of ovarian development in adult *A. suspensa*. Stages 1-4 (A-D) comprise the classes of sexually immature females, and describe sequential steps in the ovary maturation phase. Stages 5 and 6 represent mature ovaries, before and after the onset of oviposition, respectively. See text for detailed descriptions of developmental stages. (All whole ovary images at same magnification; scale unit = 0.1 mm.)

Ovarian index has been used previously for assessment of sexual maturity in this strain of *A. suspensa*, and Kendra et al. (2005b) concluded that peak EAG response to ammonia occurred in immature flies (4-6 days old) while peak response to carbon dioxide occurred in sexually mature flies (10-12 days old). Clas-

sification by developmental stage now provides further interpretation of those results. Maximal antennal response to ammonia was measured from females with stage 3 ovaries actively undergoing vitellogenesis (deposition of yolk proteins), and this coincides with the age of peak protein consumption reported by Landolt and Davis-Hernandez (1993). Maximal response to carbon dioxide was found in stage 6 females during the ovipositional phase, which is consistent with the theory proposed by Stange (1999) that carbon dioxide serves as a close-range oviposition attractant for tephritid fruit flies.

The presence of mature oocytes in an ovary is regarded as the definitive character for female sexual maturity (Nation 1972, Aluja et al. 2001). Some 7 day old females had mature oocytes, but by day 8 all females had mature oocytes under laboratory conditions. The mean egg load (Fig. 3B) fluctuated over time, suggesting that eggs are laid in batches initially, when ovarioles are most synchronous. Once a female is sexually mature, with fully developed eggs, she may switch from food-seeking behaviors, which allow her to obtain protein for egg development, to oviposition-site seeking behaviors, which enable her to locate suitable host fruit. Predominance of these two activities may alternate throughout stage 6, and a mature female might not engage in host seeking until she possesses an appropriate egg load. Thus, determination of egg load in mature females may provide further discrimination among flies captured in field trials. All four characters were positively correlated with egg load, but the two best indicators were ovary index ($r = 0.7832$, $P < 0.0001$; Fig. 3C) and ovary width ($r = 0.7464$, $P < 0.0001$). Use of these parameters to assess egg load would eliminate the need to separate ovarioles and count mature eggs, thereby providing an efficient method for processing large samples of flies. Adjustment for female size was not significant for any of the ovary characters examined in this

laboratory strain, and therefore did not improve assignment of females to a particular developmental stage. The greatest effect was observed with ovary length ($F = 2.06$; $df = 5, 268$; $P = 0.0714$), indicating that in tests of flies that are more variable in size, accounting for individual size may improve use of ovary length measurements as an indicator of sexual maturity.

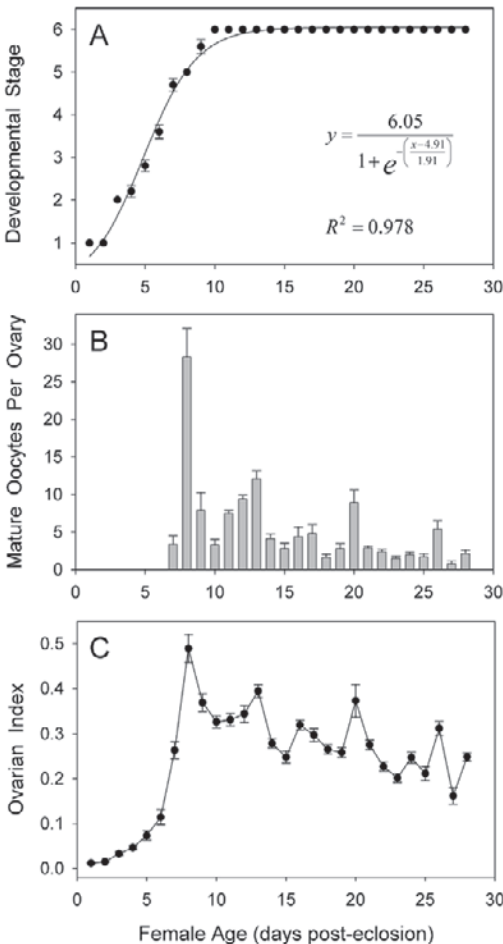


Figure 3. Three methods for assessing reproductive status in female *A. suspensa*. (A) Ovarian maturation depicted by developmental stages. (B) Number of mature oocytes per ovary (egg load). (C) Ovarian index (ovary length multiplied by width), standardized relative to length of forewing. All three temporal profiles present mean values (\pm SE) recorded from ovaries dissected 1-28 days after adult emergence; $n = 10$ females per day.

ACKNOWLEDGMENTS

The authors are grateful for technical assistance provided by Monica Schiessl and Micah Gill (USDA-ARS, Miami, FL), and for reviews of an earlier version of this manuscript provided by Don Thomas (USDA-ARS, Weslaco, TX) and Rui Pereira (University of Florida, Gainesville, FL). This article reports the results of research only; mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

REFERENCES

- Aluja, M. 1994. Bionomics and management of *Anastrepha*. Annual Review of Entomology 39: 155-178.
- Aluja, M., F. Diaz-Fleisher, D. R. Papaj, G. Lagunes, and J. Sivinski. 2001. Effects of age, diet, female density, and the host resource on egg load in *Anastrepha ludens* and *Anastrepha obliqua* (Diptera: Tephritidae). Journal of Insect Physiology 47: 975-988.
- Bateman, M. A., and T. C. Morton. 1981. The importance of ammonia in proteinaceous attractants for fruit flies (Family: Tephritidae). Australian Journal of Agricultural Research 32: 883-903.
- Box, G. E. P., W. G. Hunter, and J. S. Hunter. 1978. Statistics for experimenters. An introduction to design, data analysis, and model building. J. Wiley & Sons, New York, NY, USA.
- Dodson, G. 1978. Morphology of the reproductive system in *Anastrepha suspensa* (Loew) and notes on related species. Florida Entomologist 61: 231-239.
- Dodson, G. 1982. Mating and territoriality in wild *Anastrepha suspensa* (Diptera: Tephritidae) in field cages. Journal of the Georgia Entomological Society 17: 189-199.
- Epsky, N. D., P. E. Kendra, and R. R. Heath. 2004. Development of lures for detection and delimitation of invasive *Anastrepha* fruit flies, pp. 84-89. In W. Klassen, W. Colon, and W. I. Lugo (eds.), Proceedings of the 39th Annual Meeting of the Caribbean Food Crops Society. July 2003, Grenada.
- Fletcher, B. S. 1989. Temperature-development rate relationships of the immature stages and adults of tephritid fruit flies, pp. 283-289. In A. S. Robinson and G. Hooper (eds.), Fruit Flies – Their Biology, Natural Enemies and Control, Vol 3A. Elsevier, Amsterdam.
- Greany, P. D., and C. Riherd. 1993. Preface: Caribbean fruit fly status, economic importance, and control (Diptera: Tephritidae). Florida Entomologist 76: 209-211.

- Heath, R. R., N. D. Epsky, A. Guzman, B. D. Dueben, A. Manukian, and W. L. Meyer. 1995. Development of a dry plastic trap with food-based synthetic attractant for the Mediterranean and the Mexican fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* 88: 1307-1315.
- Kendra, P. E., A. Vázquez, N. D. Epsky, and R. R. Heath. 2005a. Ammonia and carbon dioxide: Quantitation and electroantennogram responses of Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Environmental Entomology* 34: 569-575.
- Kendra, P. E., W. S. Montgomery, D. M. Mateo, H. Puche, N. D. Epsky, and R. R. Heath. 2005b. Effect of age on EAG response and attraction of female *Anastrepha suspensa* (Diptera: Tephritidae) to ammonia and carbon dioxide. *Environmental Entomology* 34: 584-590.
- Kendra, P. E., W. S. Montgomery, N. D. Epsky, and R. R. Heath. 2006. Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae). *Florida Entomologist* 89: 144-151.
- Landolt, P. J., and K. M. Davis-Hernandez. 1993. Temporal patterns of feeding by Caribbean fruit flies (Diptera: Tephritidae) on sucrose and hydrolyzed yeast. *Annals of the Entomological Society of America* 86: 749-755.
- Nation, J. L. 1972. Courtship behavior and evidence for a sex attractant in the male Caribbean fruit fly, *Anastrepha suspensa*. *Annals of the Entomological Society of America* 65: 1364-1367.
- Papaj, D. R. 2000. Ovarian dynamics and host use. *Annual Review of Entomology* 45: 423-448.
- Pereira, R., P. E. A. Teal, J. Sivinski, and B. D. Dueben. 2006. Influence of male presence on sexual maturation in female Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Journal of Insect Behavior* 19: 31-43.
- Raghu, S., P. Halcoop, and R. A. I. Drew. 2003. Apodeme and ovarian development as predictors of physiological status in *Bactrocera cacuminata* (Hering) (Diptera: Tephritidae). *Australian Journal of Entomology* 42: 281-286.
- SAS Institute. 1985. SAS/STAT guide for personal computers, version 6. SAS Institute, Cary, NC, USA.
- Sivinski, J. M. 1993. Longevity and fecundity in the Caribbean fruit fly (Diptera: Tephritidae): Effects of mating, strain and body size. *Florida Entomologist* 76: 635-644.
- SPSS, Incorporated. 2002. SigmaPlot 8.0 user's guide. SPSS, Inc., Chicago, IL, USA.
- Stange, G. 1999. Carbon dioxide is a close-range oviposition attractant in the Queensland fruit fly, *Bactrocera tryoni*. *Naturwissenschaften* 96: 190-192.
- Thomas, D. B., T. C. Holler, R. R. Heath, E. J. Salinas, and A. L. Moses. 2001. Trap-lure combinations for surveillance of *Anastrepha* fruit flies (Diptera: Tephritidae). *Florida Entomologist* 84: 344-351.
- Wheeler, D. 1996. The role of nourishment in oogenesis. *Annual Review of Entomology* 41: 407-431.