Early developmental stages of Ceratitis capitata embryos

R.N. Stefani, D. Selivon* & A.L.P. Perondini
Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, R. do Matão 277, 05508-900 São Paulo, Brazil

Early stages of embryonic development of the Medfly Ceratitis capitata are described following in vivo observations from egg deposition up to germ band extension. Additionally, nuclear behaviour was studied in embryos stained in toto with the fluorochrome DAPI. It was found that embryonic development follows the general pattern known for other high dipteran species, in relation to nuclear multiplication, displacement of nuclei up to blastoderm formation, gastrulation and germ band extension. The embryos of C. capitata do not present the phenomenon of extrusion of part of the egg-cell which occurs in species of fruit flies of genus Anastrepha. It was also observed that the duration of the examined period of embryonic development in C. capitata is about three times longer than in Drosophila and slightly shorter than in Anastrepha, but the relative timing and duration of early stages are similar in all the three species.

INTRODUCTION

Comparative studies on ontogenesis have been one of the main approaches to understanding evolutionary processes in the last decades. In relation to insects, new insights have been achieved mainly by studies on embryogenesis of Drosophila, which have stimulated a large number of analysis in other insects (Sander 1996; Tautz et al. 1994).

The tephritid flies are a group of insects poorly studied in regard to embryonic development. Embryogenesis of Bactrocera (Dacus) tryoni was described by Anderson (1962), and recently, studies have been made on the development of the genus Anastrepha (Selivon et al. 1996, 1997; Perondini et al. 1998; Ribas 1999). In this genus, a peculiar phenomenon was found during the early stages of development. At initiation of the germ band extension, the embryos eliminate part of the posterior end of the egg-cell. Later on, in some species, there occurs an additional elimination by the anterior pole during head lobule development. Perondini et al. (1998) suggested that the phenomenon of elimination might be restricted to the genus Anastrepha since it was not observed in Bactrocera tryoni (Anderson 1962), B. carambolae (Selivon & Perondini, unpubl.) nor in C. capitata (Perondini et al. 1998).

These observations point to the necessity of further comparative studies on the initial stages of embryonic development in fruit flies in which the phenomenon of elimination is or is not present. The comparisons would be useful to elucidate not only the mechanism involved in this peculiar phenomenon, but also will contribute to a more comprehensive understanding of insect embryology. In this report we describe the early stages of embryonic development of Ceratitis capitata, in comparison with similar stages of Anastrepha fraterculus and Drosophila melanogaster.

MATERIALS AND METHODS

A laboratory strain of Ceratitis capitata was used in the present study. For in vivo observations of embryonic development, eggs were collected at hourly intervals, dechorionated in a 3% chlorox solution and immersed in water. Embryogenesis was followed from oviposition up to the initial stages of germ band elongation.

For analysis of nuclear divisions, dechorionated eggs were fixed by phase partition according to Zalokar & Erk (1976), and after removal of the vitelline membrane the embryos were stained with the fluorochrome DAPI according to Foe & Alberts (1983). The stained embryos were placed in a drop of glycerol:PBS (1:1) containing the antifading n-propylgallate. For embryos at initial cleavages the number of nuclei was counted directly. At later stages, owing to the impossibility of direct counting, the number of nuclei was estimated according to Perondini et al. (1986).

Images were obtained using a Leica DC100 CCD device coupled to an Olympus fluorescence microscope.

RESULTS AND DISCUSSION

Nuclear multiplication

As expected, embryos stained by the fluorochrome DAPI showed an increasing number of nuclei during embryonic development. Based on analysis of Drosophila embryos, a terminology to designate the early embryonic stages according to the number of mitotic cycles was introduced by Zalokar & Erk (1976), and refined by Foe & Alberts...
The first stage corresponds to the fertilized egg after nuclear syngamy, thus showing a single nucleus. Each new interphase determines the next stage, hence, each one has a characteristic number of nuclei corresponding to $2^{n-1}$, where $n$ is the number of the stages.

Figure 1A shows an embryo during the maturation division of the female pronucleus. In the next stages, embryos depicting 1 to 64 nuclei always showed a number of nuclei as a power of 2, according to a geometric progression. Beyond 64 nuclei, a variation in the number of nuclei per embryo was found, but when the data were arranged in classes, the modal number of the classes followed a geometric progression up to embryos with 256 nuclei. Hence, up to this stage, eight nuclear divisions must have occurred. During these preblastodermic stages, the nuclei divide synchronously immersed into the endoplasm, and show characteristic displacements. At stage 5, the nuclei are distributed in the anterior two-thirds of the embryo, and lie parallel to the surface, as a hollow monolayer ellipse (Fig. 1B). During the next four divisions, the nuclei undergo two movements: migration toward the posterior pole (the ellipse elongates) as shown in Fig. 1C, and a movement toward the egg's surface (Fig. 1D). When the embryos are in stage 9, a large proportion of the 256 nuclei reaches the periplasm of the egg, marking the beginning of the syncytial blastoderm. The rest of the nuclei do not migrate, remaining inside the endoplasm, forming the so-called yolk nuclei. They give rise to the vitellophages, and their ensuing multiplication occurs out of cycle with the nuclei in the periplasm. In the periplasm, the nuclei undergo five new rounds of divisions, forming about 5000 nuclei when cellularization of the blastoderm occurs (Fig. 1E).

During the syncytial blastoderm stage, the divisions of the nuclei at the periplasm are no longer synchronous within each embryo. An incipient parasynergy may already be seen at stage 10, and it is quite evident in most of the embryos after nuclear cycle 11. In Ceratitis capitata, most embryos show two or three distinct phases of mitosis, as in other Diptera (Zalokar & Erk 1976; Perondini et al. 1986). The 'wave of mitosis' generally starts at the anterior pole since the most advanced phases of the mitotic cycle are usually found at the anterior pole of the embryos.

Nuclear divisions and migration observed in Ceratitis capitata are similar to those described in Drosophila melanogaster (Zalokar & Erk 1976; Foe & Alberts 1983) and Anastrepha fraterculus (Ribas 1999), where 13 nuclear divisions (14 stages) occurred before cellularization of the blastoderm.

**Early embryonic stages**

At oviposition the egg-cell of Ceratitis capitata fills the space delimited by the external coverings, the vitelline membrane and the chorion. Typically, two basic regions may be observed in the egg-cell structure by an *in vivo* analysis: a thin translucent periplasm and the internal region filled by vitellum, the endoplasm. Soon after oviposition the egg-cell starts a retraction at both of its extremities, leaving reduced spaces between the cell and the egg coverings (Fig. 2A). Most of eggs between 0 to 1 h after oviposition (a.o.) are at stage 2 (two nuclei), while some eggs are still eliminating the second polar body (Fig. 1A), and others at stage 1 (the...
single nucleus of the zygote). Up to 2.5 h a.o., few alterations are observable by an *in vivo* analysis. However, the embryos have progressed, actively multiplying their nuclei, the most advanced embryos possessing 64 nuclei (stage 7). The nuclei at this stage have already dispersed themselves along the embryo, but are still far from the surface. At about 3.5 h a.o., the surface of the embryos became irregular with a series of small protuberances, and clear spots appears underneath these protuberances, caused by the arrival of the nuclei surrounded by yolk-free cytoplasm at the surface. At this moment, which marks the beginning of the syncytial blastoderm, the embryos are in stage 10, and about 156 nuclei arrive at the surface, while the other nuclei (about 100) remain inside the endoplasm. At the posterior pole, the pole cells appear at about 4 h a.o. (embryos at stage 11), as shown in Fig. 2B. The number of nuclei continues to grow and by 8.5 h a.o. cellularization of the blastoderm occurs (Fig. 2C).

By 9.5 h a.o. the cephalic furrow begins to form, indicating that gastrulation was initiated (Fig. 2D). Embryos at this period showed full development of the cellular blastoderm and were well advanced into stage 14. In the next hour, 10.5 h a.o., most of the embryos have started the process of germ band elongation.

**Comparison of initial development.** Since the embryonic development of *Drosophila* is better understood and has been used as paradigm of development, at least for dipterans, in the next analysis the data on *C. capitata* described here will be compared with the information on *Drosophila* (Foe & Alberts 1983). It will also include a comparison with the data on *Anastrepha fraterculus*, a species of fruit fly for which data are also available (Selivon et al. 1996; Ribas 1999).

In this analysis, six unequivocal events of early embryonic development were used: migration of nuclei toward the posterior pole, initiation of syncytial blastoderm, appearance of pole cells, cellularization of the blastoderm, appearance of the cephalic furrow and beginning of germ band elongation. The data on the species were correlated in graphic plots of *Ceratitis* versus *Drosophila* and *Ceratitis* versus *Anastrepha* (Fig. 3). As can be seen, in both cases, the points fall on almost straight lines, showing highly significant correlation (*Ceratitis/Drosophila*, \( r^2 = 0.9847 \); *Ceratitis/Anastrepha*, \( r^2 = 0.9802 \)). The main differences among the three species is relative to the slopes of the curves, 3.0215 and 0.8978, respectively, meaning that the duration of initial embryonic development of *Ceratitis* is about three times longer than in *Drosophila* and slightly shorter than in *Anastrepha*. Despite these differences, the relative timing and duration of each stage is similar for all three genera, as shown by the significant correlations of the data.

The fact that the timing of the early embryonic stages of *C. capitata* described here is similar to that of other fruit flies, namely *Bactrocera tryoni* (Anderson 1962) and *Anastrepha fraterculus* (Selivon et al. 1996; Ribas 1999), and *Drosophila*, which belong to a different dipteran family (*Drosophilidae*), is not unusual since similarities in these stages are known to be conserved even in more distantly related species of flies (Tautz et al. 1994; Sander 1996). The comparison also indicates that the phenomenon of elimination of part of the egg-cell during germ band elongation which occurs in *Anastrepha* (Selivon et al. 1996, 1997; Perondini et al. 1998), does not result in gross modifications in the
pattern of initial development. Whether or not more subtle differences in specific mechanisms, like pole cell formation in Ceratitis capitata, are present in contrast to Drosophila (Riparbelli et al. 1996), remains to be demonstrated.

ACKNOWLEDGEMENTS
This study was supported by FAPESP (98/10701-4, 01/07049-8). R.N.S. has a doctoral scholarship from CAPES, and D.S. and A.L.P.P. are research fellows of CNPq.

REFERENCES


