DESCRIPTION OF *ANASTREPHA SORORCULA* AND *A. SERPENTINA* (DIPTERA: TEPHRITIDAE) EGGS

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ABSTRACT

The morphology of *Anastrepha sororcula* Zucchi and *A. serpentina* (Wiedemann) eggs are described by scanning electron microscopy. *A. sororcula* eggs present a conspicuous sculpturing of the chorion at the micropylar end while the eggs of *A. serpentina* are devoid of such ornamentation. The sculpturing of the *A. sororcula* eggs, represented by ridges in a polygonal arrangement, is more developed in the ventral than in the dorsal side of the egg. The micropyle is located in the dorsal side near the egg apex and is surrounded by a row of chorionic polygons. In *A. serpentina* the micropyle is located in a prominent rim very near the extremity of the egg. Aeropyles are found at the anterior end usually at the ventral side of the eggs. Fractured eggs of both species showed that the chorion is cavernous throughout the egg body. It is suggested that these eggs may be able to perform a plastron-mediated respiration. The results suggest that egg morphology may be useful to elucidate taxonomic and phylogenetic relationship among *Anastrepha* species.

Key Words: Fruit flies, chorion, eggshell, plastron

RESUMEN

La morfología de los huevos de *Anastrepha sororcula* Zucchi y de *A. serpentina* (Wiedemann) es descrita por microscopía electrónica. El corion de los huevos de *A. sororcula* presenta una escultura conspicua en el extremo micropilar mientras que los huevos de *A. serpentina* están desprovistos de tal ornamentación. La ornamentación de los huevos de *A. sororcula*, representada por pliegues en un arreglo poligonal, está más desarrollada en el lado ventral que en el lado dorsal. El micrópilo se localiza en el lado dorsal cerca del ápice del huevo y está rodeado por una hilera de polígonos coriónicos. En *A. serpentina* el micrópilo se localiza en una prominencia muy cerca del ápice del huevo. Normalmente los aerópilos se encuentran en el extremo anterior, lado ventral, de los huevos. Los huevos fracturados de ambas especies mostraron que el corion es cavernoso a lo largo del cuerpo del huevo. Se sugiere que estos huevos pueden realizar la respiración por medio de un plastrón. Los resultados sugieren que la morfología del huevo puede ser útil para elucidar las relaciones taxonómicas y filogenéticas entre las especies de *Anastrepha*.

The genus *Anastrepha* comprises about 180 species which occur in South and Central America, in the West Indies, and with few species being found in southern USA (White & Elson-Harris 1992). Although numerous works have been focused on several biological parameters of these flies, description of the eggs were made for only a few species, despite the fact that egg morphology may have taxonomic applications (Norrbom 1985).

Similar to eggs of other Tephritidae and Diptera Cyclorrhapha (see Ferrar 1987), the eggs of *Anastrepha* species may present a sculptured or smooth chorion. In the

former class are included *A. fraterculus*, *A. bistrigata*, *A. striata*, *A. suspensa*, *A. obliqua*, *A. ludens* (Emmart 1933, Sein 1933, Lawrence 1979, Norrbom 1985, Steck & Malavasi 1988, Carroll & Wharton 1989, Murillo & Jirón 1994, Selivon & Perondini 1998), although variations in the two last ones were detected (Norrbom 1985, Carroll & Warthon 1989, Norrbom & Foote 1989, Murillo & Jirón 1994). On the other hand, the eggs of *A. serpentina*, *A. cordata*, *A. grandis*, *A. leptozona* and *A. pittieri*, present a chorion without decoration (Emmart 1933, Norrbom 1985, Steck & Wharton 1988). Another morphological feature is the presence of respiratory horns or appendages in some species, such as *A. obliqua* (Emmart 1933, Norrbom 1985, Murillo & Jirón 1994), *A. pittieri* and *A. nigrifascia* (Norrbom 1985).

Herein, we describe the morphology of the eggs of *Anastrepha sororcula* Zucchi and present also a redescription by scanning electron microscopy of *A. serpentina* (Wiedemann) eggs, previously studied by Emmart (1933).

Materials and Methods

The laboratory strain of *Anastrepha sororcula* Zucchi used in the present work, derived from infested guava fruits (*Psidium guajava*) which were cultivated in Conceição do Almeida (12°30'S, 39°10'W), the state of Bahia. The strain of *A. serpentina* (Wied.) was initiated with flies derived from "abricó" fruits (*Manilkara zapotilla)* collected in São Sebastião (23°40'S, 45°20'W), the state of São Paulo. The flies were fed water and a 3:1 mixture of sugar and corn protein hydrolysate. For oviposition, guavas and papayas (*Carica papaya)* were furnished, respectively for *A. sororcula* and *A. serpentina*.

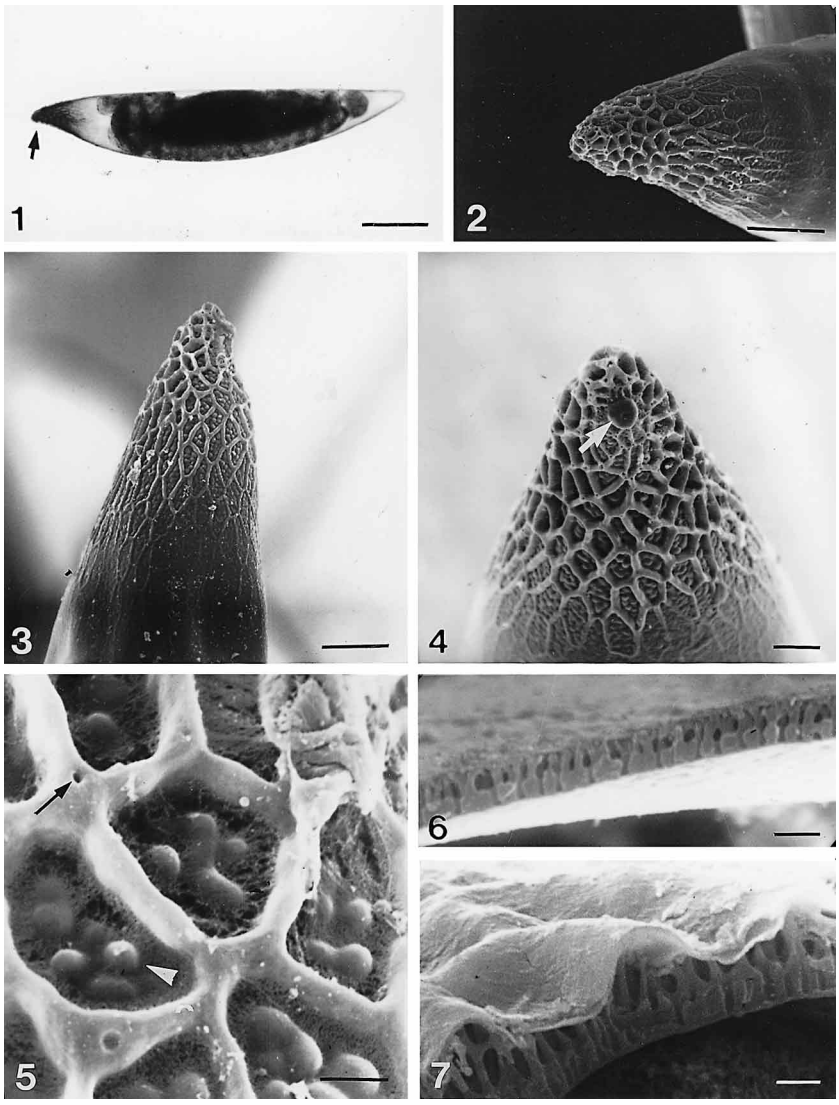
For the collection of *A. sororcula* eggs, the guavas were replaced by artificial substrates, which consisted of 2% agar hemispheres, stained with red aniline and wrapped in Parafilm® "M" (mod. Boller 1968). The eggs of *A. serpentina* were obtained directly from the papayas. The eggs were transferred to depression slides and immersed in fixative (2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4), post fixed in 1% osmium tetraoxide and dehydrated in an alcohol series. After critical point drying and "sputtering", the gold covered specimens were examined in a Zeiss DMS 940 scanning electron microscope.

Results

Anastrepha sororcula

The eggs of *A. sororcula* (n = 20) have an average length of 1.11 ± 0.12 mm and a mean diameter (in the broadest region) of 0.19 ± 0.01 mm. They are whitish, slightly curved, the curvature of the ventral side being more pronounced than the dorsal one, which is almost straight. The egg is broader from the middle toward the anterior third and tapers gradually toward the posterior pole (Fig. 1). At the anterior end the ventral side curves more abruptly than the dorsal, causing the egg apex to be asymmetric. The apex is formed by a pointed papilla, which bends to the ventral side (Figs. 1, 2). In cross section the egg is symmetrical and almost circular.

The chorion presents a well-developed lattice of ridges in a polygonal arrangement covering the anterior pole of the egg (Figs. 2, 3, 5). The sculpturing fades posteriorly and no ornamentation is found elsewhere on the eggs. Starting from the apex and around the egg circumference, the lattice is made of several tiers of polygons of different sizes but showing a regular shape, that is, in each one the sides are of similar length (Figs. 2, 3). However, the regular polygons are followed by several rows of elon-



Figs. 1-7. *Anastrepha sororcula* eggs: 1, light microscope view of an egg submerged in water. The arrow points to the papilla at the anterior end; 2-5, Scanning electron microscope (SEM) views of the anterior end of eggs: 2,shows that the ornamentation covers a larger area in the ventral side (at bottom), 3, the polygons ridges are more developed on ventral (left) than the dorsal side,4, the micropyle at the dorsal side near the apex (arrow), 5, enlargement of an area at the anterior end showing the polygons, the protuberances (arrowhead) and aeropyles (arrow); 6, 7, fractured eggs showing the internal structure of the chorion at a median (6) and anterior region (7) of the egg with the folds of the polygon ridges. Scale bars in (1) = 200 μm; (2,3) = 50 μm; (4) = 20 μm; (5) = 5 μm; (6, 7) = 2 μm.

gated ones, before they disappear. The sculpturing is asymmetric, the polygon ridges on the ventral side being more developed than those in the dorsal of the eggs (Fig. 2).

The micropyle is located at the dorsal side of the papilla and there is a row of small chorionic polygons around it, as shown in Fig. 4. Circular openings, the aeropyles, occur near the papilla. These orifices are usually found in the corners of the polygons were two or three ridges meet (Figs. 2, 4), and around the egg circumference.

The chorion in the internal areas of the polygons, present an irregular surface with round protuberances (Figs. 3, 5). These protuberances are fused together in the polygons more distal from the apex. However, in the proximal ones, the protuberances seem to be detached from each other and from the walls of the polygons. They are connected through a network of fibrilles (Fig. 5).

The morphological features above described were observed in a sample of 15 eggs, and conspicuous differences were not detected.

The chorion is cavernous exhibiting a continuous tunneling of open spaces over the entire egg body. This can be seen in a fractured egg, as shown in Fig. 6. Internally, the chorion seems to be made of a basal layer traversed by channels, an intermediate region of pillars and large open spaces, and another layer formed by branching of the pillars. On the external side, there is a fine layer of irregular appearance covering the egg surface.

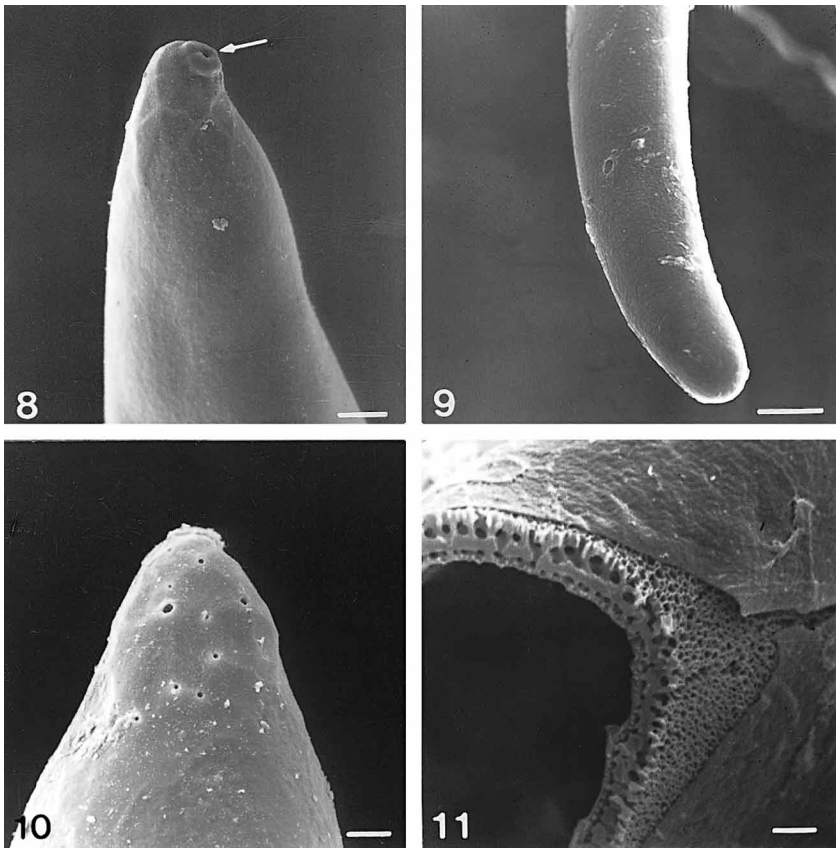
Anastrepha serpentina

The eggs of *A. serpentina* (n = 20) show an average length of 1.66 ± 0.08 mm and a mean broadest diameter of 0.21 ± 0.01 mm. They are creamy white, with a anterior end and a slender tapering toward the posterior pole. The eggs are curved, the convex side being ventral. The micropyle is located almost at the anterior tip and is surrounded by a salient rim (Fig. 8). The entire egg surface is smooth chorion without sculpturing (Figs. 8, 9). These characteristics are in accordance with the description of the eggs of this species made by Emmart (1933). The present study by SEM also shows that at the anterior end polygonal marks are found, although barely visible (Figs. 8, 10). A number of aeropyles occur in a region at the ventral surface near the anterior pole. These orifices were never observed in the dorsal side of the eggs (Fig. 10). No conspicuous morphological differences were found in a sample of 15 eggs.

In fractured eggs, five layers could be distinguished forming the internal structure of the chorion (Fig. 11). The most internal one, is thin and makes projections toward the surface which expand considerably in the median region forming a thick intermediate layer. From this layer up, new projections are found branching near the egg surface. In the two regions occupied by the projections, open spaces are found. These two areas of open spaces communicates with each other by tunnels that cross the intermediate layer of dense material. These open spaces are also continuous to the open areas among the branches of dense material nearest to the egg surface. These outermost branches fuse to each other actually forming a perforated layer. On the outside, there is a layer of uniform consistency covering the egg surface (Fig. 11).

DISCUSSION

The eggshell in *A. sororcula* is, in several aspects, similar to those found in other species of the genus by presenting an area of sculpturing of the chorion covering a region around the micropylar end of the eggs. On the other hand, they differ from the eggs of other fruit flies in which no decoration of the chorion is present, such as *A. serpentina*, described earlier by Emmart (1933) and restudied in the present work. These



Figs. 8-11. SEM views of *Anastrepha serpentina* eggs. 8, side view of anterior end showing the micropyle (arrow) at the dorsal side; 9, posterior end without ornamentation; 10, ventral view of anterior end with aeropyles; 11, fractured chorion showing the complex structure of pillars and open spaces. Scale bars in (8) = 20 μm; (9) = 50 μm; (10) = 10 μm; (11) = 2 μm.

two general classes of eggs, are found not only in genus *Anastrepha,* but also in other frugivorous (Persson 1963, Margaritis 1985) and non-frugivorous Tephritidae species (e.g., Haseler 1965, Novak & Foote 1968, Headrick & Goeden 1990), being, actually, a common feature of insect eggs (see Hinton, 1969, 1981; Ferrar, 1987).

Another feature of the eggs of both species here analysed, is the absence of a conspicuous respiratory appendage on the anterior end, like those found in other frugivorous fruit flies, such as *A. obliqua* (Emmart 1933, Norrbom 1985, Murillo & Jirón 1994), *A. nigrifascia* and *A. pittieri* (Norrbom 1985) and in non-frugivorous species, such as *Paracantha gentilis* (Headrick & Goeden 1990) among others. However, the sculpturing, the internal tunneling and the aeropyles probably represent morphological adaptations of the chorion to provide atmospheric air to the developing embryos, as is known to occur in eggs of other insect species (Hinton 1969, 1981). The open in-

ternal spaces may hold a layer of gas that extends throughout the egg body. These adaptations could provide the eggs of fruit flies with the capacity for a plastron-mediated respiration, if they are submerged in water. This situation might be met by these eggs inside the fruit tissues. Even for the eggs of *A. serpentina*, in which no sculpturing is found, the presence of aeropyles and the open spaces within the chorion could also provide conditions for a plastron respiration. However, these suggestions must await further studies since no experimental demonstration of plastron respiration in Tephritidae eggs is found in the literature. The internal structure of the chorion of *A. sororcula* and *A. serpentina* eggs seems to present a complexity similar to that of other *Anastrepha* species (Murillo & Jirón 1994, Selivon & Perondini 1998), and of *Ceratitis capitata* (Margaritis 1985) by showing several layers and open spaces, but is different from the chorion of *Bactrocera (Dacus) oleae* (Margaritis 1985), and *Rhagoletis cerasi* (Mouzaki & Margaritis 1991) eggs which show less complexity of layers.

The morphological similarities of *A. sororcula* eggs to those of the other species of the "*fraterculus* group" so far studied, *A. fraterculus, A. ludens, A. obliqua* and *A. suspensa*, reflect the close taxonomic relationships among these species showed not only by morphological characteristics (Norrbom & Kim 1988, White & Elson-Harris 1992) but also by genetic data (Morgante et al. 1980, Selivon 1996). Conversely, its distance to *A. serpentina* is corroborated by the same criteria. Thus far, egg morphology seems to be a character that may indeed be useful to elucidate taxonomic relationships among fruit flies, as suggested by Norrbom (1985).

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References Cited

BOLLER, E. F. 1968. An artificial oviposition device for the European cherry fly, *Rhagoletis cerasi*. J. Econom. Entomol. 61: 1227-1234.

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

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

FERRAR, P. 1987. A guide to the breeding habits and immature stages of Diptera Cyclorrhapha. Entomonograph 8: 1-907.

HASELER, W. H. 1965. Life-history and behaviour of the crofton weed gall fly *Procecidochares utilis* Stone (Diptera: Trypetidae). J. Ent. Soc. Qd. 4: 27-32

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

HINTON, H. E. 1969. Respiratory system of insect eggshell. Annu. Rev. Entomol. 14: 343-368.

HINTON, H. E. 1981. The biology of insect eggs. Pergamon Press, Oxford.

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

MARGARITIS, L. H. 1985. Comparative study of the eggshell of the fruit flies *Dacus oleae* and *Ceratitis capitata* (Diptera: Tephritidae). Canadian J. Zool. 63: 2194-2206.

MORGANTE, J. S., A. MALAVASI, AND G. L. BUSH. 1980. Biochemical systematics and evolutionary relationships of neotropical *Anastrepha*. *Ann. Entomol. Soc. Amer.* 73: 622-630.

MOUZAKI, D. G., AND L. H. MARGARITIS. 1991. The eggshell of the cherry fly *Rhagoletis cerasi*. Tissue and Cell 23: 745-754.

MURILLO, T. AND L. F. JIRÓN. 1994. Egg morphology of *Anastrepha obliqua* and some comparative aspects with eggs of *Anastrepha fraterculus* (Diptera, Tephritidae). Florida Entomol. 77: 342-348.

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 State University, Norristown.

NORRBOM, A. L., AND L. C. KIM. 1988. A list of the reported host plants of the species of *Anastrepha* (Diptera; Tephritidae). USDA, APHIS 52-81.

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 [ed.] Fruit flies: their biology, natural enemies and control. Vol. 3A. Elsevier N.Y. 373 pp.

NOVAK, J. A., AND B. A. FOOTE. 1968**.** Biology and immature stages of fruit flies: *Paroxyna albiceps* (Diptera: Tephritidae). J. Kansas Ent. Soc. 41: 607-618.

PERSSON, P. I. 1963**.** Studies on the biology and larval morphology of some Trypetidae (Dip.). Opusc. Ent. 28: 33-69.

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

SELIVON, D. 1996. Estudo sobre a diferenciação populacional em *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae). Ph.D. Thesis. Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo.

SELIVON, D., AND A. L. P. PERONDINI. 1998. Eggshell morphology in two cryptic species of the *Anastrepha fraterculus* complex (Diptera: Tephritidae). Ann. Entomol. Soc. Amer. 91: 473-478.

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

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

WHITE, I. M., AND M. M. ELSON-HARRIS. 1992. *Fruit flies of economic significance: their identification and bionomics*. CAB International, Wallingford, UK.