

SHORT COMMUNICATION

Extrusion of yolk masses by hybrid embryos of two cryptic species of *Anastrepha fraterculus* (Diptera, Tephritidae)

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ABSTRACT

Embryos of two cryptic species of *Anastrepha fraterculus* (type I and type II) differ in the frequencies at which masses of yolk are extruded from their extremities. In embryos from type I flies, the elimination of masses from the anterior pole occurs at a lower frequency than in type II embryos. No significant differences in extrusion at the posterior pole were found. In hybrid embryos produced by reciprocal crosses between type I and type II flies, extrusion of yolk masses at the anterior pole occurred at a frequency intermediate between those observed for the embryos of the parental populations. At the posterior pole there were no alterations. These results add evidence that genetic control might be involved in the phenomenon of extrusion of yolk masses by the embryos of *Anastrepha* species.

INTRODUCTION

Many studies suggest that the nominal species *Anastrepha fraterculus* comprises cryptic species (Morgante *et al.*, 1980; Solferini and Morgante, 1987; Steck, 1991). Recently, based on genetic, chromosomic, morphometric and intercrossing studies, two distinct forms were recognized which were temporarily denominated by Selivon (1996) as *A. fraterculus* type I and *A. fraterculus* type II. Additionally, it was found that the two types differ in the frequency at which the extrusion of yolk masses occurs in the embryos (Selivon *et al.*, 1996a). This peculiar mechanism of elimination of yolk by the embryos of *Anastrepha* species was described earlier by Selivon *et al.* (1994, 1995, 1996b) and evidence was obtained suggesting that genetic com-

ponents are involved in the control of these processes (Selivon *et al.*, 1996a).

MATERIAL AND METHODS

A. fraterculus type I came from larvae collected in guavas (*Psidium guajava*) from Santa Isabel, SP, while *A. fraterculus* type II was collected in oranges (*Citrus sinensis*) from the same locality (Selivon, 1996). The flies were kept in population cages (30 x 30 x 60 cm) and fed with water and a 3:1 mixture of sugar and corn protein hydrolysate. Guavas were provided routinely for oviposition but were substituted by 2% agar hemispheres, stained with red anilin and wrapped in Parafilm^R "M" (mod. Boller, 1968), when samples of eggs were needed for analysis (Selivon *et al.*, 1996b). For the study of hybrid embryos, two cages were made, containing flies derived from the original populations.

In one, recently emerged, virgin type I females were crossed to type II males, and in the other, the reciprocal crosses were made. Each cage was initiated with 10 pairs of matings. Samples of eggs were collected from the original populations as well as from the intertype crosses. The eggs were transferred to plain agar plates and left to develop. After head lobule formation, about 32 h after oviposition at 25°C, the embryos were covered by a drop of water and examined under a stereomicroscope. It was not necessary to remove the chorion since the egg covers are transparent. The embryos of *Anastrepha* species may or may not extrude yolk masses at the anterior and posterior poles (Figure 1). Selivon *et al.* (1994, 1996b) recognized four classes of embryos, into which the embryos of the present analysis were classified: class 1, embryos which extrude masses at the anterior and posterior poles; class 2, embryos with extruded mass only at the anterior pole; class 3, embryos with mass only at the posterior pole and class 4, embryos that do not extrude any mass.

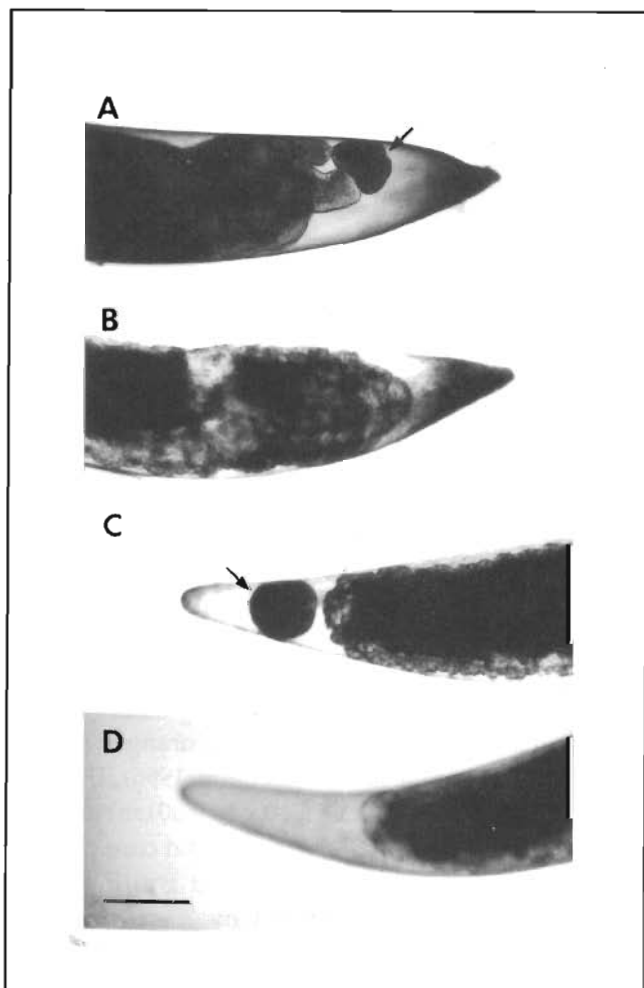


Figure 1 - Anterior (A, B) and posterior (C, D) tips of *Anastrepha fraterculus* eggs showing the presence (arrows) and absence of extruded masses of yolk. Bar = 150 μ m.

RESULTS AND DISCUSSION

The data obtained from the analysis of both original populations confirm the previous observations of Selivon *et al.* (1996a) that the two types of *A. fraterculus* differ in the frequencies of the embryonic classes. In the type I population, class 3 embryos were more frequent, followed by classes 1, 4 and 2, while type II populations exhibited class 1 embryos at the highest frequency, and embryonic classes 2, 3 and 4 occurred in a decreasing sequence, as shown in Figure 2. Since these patterns were found in several samples of the type I and type II populations (Selivon *et al.*, 1994, 1995, 1996a,b) they were taken as true differences between the two types of *A. fraterculus* (Selivon *et al.*, 1996a).

Modifications of these patterns were observed in the hybrid embryos produced in the intertype crosses. For both reciprocal crosses, a similar, intermediate pattern of embryonic class frequencies was observed: classes 1 and 3 embryos were more frequent and classes 2 and 4 occurred at lower frequencies (Figure 2).

The main difference between embryos from type I and type II flies resides in the lower frequency at

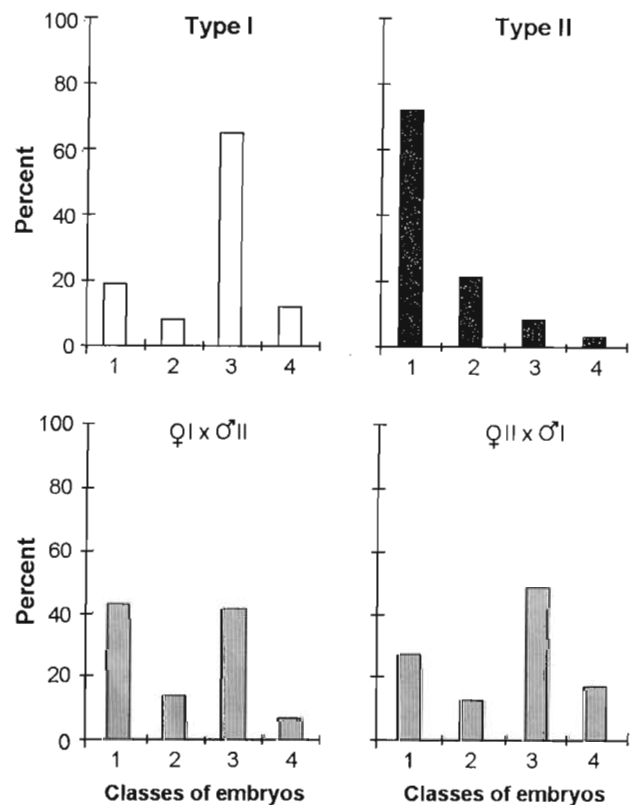


Figure 2 - Frequencies of embryonic classes observed in samples from the *Anastrepha fraterculus* type I (N = 124) and type II (N = 145) populations, and in reciprocal crosses, Female I x Male II (N = 80) and Female II x Male I (N = 310). Class 1, embryos with yolk masses at both poles; class 2, masses at the anterior pole; class 3, mass only at the posterior pole, and class 4, embryos without extruded masses.

which masses are extruded from the anterior pole of type I embryos, with no significant differences in the frequency of extrusion at the posterior pole (Figure 3). In the hybrid embryos resulting from both reciprocal crosses, there were no alterations in relation to the posterior pole, as would be expected since type I and type II embryos do not differ in this aspect. The main modification observed in the hybrids concerns the extrusion of masses at the anterior pole: the extrusion of masses at this pole occurred at frequencies intermediate between those of the parental, type I and II populations.

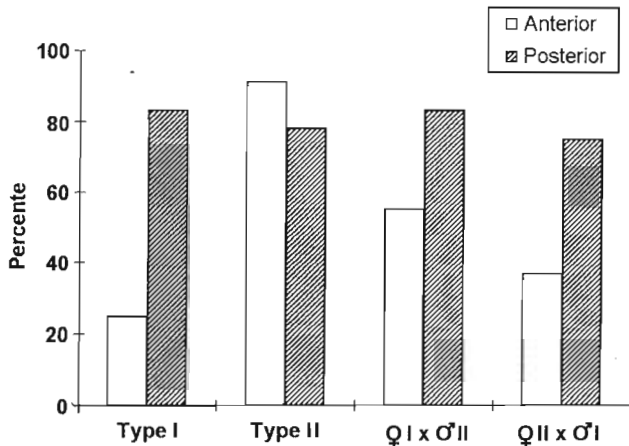


Figure 3 - Frequencies of embryos exhibiting extrusion of yolk masses at the anterior and posterior poles in the original type I and II populations, and in hybrids produced in crosses between type I and type II flies.

The controlling mechanism involved in the extrusion of yolk masses by the embryos of *Anastrepha* is still unknown. The present results, in addition to data obtained by Selivon *et al.* (1994, 1995, 1996a,b), indicate that this phenomenon is not an occasional, randomly occurring event in these embryos, but it is a regular process that occurs during embryogenesis of *Anastrepha* species. The specificity of class frequencies observed in the type I and II populations and the modifications which occurred in the hybrids are indicative that genetic control is involved in the phenomenon of yolk mass extrusion in *Anastrepha* species.

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RESUMO

Embriões de duas espécies crípticas de *Anastrepha fraterculus* (tipos I e II) diferem quanto às frequências de

extrusão de massas de vitelo pelos pólos anterior e posterior. A extrusão pelo pólo anterior ocorre com menor frequência em embriões do tipo I, não existindo diferenças significativas em relação às frequências de extrusão pelo pólo posterior. Nos embriões híbridos, produzidos por cruzamentos recíprocos entre as duas espécies crípticas, nenhuma alteração foi observada com respeito à extrusão pelo pólo posterior dos embriões. Entretanto, a frequência de eliminação de massas pelo pólo anterior dos embriões foi alterada, apresentando valor intermediário em relação aos observados nas espécies parentais. Estes dados, somados aos anteriormente obtidos, sugerem a existência de um componente genético, ainda não determinado, no fenômeno de extrusão de vitelo pelos embriões de *Anastrepha*.

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