EGG MORPHOLOGY OF *ANASTREPHA OBLIQUA* AND SOME COMPARATIVE ASPECTS WITH EGGS OF *ANASTREPHA FRATERCULUS* (DIPTERA:TEPHRITIDAE)

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ABSTRACT

The egg morphology of *Anastrepha obliqua* (Macquart) is described utilizing both light and scanning electron microscopy. Other observations include oviposition depth by *A. obliqua* on mango and comparative analysis with *A. fraterculus* (Wiedemann) egg morphology. *A. obliqua* eggs bear a conspicuous structure at the anterior pole, which is called a respiratory horn. A discussion of this term and definitions given by other authors for similar structures found in other fruit fly eggs is given. We suggest that the occurrence of the respiratory horn outside the mango fruit peel by *A. obliqua* may be a useful character for post-harvest inspection and mango fruit disinfestation.

Key Words: Mango, fruit flies, insect egg anatomy, insect respiration

RESUMEN

La morfología de *Anastrepha obliqua* (Macquart) es descrita por medio de microscopia de luz y electrónica de barrido. Otras observaciones incluyen la profundidad de ovoposición por *A. obliqua* en mangos y el analisis comparativo con la morfología del huevo de *A. fraterculus* (Wiedemann). Los huevos de A. obliqua poseen una notable estructura en el polo anterior, que es llamada cuerno respiratorio. Se ofrece una discusion acerca de este termino y de definiciones dadas por otros autores para estructuras similares en otros huevos de moscas fruteras. Sugerimos que la aparición del cuerno respiratorio fuera de la cáscara del mango en *A. obliqua* puede ser una caracteristica util para la inspección post cosecha y la desinfección de los frutos.

The taxonomy of *Anastrepha* fruit flies is based primarily on adult forms; however, egg morphology also has taxonomic value, and more descriptive work needs to be done (Norrbom & Foote 1989). Emmart (1933) published descriptions of the eggs of *A.ludens* (Loew), *A. obliqua* (Macquart) (confused with *A. fraterculus* (Wiedemann)), *A. striata* Schiner and *A. serpentina* (Wiedemann). Sein (1933) reported that a portion of the egg of *A. obliqua* remains outside of the fruit peel after oviposition. Later, Lawrence (1979) described the eggs of *A. suspensa* (Loew). More recently, Norrbom (1985) described the general characters of the eggs of eight *Anastrepha* species, including *A. obliqua*. Additional descriptions of the eggs of *A. bistrigata* Bezzi (Steck & Malavasi 1988), *A. grandis* (Macquart) (Steck & Wharton 1988) and *A. ludens* (Carroll & Wharton 1989) have appeared since then.

Based on the above mentioned descriptions, there is some confusion concerning the morphology of *A. obliqua* eggs. Emmart (1933) described a twisted anterior end of the egg, hairs around the micropyle and on the posterior end, and a sculptured surface on the anterior (micropylar) end. Sein (1933) described a smooth surface over the entire egg. Norrbom (1985) described *A. obliqua* eggs as having an anterior lobe covered by pore-like openings and a reticulated surface at the base of this lobe. The latter author made these observations on ova dissected from preserved females and expressed some reservations about the appearance of the egg after oviposition.

Here, we describe the morphology of the eggs of *A. obliqua* with light and scanning electron microscopy (SEM). The position of the egg after oviposition into mango fruit is also described with SEM. We also make a comparative description with *A. fraterculus* egg morphology.

MATERIALS AND METHODS

Adults of *A. obliqua* were reared at the laboratories of Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) in Guadalupe (San José Prov., elev. 1200 m.) obtained from field infested mangoes collected at Orotina (Alajuela Prov., elev. 200 m.). About 300 of these adults (both sexes) were held in a 1 m^3 screened cage and allowed to oviposit into an artificial medium consisting of a 10 cm diam agar hemisphere coated with a beeswax-paraffin mixture. Thirty eggs were retrieved and placed in glycerin on depression slides and measured under a compound light microscope. General features were observed with light microscopy by mounting another thirty eggs in Hoyers temporary mounting medium. Adults of *A. fraterculus* were obtained from a laboratory colony (generation F23), and eggs were obtained by placing a piece of black cloth externally on the upper wall of the colony cage for oviposition. They were treated as described above.

Eggs of both *Anastrepha* species were fixed for SEM in 2.5% glutaraldehyde and 2.0% paraformaldehyde, in phosphate buffer (0.1 M, pH 7.4), post-fixed in 1% osmium tetraoxide, dehydrated in graded alcohol, and critically point dried. Stub mounted specimens were examined with a scanning electron microscope (Hitachi S-570). Preparation for cryofracture was carried out, as an intermediate step after alcoholic dehydration, using a substitution graded mixture up to 1:1 alcohol: DMSO (dimethyl sulfoxide). An aluminum surface frozen by liquid nitrogen was the support for the sample, which was cryofractured by a stroke with a razor blade. After the SEM preparation process, we observed some problems keeping the egg in turgid form; however, the anterior pole maintained its shape, thus facilitating its observation.

Five mature mango fruits of Yellow, Mora and Tommy Atkins varieties were exposed to oviposition by about 300 *A. obliqua* adults (both sexes) in a 1 m^3 screened cage for twelve hours. Thirty 1 cm^3 pieces of fruit rind, each containing one egg, were taken from each variety for microscopic examination and measurement of the exposed portion of the egg with a dissecting microscope. Oviposition depth into mango fruit by *A. obliqua* was calculated as the difference between the average egg length and the length of each of the exposed respiratory horns. Ten pieces of each mango variety, each containing one egg, were similarly treated for SEM as described above to examine the exposed egg horn after oviposition.

RESULTS AND DISCUSSION

Anastrepha obliqua and *A. fraterculus* are closely related species belonging to the *fraterculus* subgroup (Norrbom 1985); however, their egg morphologies are clearly different.

Anastrepha obliqua eggs observed by light microscopy are creamy white with the anterior end drawn into a respiratory horn and the posterior end bluntly rounded (Fig. 1). Average measurements of thirty *A. obliqua* eggs were: total length 1.447 mm (1.181-1.584 mm), greatest width 0.225 mm (0.202-0.259 mm) and respiratory horn length 0.142 mm (0.086-0.245 mm).

The respiratory horn is a projection of the chorion and aeropyles are grouped on the apex; this structure obviously functions in gaseous exchange. It has been observed that eggs hatch after being in bubbling water for 48 hours (for mass rearing purposes) (Aagesen, personal communication). Thus, it seems possible that the egg's respiratory system can function as a plastron. At the base of the respiratory horn, we observed a micropyle (Fig. 3). No hair-like structures were found as Emmart (1933) previously reported.

Our use of the term respiratory horn is made in the same sense as that of Hinton (1981) who used it to describe structures on eggs which are used in atmospheric respiration. In the case of *A. obliqua*, this structure seems obviously to function in respiration, and the term respiratory horn seems preferable to other terms commonly used to describe similar structures on tephritid eggs such as "pedicel" (Headrick & Goeden 1990), "stalk" (Benjamin 1934, Cavender & Goeden 1984), or "lobe" and "appendage" (Norrbom 1985). Pedicel, for example, is the diminutive of the latin *ped* or *pedis* and means "little foot"; stalk refers to a lengthened part on which an organ grows or is supported; thus, neither of the former terms is appropriate to the structure seen here, and other terms are less descriptive of its apparent function.

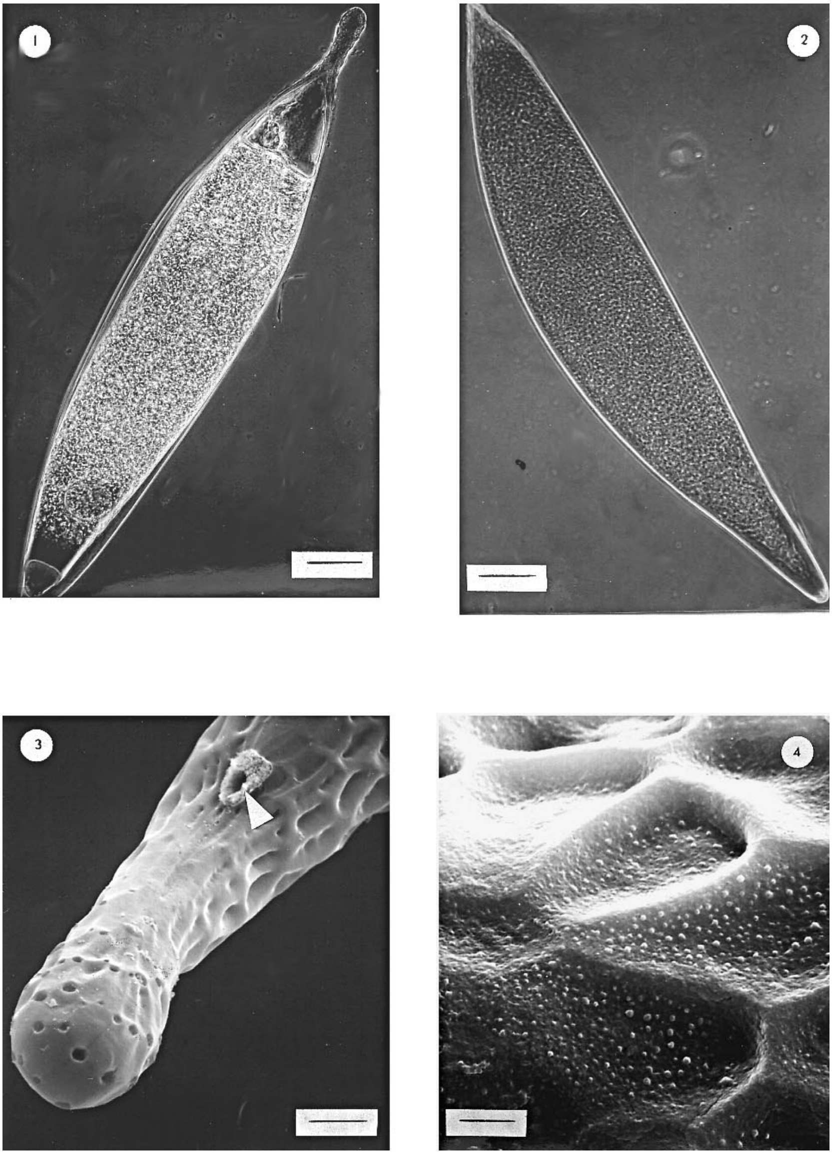
Most of the egg's surface is smooth with no obvious structures or "sculpturing" on it. However, it is reticulated near both ends and these areas have a granular texture (Fig. 4). The reticulation at the respiratory horn's base is more strongly defined, but less polygonal than at the posterior end.

The entire chorion as well as the respiratory horn is cavernous; thus, there is a continuous network of open spaces extending from the respiratory horn to the rest of the chorion over the entire egg body. The internal structures of the chorion and respiratory horn are shown in Fig. 5 and 6, respectively.

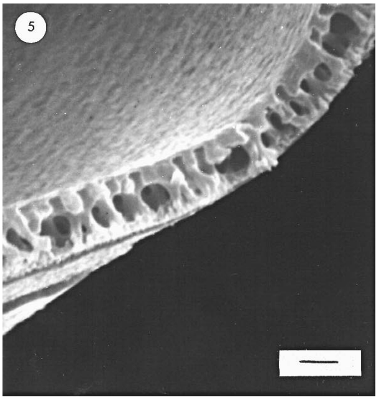
Bacteria were observed on recently oviposited eggs of *A. obliqua*. Previous observations by Murillo et al. (1990) showed that some bacterial flora, which they called "indigenous microflora", are associated with *A. obliqua* eggs just after oviposition. These bacterial species belong to the Enterobacteriaceae group, which also have been reported on the eggs of other tephritid species (Drew & Lloyd 1989, Howard et al. 1985).

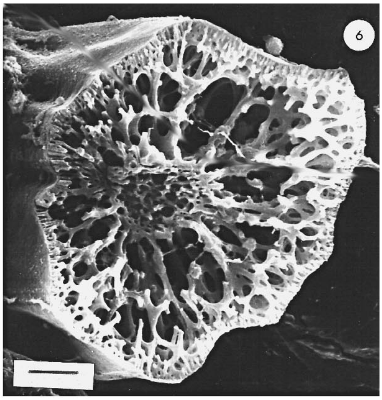
Anastrepha fraterculus eggs are elongated, creamy white, with a very short chorionic extension anteriorly and bluntly rounded posteriorly (Fig. 2). The micropyle is more conspicuous than that observed in *A. obliqua*, and it is located almost at the anterior apex of the egg (Fig. 8). The eggs of *A. fraterculus* bear polygonal reticulations over the anterior pole and subdistally to the posterior pole, and they are more clearly defined than those of *A. obliqua* eggs. There are aeropyles on the borders of the reticulations on the anterior pole, surrounding the micropyle (Fig. 9).

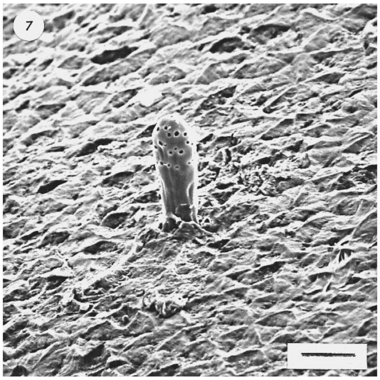
Anastrepha obliqua lay their eggs individually with the respiratory horn remaining at the fruit's surface from the micropyle upward (Fig. 7). This suggests that the *A. obliqua* egg needs unobstructed air exchange with the atmosphere before the larva hatches. The angle of the respiratory horn relative to the fruit surface may change, but it remains exposed. Average oviposition depth was 1.34 mm in the three mango varieties studied, and there were no statistically significant differences among them (1.347 mm oviposition depth for Yellow and Tommy Atkins, and 1.334 mm for Mora; SE 0.022, 0.032 and 0.040, respectively; $P \leq 0.05$).

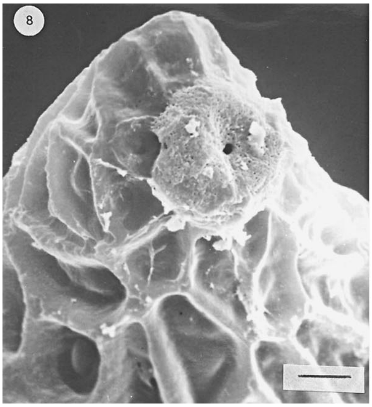


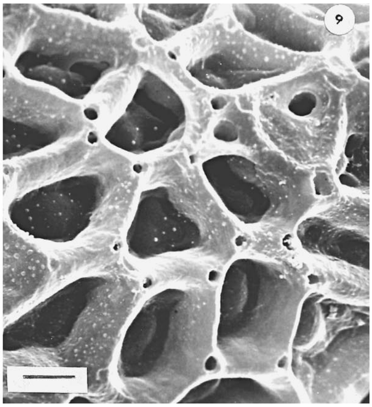
Figs. 1-4.
(1) The egg of *Anastrepha obliqua* (Bar = 120 µm).
(2) The egg of *Anastrepha fraterculus* (Bar = 115 µm).
(3) Respiratory horn of the egg of *A. obliqua*, aeropyles at the apex and micropyle at the base (arrow) (SEM) (Bar = 14 µm).
(4) Granular surface of the anterior reticulated area of *A. obliqua* egg (SEM) (Bar = 3 µm).











Figs. 5-9.
(5) Cryofracture of the chorion of the *A. obliqua* egg (SEM) (Bar = 1 µm).
(6) Cryofracture of the respiratory horn of the *A. obliqua* egg (SEM) (Bar = µm).
(7) Respiratory horn of *A. obliqua* egg emerging from the mango peel (SEM) (Bar = 50µm).
(8) Micropyle at the anterior apex of *A. fraterculus* egg (SEM) (Bar = 5 µm).
(9) Aeropyles on the borders of reticulations in the area surrounding the micropyle of *A. fraterculus* egg (SEM) (Bar = 5 µm).

Plastron-bearing respiratory horns have been reported in species belonging to seven dipteran families (Dryomyzidae, Sepsidae, Coelopidae, Sphaeroceridae, Drosophilidae, Cordiluridae and Muscidae) (Hinton 1981). However, so far there has been no report of its occurrence in Tephritidae. Mouzaki & Margaritis (1987) suggested that the respiratory structures present in the family Drosophilidae have been replaced in Tephritidae by: a) an area for respiration on the main body of the egg, b) later by the whole egg's surface, and c) very limited areas on the main body and the anterior cup of the egg. However, at least in *A. obliqua* a respiratory horn is present, and the structures found in *A. nigrifascia* (Stone), *A. pittieri* (Caravallo) (Norrbom 1985), *Paracantha gentilis* (Hering) (Headrick & Goeden 1990), *P. cultaris* (Coquillett) (Cavender & Goeden 1984), *P. culta* (Wiedemann), *P. forficula* (Benjamin), *Toxotrypana curvicauda* Gerstaecker, and *Zonosemata electa* (Say) (Benjamin 1934) eggs also may serve a similar function.

In addition to a respiratory function, the egg horn of *A. obliqua* may play a role in the recognition of infested hosts (fruits) by gravid females. Takasu and Hirose (1988) found that *Ooencyrtus nezarae* Ishii (Encyrtidae: Hymenoptera) utilizes the egg stalk (as they named it) as an external marker, which permits the female to recognize hosts which are already infested. It is possible that *A. obliqua* females, before oviposition, also check suitable fruit for a physical marker (exposed horn), and as a parasitism indicator complementary to oviposition deterring pheromones (chemical marker) (Prokopy & Roitberg 1984). However, this function needs to be demonstrated.

After the larva hatches it migrates into internal fruit tissues. The respiratory horn maintains its physical integrity attached to the fruit peel, unless it is broken off by rubbing or handling.

Perhaps the exposure of the *A. obliqua* respiratory horn, outside the mango fruit peel, could be an aspect to consider when planning post-harvest technology for fresh fruits. There may be value in developing a chemical, physical or biological method to detect the presence of the respiratory horn. These methods could be developed also for use in fruit disinfestation, e.g., addition of a surfactant to a fruit bath may allow water to enter the chorion interior and limit egg respiration.

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REFERENCES CITED

BENJAMIN, F. H. 1934. Descriptions of some native trypetid flies with notes on their habits. United States Dept. Agric. Technical Bulletin 401.

CARROLL, L. E., AND R. A. WHARTON. 1989. Morphology of the immature stages of *Anastrepha ludens* (Diptera: Tephritidae). Ann. Entomol. Soc. America 82: 201-214.

CAVENDER, G. L., AND R. D. GOEDEN. 1984. The life history of *Paracantha cultaris* (Coquillet) on wild sunflower, *Helianthus annus* L. ssp. *lenticularis* (Douglas)

Cockerell, in southern California (Diptera: Tephritidae). Pan-Pacific. Entomol. 60:213-218.

DREW, R. A. I., AND A. C. LLOYD. 1989. Bacteria associated with fruit flies and their host plants, pp. 131-140 *in* A.S. Robinson and G. Hooper, [eds], World Crop Pests. Fruit Flies, their biology, natural enemies and control. Amsterdam, Elsevier Science. v. 3A.

EMMART, E. W. 1933. The eggs of four species of the fruit flies of the genus *Anastrepha*. Proc. Entomol. Soc. Washington 35: 184-191.

HEADRICK, D., AND R. D. GOEDEN. 1990. Description of the immature stages of *Paracantha gentilis* (Diptera: Tephritidae). Ann. Entomol. Soc. America 83: 220-229.

HINTON, H. E. 1981. Biology of insect eggs. Pergamon Press, Oxford. v. I, 316 p.

HOWARD, D. J., G. L. BUSH, AND J. A. BREZNAK. 1985. The evolutionary significance of bacteria associated with *Rhagoletis*. Evolution 39:405-417.

LAWRENCE, P. O. 1979. Immature stages of the Caribbean fruit fly *Anastrepha suspensa*. Florida Entomol. 62: 214-219.

MOUZAKI, D. G., AND L. H. MARGARITIS. 1987. Comparative structural study of the egg-shell (chorion) in *Dacus oleae, Rhagoletis cerasi, Ceratitis capitata,* and *Eurytoma amygdali*. Fruit Flies Procs. II International Symposium, Crete, Sept. 1986. p. 79-87.

MURILLO, T., P. RIVERA, F. HERNANDEZ, AND L. F. JIRON. 1990. Indigenous microflora of the West Indies fruit fly, *Anastrepha obliqua* (Diptera Tephritidae). Fruits (France) 45: 629-631.

NORRBOM, A. L. 1985. Phylogenetic analysis and taxonomy of the *cryptostrepha, daciformis, robusta* and *schausi* species groups of *Anastrepha* Schiner (Diptera: Tephritidae). Ph.D. Thesis. Pennsylvania Sta. Univ. 359 p.

NORRBOM, A. L., AND R. H. FOOTE. 1989. The taxonomy and zoogeography of the genus *Anastrepha* (Diptera: Tephritidae), pp. 15-26 *in* A.S. Robinson and G. Hooper, [eds.], World Crop Pests. Fruit Flies, their biology, natural enemies and control. Amsterdam, Elsevier Science. v. 3A.

PROKOPY, R. J., AND B. ROITBERG. 1984. Foraging behavior of true fruit flies. American Scientist 72: 41-49.

SEIN, F. 1933. *Anastrepha* (Trypetidae, Diptera) fruit flies in Puerto Rico. J. Dept. Agric. Puerto Rico 17:183-196.

STECK, G. J., AND A. MALAVASI. 1988. Description of immature stages of *Anastrepha bistrigata* (Diptera Tephritidae). Ann. Entomol. Soc. America 81: 1004-1009.

STECK, G. J., AND R. A. WHARTON. 1988. Description of immature stages of *Anastrepha interrupta, A. limae, A. grandis* (Diptera: Tephritidae). Ann. Entomol. Soc. America 81: 994-1003.

TAKASU, K., AND Y. HIROSE. 1988. Host discrimination in the parasitoid *Ooencyrtus nezarae*: the role of the egg stalk as an external marker. Entom. Exper. & Appl. 47:45-48.