

Egg size, yolk mass extrusion and hatching behavior in two cryptic species of *Anastrepha fraterculus* (Wiedemann) (Diptera, Tephritidae)

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ABSTRACT

Variations in egg length were observed for two populations of cryptic species of *Anastrepha fraterculus* (Wiedemann). The eggs of type I flies were smaller than those of type II individuals. For both types, in regard to yolk mass extrusion, four classes of embryos were detected. Class 1: embryos that extrude masses at both extremities; class 2: embryos in which extrusion occurs only at the anterior pole; class 3: embryos that eliminate mass only at the posterior pole, and class 4: embryos that do not extrude any mass. Embryo class frequencies were similar for populations belonging to the same type, but different between types. Individual females may produce eggs from different embryo classes, but for any given female the pattern remains constant during a long period of oviposition. Variation in size of the extruded masses was similar for both populations. Individual females produced embryos with a small range of mass diameters, and different females produced masses of different mean size. However, individual mass size remained constant during oviposition. The results suggest the existence of genetic components involved in the control of this unusual process. Larvae of both types presented, just before eclosion, similar unusual behaviors: they ingest the anterior extruded mass, rotate 180°, absorb the posterior mass and eclose near the posterior pole. Data show that cryptic *A. fraterculus* type I and type II differs in regard to egg size as well as to the phenomenon of yolk mass extrusion.

INTRODUCTION

The fruit fly *Anastrepha fraterculus* is known to exhibit extensive morphological variation within its geographic distribution (Stone, 1942; Baker *et al.*, 1944). Moreover, variation in isozymes, chromosomes and mitochondrial DNA suggests that a complex of cryptic species is actually involved (Morgante *et al.*, 1980; Solferini and Morgante, 1987; Steck, 1991; Steck and Sheppard, 1993).

Recently, comparison of *A. fraterculus* collected in guavas and oranges from a single orchard in Santa Isabel, State of São Paulo, Brazil, showed differences in

isozymes, chromosome complement, adult morphology, intercross outcomes (Selivon and Morgante, 1995; Selivon *et al.*, 1995; Selivon, 1996), as well as demographic parameters (Ometto and Morgante, 1995). Further analysis of the same parameters in samples collected from several host plants from other locations showed that the above differences were constant (Selivon, 1996). These results allowed the recognition of at least two cryptic species, which were temporarily called *A. fraterculus* type I and *A. fraterculus* type II. The populations collected in guavas and oranges from Santa Isabel were, respectively, representative of the two types (Selivon, 1996).

We have described unusual phenomena in the embryonic development of individuals from the Santa Isabel (orange) population of *A. fraterculus* type II

(Selivon *et al.*, 1996). During gastrulation and head lobules development, yolk masses may be extruded from the posterior and/or anterior poles of the embryo. These masses contain yolk material, vitellophages, large number of bacteria and are enveloped by the plasma membrane, as shown by electron microscopy. Moreover, the larvae had unusual behavior just before eclosion: they ingested the yolk mass extruded at the anterior pole, then rotated inside the egg and ingested the posterior extruded mass. Soon afterwards, the larvae eclosed near the posterior pole of the egg (Selivon *et al.*, 1996). Preliminary data, on the other hand, indicated that differences may exist between the two cryptic types of *A. fraterculus* in relation to the yolk mass extrusion (Selivon *et al.*, 1995) and analysis of hybrid embryos reinforced the suggestion that genetic control may be operative in this phenomenon (Selivon and Perondini, 1997).

Egg and yolk mass extrusion studies were extended to encompass other populations of the two cryptic species. The present report shows that type I and type II cryptic forms of *A. fraterculus* do indeed differ in the egg length as well as frequency of yolk mass extrusion. Moreover, the analysis of a single pair of matings suggests that genetic components are involved in the mechanism controlling yolk mass extrusion.

MATERIAL AND METHODS

Laboratory populations of *Anastrepha fraterculus* type I and type II were established from adult flies emerged from fruits collected from three sites. In Santa Isabel, State of São Paulo (23°18'S, 46°13'W), type I individuals were collected from guavas (*Psidium guajava*) and type II were found in oranges (*Citrus sinensis*). All came from a single orchard and were called SIg and SIo, respectively. The other sample of type I individuals was collected from feijoa (*Feijoa sellowiana*) cultivated in Vacaria (VCf), State of Rio Grande do Sul (28°27'S, 50°48'W), and type II flies were derived from guavas collected in Conceição do Almeida (CAg), State of Bahia (12°30'S, 39°10'W).

In the laboratory, the flies were maintained in cages (30 x 60 x 30 cm) and fed water and a diet consisting of a 3:1 mixture of sugar and protein hydrolysate. Food was given at regular intervals, with the same amount given to all. Guavas were regularly furnished for oviposition. However, when eggs were required for embryo development analysis, hemispheres made of 2% plain agar, stained with red anilin and wrapped in Parafilm®"M" (modif. Boller, 1968) were provided for oviposition.

For the crosses of individual females, F1 flies derived from the original cages were left to mature for 10 days. Afterwards, couples were individually transferred to smaller cages (15 x 15 x 25 cm) and were maintained according to the procedure described above. Egg samples were collected every 15 days. Four samples were taken from each female. The first sample was gathered 15 days after initiation of oviposition; thus, about 2/3 of the female oviposition cycle was encompassed (Ometto and Morgante, 1995). The eggs as well as the population cages were maintained at a temperature of 25°C.

Embryonic development was followed by *in vivo* inspection of the eggs which were taken from the agar hemispheres and laid onto agar plates. Since the egg covers are transparent, it was not necessary to remove the chorion. Observations were made simply by covering the eggs with a drop of water.

By the time the embryos attained the last stages of head development after anterior and posterior yolk mass extrusion (Selivon *et al.* 1996), an outline of the egg and the extruded masses were made with the aid of a drawing tube coupled to a Zeiss standard microscope. Measurements of egg length and mass diameter were made from these outlines, and statistical analyses of the data were done with GraphPad InStat™ computer package. The eggs were left to develop up to hatching when the remains of the egg covers were inspected. From these analyses not only the number of hatched eggs could be scored, but also the site where the eggs were torn for larval eclosion could be determined.

RESULTS

Population cage analysis

The following analyses were done using *A. fraterculus* type I and type II flies, maintained in population cages. Since these flies were derived from larvae obtained from infested fruits brought to the laboratory, they actually represent samples of natural populations.

Egg length

A. fraterculus eggs are elongated and curved, with a decreasing diameter at the anteroposterior axis (Figure 1A). The anterior apex presents a papilla where the micropyle is located and is decorated by folds of the chorion with a polygonal arrangement (Murillo and Jirón, 1994). In the present analysis, eggs from four Brazilian populations of *A. fraterculus* were measured. Analysis concentrated on egg length since no

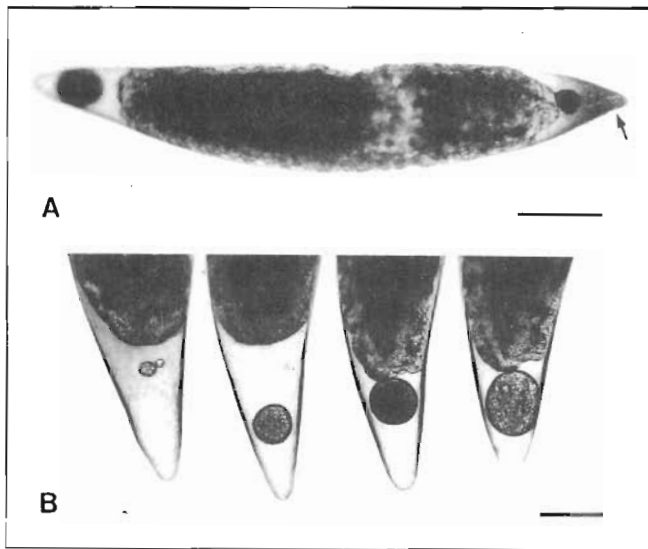


Figure 1 - Photomicrographs of living embryos of *A. fraterculus* after germ band retraction. (A) Lateral view of an embryo showing the papilla (arrow) at the anterior end. The extruded masses at the anterior and posterior poles are quite evident. The convex surface is the ventral side of the embryo. Bar = 200 µm. (B) Posterior end of four embryos depicting the size differences of the extruded mass. Bar = 100 µm.

appreciable variation was found for cross diameters, which measured on average 0.24 ± 0.02 mm. In samples of 20 eggs from each population, the average egg length was 1.35 ± 0.05 mm and 1.34 ± 0.03 mm for type I populations of SIg and VCf, respectively. On the other hand, type II populations of CAg and SIo showed mean values of 1.42 ± 0.07 mm and 1.45 ± 0.05 mm, respectively. The data were analyzed by the non-parametric Kruskal-Wallis test, resulting in a very significant value (KW = 41.922 (corrected for ties); $P < 0.0001$). The Dunn multiple comparison test (data not shown) indicated that there were no significant differences in egg length from populations of the same type, but *A. fraterculus* type I eggs were shorter than those of type II ($P < 0.001$).

Extrusion of yolk mass: classes of eggs

Egg samples from the four populations were left to develop and were examined *in vivo* in relation to

mass extrusion and hatching. Table I shows the number of examined embryos. The hatching rate varied from 61 to 77% for the four populations. As previously described (Selivon *et al.*, 1996), embryos with detached yolk mass were found in the present analysis, exemplified in Figure 1A by an embryo with masses at both extremities. Observations made in the present study confirmed that posterior mass is extruded at the beginning of gastrulation, and that anterior mass is extruded during the final stages of head lobules development, as described by Selivon *et al.* (1996) in another sample of *A. fraterculus* type II from Santa Isabel, SP. Four embryo classes exist: 1) embryos that extrude mass at both extremities; 2) embryos with mass only at the anterior pole; 3) embryos with extruded mass at the posterior pole, and 4) embryos which do not extrude mass. In order to evaluate whether there are differences among the four populations in relation to relative frequency of the embryo classes, the data were plotted in Figure 2. It is clear that the pattern of class frequencies is similar between the two populations belonging to the same type, but that they differ between the types. Class 3 embryos were more frequent in type I populations (followed by classes 1, 4 and 2), while in type II populations, class 1 was more frequent followed by classes 2, 3 and 4 in a decreasing sequence.

Size of extruded yolk mass

As observed before (Selivon *et al.*, 1996), the extruded masses show a large variation in size, from small spherules to large blocks of yolk, as shown in Figure 1B. Most of the time, they present a spherical shape, except when they are so large that they became pressed against the egg covers. In order to quantify this variation, the diameter of extruded mass was measured in samples of eggs from type I and type II populations. Diameters spanned from 20 to 220 µm for the anterior and posterior pole mass, in both types of eggs. As there were no differences between homologous mass within populations belonging to the same type, the data were

Table I - Number and hatching of the different classes of embryos from the four populations of *A. fraterculus*.

Populations	No. of embryos						Class 1			Class 2			Class 3			Class 4		
	Total	U	D	H	H/D	H/T	n	h	h/n	n	h	h/n	n	h	h/n	n	h	h/n
VCf	102	37	65	62	0.95	0.61	14	14	1.0	2	2	1.0	35	35	1.0	14	11	0.78
SIg	187	47	140	127	0.91	0.68	17	15	0.88	4	4	1.0	108	102	0.94	11	6	0.55
SIo	129	29	100	97	0.97	0.75	56	56	1.0	28	27	0.96	10	9	0.90	6	5	0.83
CAg	119	26	93	92	0.99	0.77	75	75	1.0	11	10	0.91	4	4	1.0	3	3	1.0

U = Undeveloped; D = developed; H = hatching; T = total; n = no. embryos; h = hatched.

VCf, *Feijoa sellowiana* cultivated in Vacaria; SIg, *Psidium guajava* collected in Santa Isabel; SIo, *Citrus sinensis* collected in Santa Isabel; CAg, quavas collected in Conceição de Almeida.

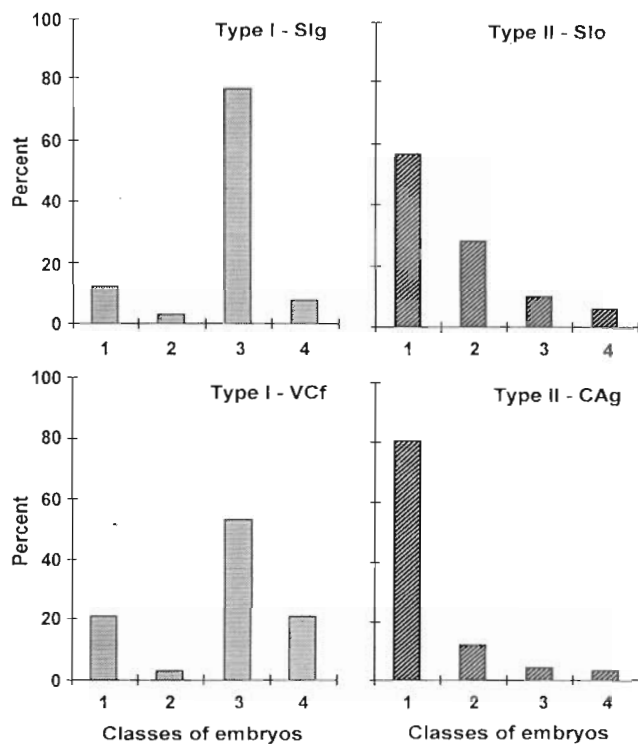


Figure 2 - Relative frequencies of embryo classes in type I and type II fly samples maintained in population cages. Note the similarities between populations of the same type and the differences between the types. For abbreviations see Table I.

pooled as type I and type II eggs. Average mass values were: type I, anterior mass, class 1 embryos (I-ant1) = $64.3 \pm 34 \mu\text{m}$; I-pos1 = $97.7 \pm 23 \mu\text{m}$; I-pos3 = $91.1 \pm 22 \mu\text{m}$, and for type II embryos, II-ant1 = $85.7 \pm 26 \mu\text{m}$; II-pos1 = $80.0 \pm 30 \mu\text{m}$ and II-ant2 = $77.9 \pm 26 \mu\text{m}$. Since the variances were homogeneous, the data were analyzed by a one-way ANOVA, which revealed significant differences among samples ($F = 4.376$; d.f. = $5/154$; $P = 0.0009$). The multiple comparison test of Tukey showed that the differences in mass diameter were not significant except for the anterior mass of class 1 embryos from type I populations, which were smaller than the posterior mass of the same type of embryos.

Pre-eclosion larval behavior

The number of analyzed embryos per population and also the frequency at which development was completed for each class of embryos are shown in Table I. Hatching occurred at a high frequency in every case, and similar rates were observed regardless of the presence or absence of extruded yolk mass. Examination of egg cover remains showed that the hatching slit was located near the posterior pole of the eggs in 92 to 95% of all eggs, regardless of class and population. Furthermore, results show that the larvae of *A. fraterculus* type I rotated inside the egg before hatching,

as Selivon *et al.* (1996) had previously shown for type II embryos. A great majority (about 95%) of the larvae from both types ingested the anterior extruded yolk mass, rotated, absorbed the posterior extruded mass and eclosed.

Analysis of single pairs of matings

Both this study and that of Selivon *et al.* (1996), on mass extrusion of *A. fraterculus* embryos, were conducted on egg samples taken from fly populations maintained in cages. From these data it is impossible to know whether the variability in egg length, embryo class frequencies and extruded mass size is due to differences in eggs laid by different females or to variation within the offspring of each female. In order to answer these questions, five single pairs of matings of each type (I or II) were studied, and eggs from individual females were collected in three to four samples at 15-day intervals. Four females stopped laying eggs after about 45 days, so only three samples were collected from them. Since the populational studies described above observed no differences between the two populations of each type, this analysis was done with flies derived from just one population of each type, SIg and SIO (type I and II, respectively).

Egg length

Egg length was measured in samples of 20 eggs collected from five individual crosses of each type. Differences among the mean length of eggs produced by different females of each type were detected (Table II), as shown by a Kruskal-Wallis ANOVA (type I, $KW = 37.35$, $P < 0.0001$; type II, $KW = 49.41$, $P < 0.0001$). The range of egg length observed in samples of individual females is smaller than the range observed for the populations. Total variability observed for type I and II agrees with the combined data of the different females, as expected (data not shown). The two groups of combined data were compared by the Mann-Whitney test, which showed that the eggs of type I flies were

Table II - Mean size (mm \pm SD) of eggs from type I and type II individual females.

Female	Type I	Type II
1	1.32 ± 0.03	1.34 ± 0.06
2	1.39 ± 0.03	1.47 ± 0.03
3	1.34 ± 0.05	1.46 ± 0.05
4	1.29 ± 0.06	1.38 ± 0.08
5	1.35 ± 0.04	1.41 ± 0.03
Type average	1.34 ± 0.05	1.41 ± 0.07

significantly smaller than those of type II females ($U' = 7940.00$; $P < 0.0001$).

Frequency of embryo classes

Figure 3 depicts embryo class frequency in the offspring from the four samples taken during the oviposition cycle of individual females from each type. It was noted that: 1) individual females may produce

embryos of different classes, e.g., female I-1 compared to I-3, or female II-1 to female II-2 or II-5, and so on; 2) when different females produce the same classes of embryos, there may be differences in the frequencies of classes, e.g., between I-1 and I-2, or II-1 and II-3, and so on; 3) the embryo class produced by any given female did not vary significantly during the oviposition cycle. It is also clear, as shown in Figure 3, that in progenies of type I females, classes 1 and 3 were the most frequent, while in type II females, embryos of classes 1 and 2 were more common.

The pooled data (not shown) of the different females of each type resulted in patterns of embryo class frequencies similar to those found in the analysis of eggs taken directly from the original type I and type II populations (Figure 2).

Diameter of the extruded mass

The diameters of anterior and posterior mass extruded by the embryos were measured, and the mean values per sample were plotted in Figure 4. The data show that the mean mass diameter, either anterior or posterior, varied among females, creating different patterns, e.g., female I-2 (small anterior/ large posterior mass), II-4 (anterior/posterior mass with similar diameters) and so on. Regardless of the pattern exhibited by individual females, it was constant throughout the oviposition cycle. The slight variations between samples were not significant (ANOVA test, data not shown).

DISCUSSION

Egg length varied within populations of *A. fraterculus*, corroborating the previous observations of Selivon *et al.* (1996). This variation, however, was larger between the two cryptic forms of *A. fraterculus*: eggs from type I flies were significantly smaller than eggs of type II females. Size differences were found in the eggs of several fruit flies, not only between species but also within populations (Emmart, 1933; Sein, 1933; Margaritis, 1985; Norrbom, 1985; Steck and Malavasi, 1988; Steck and Wharton, 1988; Carroll and Wharton, 1989; Murillo and Jirón, 1994). Boleli and Teles (1992) found in *Anastrepha obliqua* that the smaller eggs were deposited during the initial stages of oviposition, which coincided, in their experiments, with the more intense period of egg-laying. In other insects, variation in egg size was related to nutritional factors (Murphy *et al.*, 1983; Wiklund and Persson, 1983; Karlsson and Wiklund, 1984; Moore and Singer, 1987). Although such

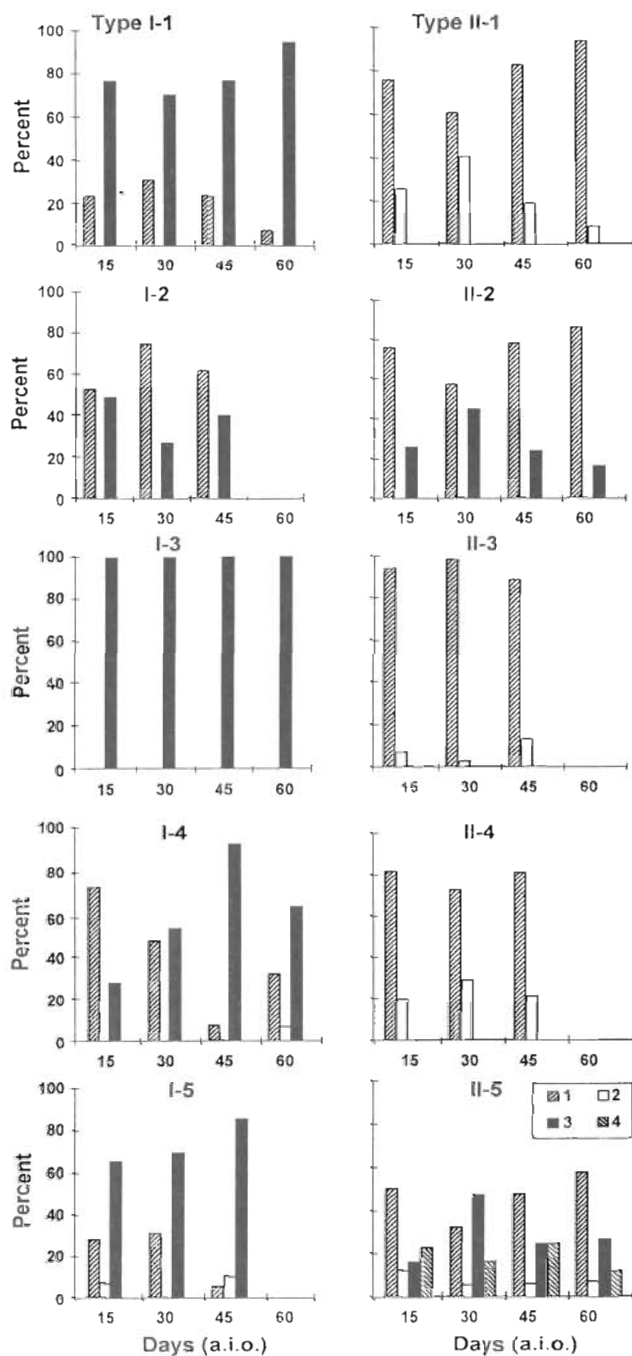


Figure 3 - Relative frequencies of embryo classes in the progenies of five individual females of each type (I-1 to I-5; II-1 to II-5) in samples taken at 15-day intervals after initiation of oviposition (a.i.o.). The inset in II-5 indicates the four embryo classes and is valid for all graphs. Number of embryos per sample varied from 17 to 38.

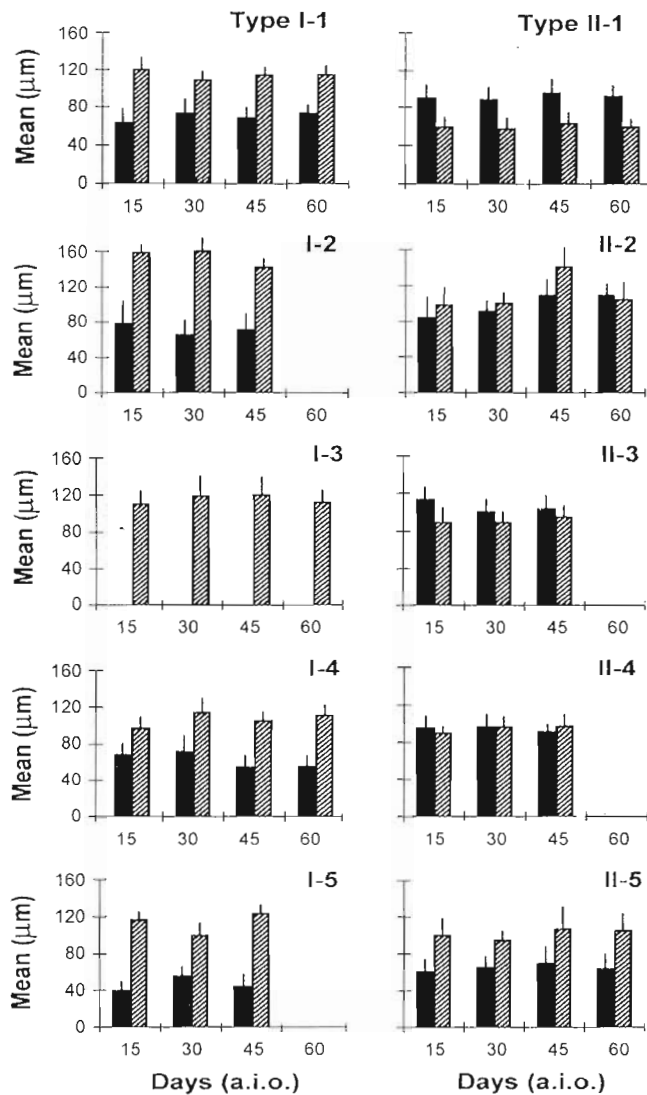


Figure 4 - Mean size (and standard deviations) of anterior (black columns) and posterior (cross hatched columns) extruded masses in the samples (days after initiation of oviposition (a.i.o.)) taken from each of the five individual type I and type II females. The number of measurements per sample for each mass varied from 5 to 25.

parameters were not studied in *A. fraterculus*, they may, perhaps, be ruled out as the cause of the observed differences between the egg length of the two cryptic species, for three reasons: first, we examined eggs for a period of about two months, during which the *fraterculus* females were in the period of maximum oviposition (Ometto and Morgante, 1995); second, the experiment began with recently emerged females, thus making it less probable that the deviations were caused by female age differences, and third, the amount of food given to the females was almost constant throughout the experiment, thus ruling out possible nutritional factors influencing egg size. In fact, the data show that the two forms of *A. fraterculus* produced eggs of different lengths. Type I eggs were generally smaller than type II. Each individual female may produce eggs

of different lengths, but the variation in each one was smaller than the total variation observed in the population. Thus, total variability resulted from the variation of the eggs produced by different females.

The present results on yolk mass extrusion by the embryos from the Santa Isabel type II population (orange) confirm the previous observations made on this same population by Selivon *et al.* (1996). This unusual phenomenon has now been found in other populations of type I and II flies (Vacaria, RS and Conceição do Almeida, BA, respectively) besides the two already known from Santa Isabel, SP. Although the four embryo classes described previously by Selivon *et al.* (1996) have been found in the four populations studied here, differences in class frequencies were observed only among populations of different types (I and II), confirming preliminary results of the Santa Isabel data (Selivon and Perondini, 1997). Individual females produced offspring with a determined embryo class pattern. This pattern, however, varied among the females. This means that the characteristic of overall population frequency for type I or II was due to the differential production of embryo classes by individual females rather than to each female producing the four classes at different frequencies. Not only the similarities between populations of the same type collected from distant sites are to be noted (type I, SIg and VCf about 800 km apart; type II, SIO and CAg about 1,400 km), but also that the two Santa Isabel populations, living in sympatry in the same orchard, maintained the differences characteristic of the two types.

Similar to the embryo class frequencies, population variability found for the diameter of the extruded masses for both type I and type II populations can also be explained in terms of the differential production by each individual female.

According to the observations of Selivon *et al.* (1996) for type II embryos, the extrusion of the posterior mass occurs at the beginning of gastrulation while the anterior mass is extruded later on during development of the head lobules. The same was detected for type I embryos in the present analysis (data not shown). These authors pointed out that the four embryo classes result, in fact, from the capacity of both egg poles to eliminate or not the yolk masses, that is, extrusion seems to be a facultative phenomenon (Selivon *et al.*, 1996; Selivon and Perondini, 1997). If one considers that up to the moment there are no indications of a mutual relationship between the poles, the frequencies at which masses were extruded from the anterior or posterior poles can be calculated from the present data. For type I individuals, anterior and posterior extrusion occurred, respectively, in 18% and 85% of the embryos; for type II

embryos, anterior extrusion occurred in 88% while posterior masses were observed in 75% of the embryos. The main difference between types I and II, as previously pointed out by Selivon and Perondini (1997), is a lower frequency of anterior mass extrusion in type I embryos.

Despite the differences described above between types I and II of *A. fraterculus*, type I larvae exhibited a similar sequence of behaviors just prior to eclosion, as described previously by Selivon *et al.* (1996) for type II individuals, which are: ingestion of the extruded yolk masses, rotation and eclosion by the posterior pole. Eclosion by the posterior pole of the egg has been described in *Anastrepha ludens* (Carroll and Wharton, 1989) and genus *Chaetorellia* (Varley, 1937; White and Marquardt, 1989). This indicates that the larvae of these species also rotate inside the egg. In other tephritid flies, for example *Bactrocera (Dacus) oleae* and *Ceratitis capitata* (Margaritis, 1985), the egg hatches near the anterior end. However, these studies did not mention yolk mass extrusion. Besides the two cryptic species of *A. fraterculus* (Selivon *et al.*, 1996; Selivon and Perondini, 1997; present work), this unusual phenomenon was found in three other species of *Anastrepha* (Selivon *et al.*, 1995), but was absent in *Ceratitis capitata* (Selivon *et al.*, 1995) and *Bactrocera tryoni* (Anderson, 1962). These preliminary observations would suggest that yolk mass extrusion may be a distinct characteristic of the genus *Anastrepha*, but other species must be studied.

The present report shows unique characteristics presented by individual females such as: embryo class constancy, mass diameter, the fact that population variability could be explained in terms of individual offspring, and the differences preserved by each cryptic species. Taken together with the modifications observed in hybrid embryos (Selivon and Perondini, 1997), they indicate that the controlling mechanism for yolk mass extrusion by the embryos of *Anastrepha* involves genetic components. The elucidation of the mechanism, however, will require a more direct approach.

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RESUMO

As duas espécies crípticas de *Anastrepha fraterculus*, tipo I e tipo II, diferiram quanto ao tamanho dos ovos, os quais

são menores no tipo I. Em ambos pode ocorrer a extrusão de vitelo pelas extremidades dos embriões, observando-se quatro classes: 1) embriões com massas extrudadas por ambos os pólos; 2) extrusão apenas pelo pólo anterior; 3) extrusão pelo pólo posterior e 4) embriões que não eliminam as massas. As frequências dessas classes são similares entre populações de um mesmo tipo, mas diferem entre as duas formas crípticas. Diferentes fêmeas podem produzir diferentes classes de embriões, mas para cada fêmea o padrão é constante e não varia durante um longo período do ciclo de oviposição. O diâmetro das massas extrudadas é variável, mas de igual magnitude para os dois tipos. Fêmeas distintas podem produzir massas de diferentes diâmetros que não se alteram em suas progênes. Os resultados sugerem que componentes genéticos estão envolvidos no controle da extrusão de vitelo pelos embriões de *Anastrepha*. As larvas, de ambos os tipos, apresentam comportamento não usual pouco antes da eclosão: ingerem a massa de vitelo do pólo anterior, giram dentro do ovo ficando com a cabeça no pólo posterior, sugam a massa de vitelo do pólo posterior e eclodem perto desse pólo.

REFERENCES

- Anderson, D.T. (1962). The embryology of *Dacus tryoni* (Frogg.), the Queensland fruit fly. *J. Embryol. Exp. Morphol.* 10: 248-262.
- Baker, A.C., Stone, W.E., Plummer C.C. and McPhail, H. (1944). A review of studies on the Mexican fruit fly and related Mexican species. *U S Dep. Agric. Misc. Publ.* 531: 1-155
- Boleli, I.C. and Teles, M.M.D.C. (1992). Egg length of *Anastrepha obliqua* Macquart (Diptera, Tephritidae) according to oviposition rate and maternal age. *Rev. Bras. Zool.* 9: 215-221.
- Boller, E.F. (1968). An artificial oviposition device for the European cherry fly, *Rhagoletis cerasi*. *J. Econ. Entomol.* 61: 1227-1234.
- Carroll, L.E. and Wharton, R.A. (1989). Morphology of the immature stages of *Anastrepha ludens* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 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.
- Karlsson, B. and Wiklund, C. (1984). Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera*. *Oikos* 43: 376-385.
- Margaritis, L.H. (1985). Comparative study of the eggshell of the fruit flies *Dacus oleae* and *Ceratitis capitata* (Diptera: Tephritidae). *Can. J. Zool.* 63: 2194-2206.
- Moore, R.E. and Singer, M.C. (1987). Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas aditha*. *Ecol. Entomol.* 12: 401-408.
- Morgante, J.S., Malavasi, A. and Bush, G.L. (1980). Biochemical systematics and evolutionary relationships of neotropical *Anastrepha*. *Ann. Entomol. Soc. Am.* 73: 622-630.

- Murillo, T. and Jirón, L.F.** (1994). Egg morphology of *Anastrepha obliqua* and some comparative aspects with eggs of *Anastrepha fraterculus* (Diptera, Tephritidae). *Fl. Entomol.* 77: 342-348.
- Murphy, D.D., Launer, A.E. and Erlich, P.R.** (1983). The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* 56: 257-263.
- Norrbom, A.L.** (1985). Phylogenetic analysis and taxonomy of the *cryptostrepha*, *daciformes*, *robusta* and *schausi* species of *Anastrepha* Schiner (Diptera: Tephritidae). Doctoral thesis, Pennsylvania State University, Norristown.
- Ometto, A.C. and Morgante, J.S.** (1995). Estudo demográfico em duas populações de *Anastrepha fraterculus*. *Braz. J. Genet.* 18 (Suppl.): 279 (Abstract).
- Sein, F.** (1933). *Anastrepha* (Trypetidae, Diptera) fruit flies in Puerto Rico. *J. Dep. Agric. Puerto Rico* 17: 183-196.
- Selivon, D.** (1996). Estudo sobre a diferenciação populacional em *Anastrepha fraterculus* (Wiedemann) (Diptera, Tephritidae). Doctoral thesis, Departamento de Biologia, Universidade de São Paulo, São Paulo, Brasil.
- Selivon, D. and Morgante, J.S.** (1995). Caracterização isozímica e cariotípica de duas populações de *Anastrepha fraterculus* (Tephritidae). *Braz. J. Genet.* 18 (Suppl.): 277 (Abstract).
- Selivon, D. and Perondini, A.L.P.** (1997). Extrusion of yolk masses by hybrid embryos of two cryptic species of *Anastrepha fraterculus* (Diptera, Tephritidae). *Braz. J. Genet.* 20: 253-255.
- Selivon, D., Morgante, J.S. and Perondini, A.L.P.** (1995). Diferenças em alguns aspectos da embriogênese de moscas-das-frutas dos gêneros *Anastrepha* e *Ceratitidis*. *Braz. J. Genet.* 18 (Suppl.): 276 (Abstract).
- Selivon, D., Morgante, J.S., Ribeiro, A.F. and Perondini, A.L.P.** (1996). Extrusion of masses of yolk during embryony development of the fruit fly *Anastrepha fraterculus*. *Invertebr. Reprod. Dev.* 29: 1-7.
- Solferini, V.N. and Morgante, J.S.** (1987). Karyotype study of eight species of *Anastrepha* (Diptera, Tephritidae). *Caryologia* 40: 229-241.
- Steck, G.J.** (1991). Biochemical systematics and population genetic structure of *Anastrepha fraterculus* and related species (Diptera, Tephritidae). *Ann. Entomol. Soc. Am.* 84: 10-28.
- Steck, G.J. and Malavasi, A.** (1988). Description of the immature stages of *Anastrepha bistrigata* (Diptera; Tephritidae). *Ann. Entomol. Soc. Am.* 81: 1004-1009.
- Steck, G.J. and Sheppard, W.S.** (1993). Mitochondrial DNA variation in *Anastrepha fraterculus*. In: *Fruit Flies: Biology and Management* (Aluja, M. and Liedo, P., eds.). Springer-Verlag, New York, pp. 9-14.
- Steck, G.J. and Wharton, R.A.** (1988). Description of immature stages of *Anastrepha interrupta*, *A. limae*, *A. grandis* (Diptera, Tephritidae). *Ann. Entomol. Soc. Am.* 81: 994-1003.
- Stone, A.** (1942). The fruit flies of the genus *Anastrepha*. *U S Dep. Agric. Misc. Publ.* 439: 1-112.
- Varley, G.C.** (1937). The life-history of some trypetid flies, with descriptions of the early stages (Diptera). *Proc. R. Entomol. Soc. Lond. (A)* 12: 109-122.
- White, I.M. and Marquardt, K.** (1989). A revision of genus *Chaetorellia* Hendel (Diptera: Tephritidae) including a new species associated with spotted kanpweed, *Centaurea maculosa* Lam. (Asteraceae). *Bull. Entomol. Res.* 79: 435-487.
- Wiklund, C. and Persson, A.** (1983). Fecundity and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? *Oikos* 40: 53-63.

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