

## SCIENTIFIC NOTE

Evaluation of Egg Morphology from Ethanol Preserved Females of *Anastrepha* sp.2 aff. *fraterculus* (Diptera: Tephritidae)

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*Depto. Biologia, Instituto de Biociências, Universidade de São Paulo, 05508-900 São Paulo, SP**Neotropical Entomology 32(3):527-529 (2003)*Avaliação da Morfologia dos Ovos de Fêmeas de *Anastrepha* sp.2 aff. *fraterculus* (Diptera: Tephritidae) Preservadas em Etanol

RESUMO - A morfologia dos ovos de *Anastrepha* sp.2 aff. *fraterculus* foi avaliada em amostras de ovariolos maduros obtidos de fêmeas preservadas em etanol, de fêmeas recém-mortas e comparada com a de ovos recém-depositados. As características da ornamentação do córion, assim como da estrutura interna dos ovos recém-depositados também puderam ser observadas nos ovos dissecados das fêmeas. Fêmeas preservadas em etanol podem ser utilizadas para análise da morfologia dos ovos, possibilitando a inclusão de espécies, cujos ovos não são obtidos facilmente, nesse tipo de análise.

PALAVRAS-CHAVE: Córion, membrana vitelínica, ovário

ABSTRACT - Features of eggshell morphology in *Anastrepha* sp.2 aff. *fraterculus* were evaluated in mature ovariole ova dissected from females preserved in ethanol, and from ovaries of fresh killed females in comparison to that of freshly laid eggs. The characteristics of sculpturing as well as of internal structure of the chorion in oviposited eggs could be observed in both samples of mature ovariole ova. Ethanol preserved females may be used as a source of eggs for morphological studies allowing this kind of analysis to species whose eggs are not readily obtained.

KEY WORDS: Chorion, vitelline membrane, ovary

The eggs of insect species present morphological peculiarities which are related to their life strategies (Hinton 1969). The relationships of these morphological adaptations to evolutive patterns were clearly demonstrated for some group of insects (Kafatos *et al.* 1987, Zeh *et al.* 1989, Kambyselis 1993).

Among the tephritid fruit flies of genus *Anastrepha*, the importance of egg morphology for taxonomy and phylogenetic inferences was predicted by Norrbom *et al.* (2000), although only 20 out of the 200 species of frugivorous tephritids had their eggs studied (Selivon & Perondini 2000). At least three difficulties are met in these studies: most of the species cannot be maintained in laboratory populations, thus preventing to obtain eggs for the analysis; eggs from species exploring unknown host-fruits may not be collected, and even in cases where the hosts are known sometimes it is difficult to find the eggs before eclosion.

Norrbom (1985) dissected adult females preserved in ethanol and described the eggs only in their general aspects avoiding more detailed descriptions. According to his reasoning, eggshell could be altered due to its maintenance in the ethanol or the eggs taken directly from the ovaries could have their chorion ornamentation not yet fully developed or deformed by manipulation, casting doubts on

a detailed morphological description.

In the present report we tested these hypotheses by comparing the chorion ornamentation of *Anastrepha* sp.2 aff. *fraterculus* eggs oviposited by the females to those taken directly from the ovaries of freshly dissected females and from females preserved in ethanol. This species was chosen because eggs can be readily obtained in the laboratory and their morphology was already described in details (Selivon & Perondini 1998). Eggs were prepared according to these authors. Briefly, the eggs were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4), washed, post-fixed in an aqueous solution of 1% osmium tetroxide, dehydrated in an ethanol series, critical-point dried, and covered with a gold film in a sputtering device. The gold-covered specimens were examined in a Zeiss DMS 940 scanning electron microscope.

After fixation in osmium, some eggs were embedded in Spurr resin, ultra thin section were prepared and examined in a Zeiss EM electron microscope, according to Perondini & Selivon (1999).

Eggs obtained in three different ways were examined. First, eggs freshly deposited in artificial substrates by individual females were collected and put into fixative. Second, after a sample of eggs was obtained from a given

female, the female was dissected, mature ova immediately taken out from its ovaries, released from the soft tissues debris and transferred to a petri dish containing a layer of plain agar to maintain humidity. The eggs were left for about half an hour to have their chorion hardened, after which they were fixed. A third group of eggs was obtained by collecting mature ova from ovaries of females which were preserved in ethanol for more than a year. The dissected ova were hydrated in a decreasing ethanol series and post-fixed in 2.5% glutaraldehyde. After further processing, 15-20 eggs from each of the three samples were analyzed by SEM.

Fig. 1A shows details of the ornamentation of the anterior end of the *A. sp.2 aff. fraterculus* freshly deposited eggs. The eggs present a blunt anterior end where a clear papillae is not discernible; around the micropyle there is a row of polygons in a rosette configuration; the micropyle is located near but not at the apex of the egg, and aeropyles were found in the vertices of polygons, mainly at the ventral surface of the eggs. Ornamentation of the chorion covers only a small area of the anterior end, fading out later. No ornamentation was found at the posterior pole of the eggs. These observations are in accordance to the description of eggshell morphology of this species by Selivon & Perondini (1998).

Similar morphological features were found for mature ova sampled directly from the ovaries of freshly dissected females, as shown in Fig. 1B. The ornamentation shows a similar development as that of fresh laid eggs considering the shape and the height of the polygons walls. Moreover, the rosette around the micropyle, and the aeropyles could clearly be observed.

An example of mature ovarioles ova dissected out from the ovaries of adult females preserved in ethanol is shown in Fig. 1C. Although some scratches were introduced in the eggshell of these ova during processing, the sculpturing of the chorion at the anterior end was clear and showed every morphological detail found in the fresh laid eggs or those dissected out from fresh ovaries of this species.

Just to ascertain that the chorion of mature ovariole ova

was already assembled, ultra-thin sections of some of these ova were examined by TEM. The chorion inner structure of the mature ovariole ova does not differ from the chorion of normally laid eggs (Fig. 2), as described by Perondini & Selivon (1999).

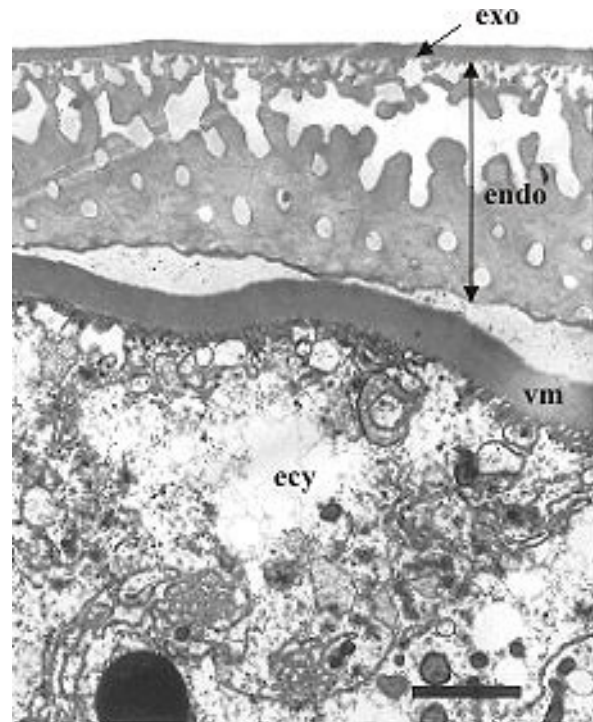


Figure 2. TEM view of a section of a mature ovariole ovum dissected from a freshly killed female. The chorion shows the same internal structure as that found in deposited eggs, accordingly to Perondini & Selivon (1999). exo, exochorion; endo, endochorion; vm, vitelline membrane; ecy, egg cytoplasm. Scale bar represent 1  $\mu$ m.

The results obtained in the present analysis showed that egg morphology in *Anastrepha* may be studied in eggs

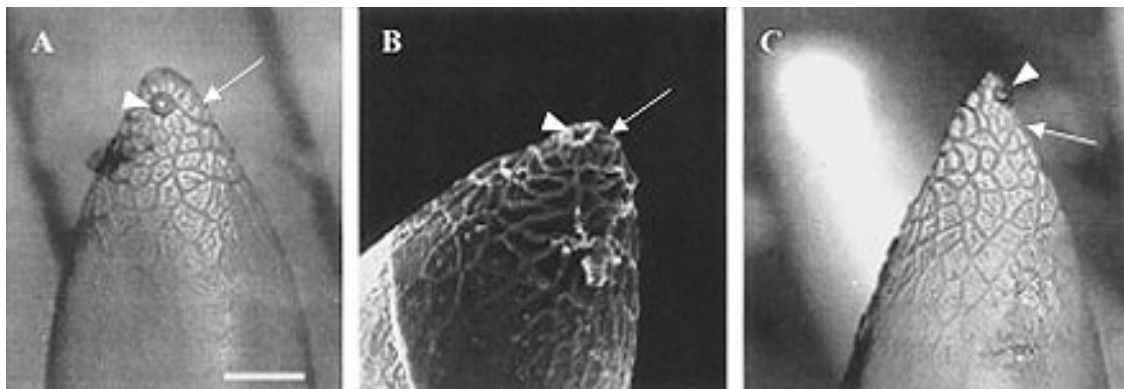


Figure 1. SEM views of the anterior end of *A. sp.2 aff. fraterculus* eggs. (A) an egg which was deposited by the female; (B) mature ovum dissected out from the ovary of a fresh killed female, and (C) ovum dissected from a female preserved in ethanol for more than a year. In the three sources, the chorion shows a similar ornamentation, the arrows pointing to the rosette of polygons around the micropyle (arrowhead). Bar in A represents 50  $\mu$ m.

sampled from adult flies preserved in ethanol or as mature ovarioles ova dissected out from freshly killed females. This certainly will make possible an improvement in the use of an important biological attribute, eggshell morphology, from a taxonomic and evolutionary point of view, by allowing the study of a larger number of species. It shows also that flies in preserved collections may similarly be screened for this characteristic, expanding the analysis, for example, for species no longer present in a given area.

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