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The isozymatic pattern of alkaline phosphatase during ontogenetic development of *Anastrepha fraterculus* (Diptera: Tephritidae)

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ABSTRACT

Eleven alkaline phosphatase (APH) electromorphs were detected during *Anastrepha fraterculus* (Diptera: Tephritidae) ontogenetic development. Specific isozymes were found in each stage analyzed (embryonic, larval, pupal and adult) and some isozymes were shared by two stages. A specific isozyme of the adult stage (Aph 0.37) occurred only in males 60 and 100 days old. Similar APH patterns were observed in third-stage larvae reared on four kinds of fruit (papaya, guava, banana and kiwi).

INTRODUCTION

The genus *Anastrepha* contains 193 described species, 78 of which occur in Brazil (Norrbom and Kim, 1988). *Anastrepha fraterculus* (Wiedemann) is the most prevalent species, infesting more than 20 species of cultivated fruit in the Brazilian states of Paraná, Santa Catarina and Rio Grande do Sul, representing 95-97% of the Tephritidae caught in traps (Salles and Kovaleski, 1990). Morgante *et al.* (1980), studying enzymatic polymorphisms, observed that sympatric populations can show a lower genetic similarity index than that of distant populations. Therefore, speciation events in *Anastrepha* have occurred with a small number of genetic differences (Morgante, 1982; Malavasi and Morgante, 1982). Low genetic variation was found at 11 presumptive loci in natural populations of ten *Anastrepha* species collected from nine different hosts and in 11 different geographical regions (number of alleles per locus = 1.31; proportion of polymorphic loci = 0.21, and mean number of heterozygotes per locus per individual = 0.040) (Malavasi and Morgante, 1982). However, isozyme analysis of eight populations of the South American fruit fly, *A. fraterculus*, from a large portion of its geographic range (from Mexico to S. Paulo-Brazil) performed by Steck (1991) showed genetic discontinuity among close populations for 18 enzymatic systems, indicating the existence of several cryptic species as well as the non-monophyletic origin of this species. All these studies focused predominantly on structural polymorphisms, whereas those affecting the amount or the time during which the enzyme is expressed received less attention, largely because their phenotypes and inheritance are generally more complex.

Alkaline phosphatase (APH) is an important enzyme in the cellular division process (Murray and Kirschner, 1991). Furthermore, in *Drosophila melanogaster*, the most abundant larval alkaline phosphatases hydrolyze o-phosphotyrosine to tyrosine, which is used in puparium formation (Harper and Armstrong, 1972). Therefore, during the ontogeny of *A.*

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fraterculus, the APH study, as well as that of several other isozymatic systems, is important because it may help understand the relatively low structural enzymatic polymorphism in spite of the broad geographical distribution and wide variety of plant hosts.

MATERIAL AND METHODS

The *A. fraterculus* population used in this work was collected from infested fruits collected in the city of Pelotas, State of Rio Grande do Sul, Brazil. It was maintained under laboratory conditions, using papaya (*Carica papaya* L.) as larval food, at a temperature of 25 ± 1°C and a 12-h photoperiod. Embryos and first-instar larvae were obtained as described by Nascimento and Oliveira (1996). The 17 embryonic stages were collected with the help of a histological needle, homogenized in water (10 µl), absorbed on 3MM Whatman paper (4 mm x 2 mm) and stored at -20°C, until the electrophoresis procedure. For embryos and first-instar larvae 150 individuals were used in each sample to provide for sufficient enzyme concentration. Only one individual was used in each sample for the remaining stages. Ten samples were analyzed for each developmental stage. The fly eggs were also placed in acrylic plate wells containing medium consisting of: 8 g agar, 200 ml concentrated grape juice and 1 ml propionic acid (M = 74.08 g/mol). Just eclosed first-instar larvae were then transferred to papaya fruit to obtain a second-instar larva (144 h) and a third-instar larva (312 h). Additional samples from the end of this last stage were placed on garden soil to obtain pupae, that were collected at 24-h intervals. Adults were collected at 4, 15, 30, 60 and 100 days and stored at -20°C until analyzed. First-instar larvae were also grown on guava (*Psidium guajava* L.), banana (*Musa spp.*) and kiwi (*Actinidia chinensis* Planch.) to obtain third-instar larvae. Oregon-R *D. melanogaster*, adults seven days old, were used as a control for electrophoretic migration.

Alkaline phosphatase isozyme patterns were studied with polyacrylamide gel electrophoresis, according to Da Cruz and Oliveira (1996). The gel was incubated for approximately 2 h at 38°C in the following staining solution: 100 ml Tris buffer, pH 8.65 (0.1 M), 100 mg alpha-naphthyl phosphate (4.06 mM), 100 mg Fast Blue BB (2.40 mM), 0.5 ml MgCl (10%), 500 mg polyvinylpyrrolidone (125 mM (PVP)) and 2 g NaCl (342 mM) (Beckman and Johnson, 1964). All chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO).

The isozymes were named according to increasing relative mobility (RM) values. The RM values are the medians of several estimations. Enzyme activity measurements were semiquantitative and the following scale was used: - = absent or not detected, + = very weak, ++ = weak, +++ = strong and ++++ = very strong. The differences between APH patterns were tested by the non-parametric Kruskal-Wallis ANOVA, followed by multiple comparisons in a fashion paralleling the Tukey test (Zar, 1984).

RESULTS

Eleven APH electromorphs were detected during the ontogenetic development of *A. fraterculus*. (Table I, Figure 1).

The anodal APH isozyme (Aph 1.18) appeared together with Aph 1.14 in some samples of the pupal stage (from 24 h to 312 h) with little space between them, and in other samples they occurred as a single band. This observation suggests that Aph 1.18 and Aph 1.14 were products of the same locus. While Aph 1.18 was detected only in the second-instar larvae and the pupal period, Aph 0.90 was observed in the three larval stages and only in 0-h pupae (Table I).

The other isozymes were stage-specific; Aph 0.96 was found in the 17th embryonic stage, whereas Aph 0.55 and Aph 0.20 were detected only in the three larval stages. Aph 1.07 and Aph 0.29 were expressed only in the pupal stage. Aph 0.51 was detected in some instars of the pupal stage with a higher intensity in 240-h and 312-h pupae. This electromorph was also observed in adults of different ages (males and females), with a higher intensity of activation from 30 until 100 days old.

Aph 0.87 occurred only in adults. In both sexes its activation was higher in 100-day old adults, and Aph 0.37 was observed exclusively in male adults (60 and 100 days old).

An isozyme (Aph 0.70) was detected only in a single sample of 15-day old females (not shown) and was not observed at other stages of development. We did not take this isozyme into account.

Differences in the activity pattern of APH isozymes were observed and analyzed by statistical tests. Significant differences in Aph 1.18, Aph 0.90 and Aph 0.20 were observed in samples of second-instar larvae, while Aph 0.55 was different at all larval stages. Among different temporal samples of the pupal stage, significant differences in activity were observed only for Aph 0.51. In the adult stage (males and females) significant differences were observed in all three APH isozymes, with Aph 0.37 occurring only in males and showed the same pattern until 30 days old. The activity of Aph 0.51 became constant from the 30th day on. The

Table I - Comparison of alkaline phosphatase (APH) activation patterns during *Anastrepha fraterculus* development.

Stages	Isozymes (RM values)										
	1.18	1.14	1.07	0.96	0.90	0.87	0.55	0.51	0.37	0.29	0.20
Embryonic											
1st - 16th	-	-	-	-	-	-	-	-	-	-	-
17th	-	-	-	++	-	-	-	-	-	-	-
Larval											
1st	-	-	-	-	+++	-	++	-	-	-	++
2nd	+	-	-	-	++	-	+++	-	-	-	+++
3rd	-	-	-	-	+++	-	++	-	-	-	++
Pupal (in hours)											
0	-	-	++	-	++	-	-	-	-	-	-
24	+	+	++	-	-	-	-	++	-	-	-
48	+	+	++	-	-	-	-	-	-	++	-
72	+	+	++	-	-	-	-	-	-	++	-
96	++	++	+	-	-	-	-	++	-	-	-
120	++	++	+	-	-	-	-	+	-	-	-
144	++	++	++	-	-	-	-	+	-	++	-
168	++	++	+	-	-	-	-	+	-	++	-
192	+	+	-	-	-	-	-	+	-	-	-
216	++	++	-	-	-	-	-	++	-	-	-
240	++	++	++	-	-	-	-	+++	-	-	-
264	++	++	++	-	-	-	-	++	-	-	-
288	+	+	+	-	-	-	-	++	-	-	-
312	+	+	+	-	-	-	-	+++	-	-	-
Adults (days)											
Males											
4	-	-	-	-	-	+	-	-	-	-	-
15	-	-	-	-	-	++	-	++	-	-	-
30	-	-	-	-	-	++	-	+++	-	-	-
60	-	-	-	-	-	+	-	+++	+++	-	-
100	-	-	-	-	-	+++	-	+++	++++	-	-
Females											
4	-	-	-	-	-	+	-	+	-	-	-
15	-	-	-	-	-	+	-	++	-	-	-
30	-	-	-	-	-	++	-	+++	-	-	-
60	-	-	-	-	-	+	-	+++	-	-	-
100	-	-	-	-	-	+++	-	+++	-	-	-

(++++) Highest to (+) lowest detectable APH expression; (-) not detected.

Aph 0.87 varied its activity depending on adult age (Table II).

Third-instar larvae reared on the four kinds of fruit tested (papaya, guava, banana and kiwi) had no differences in the electrophoretic pattern.

DISCUSSION

Anastrepha fraterculus showed only one electromorph (0.96) during embryogenesis. Its occurrence at the end of this stage (17th) is surprising because phosphatases are important enzymes in tyrosine phosphorylation, which has been shown to be involved in the control of the cell cycle, in cell-cell communication, in cellular responses to growth factor, and in embryogenesis (Glenney, 1992). Thus, PAGE is probably not a good method to detect APH in embryonic development, and we suggest the use of other approaches such as isoelectric focusing (IEF), using purified samples to access its occurrence, since cellular division is the main process at this stage [the increased number of cells in *Drosophila melanogaster* is approximately 6000 (Campos-Ortega and Hartenstein, 1985)].

Furthermore, APH presented a high intensity of activation during the larval and adult period. In

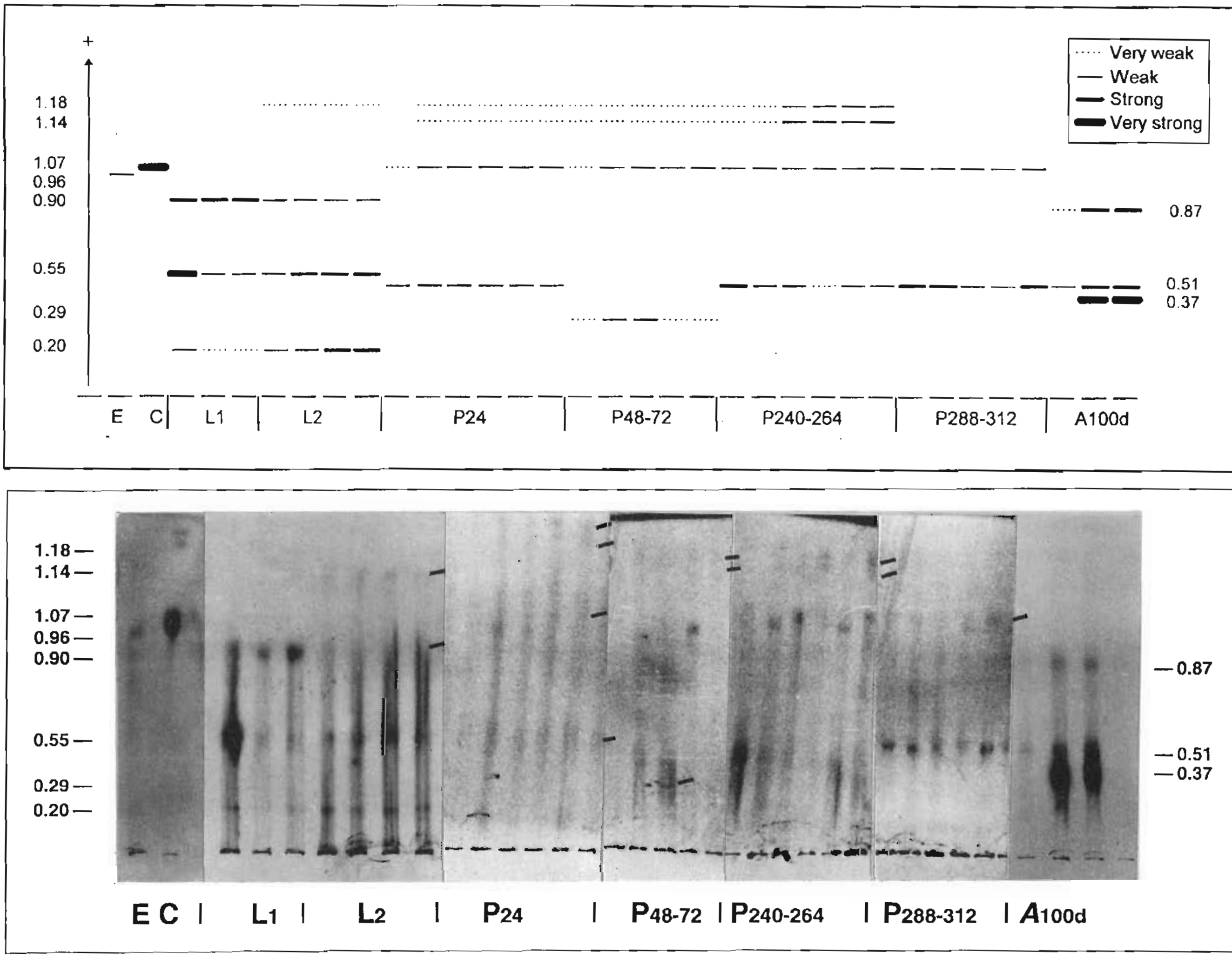

Figure 1 - Alkaline phosphatase electrophoretic pattern during the ontogenetic development of *Anastrepha fraterculus*: (E) embryo in 17th stage; (C) *Drosophila melanogaster* as control; (L1) first-instar larvae; (L2) second-instar larvae; (P24) pupae with 24 h of development, (P48-72) pupae with 48 and 72 h, (P240-264) pupae with 240 and 264 h, (P288-312) pupae with 288 and 312 h. A100d are the 100-day old adult males.

Table II - Chronological electrophoretic alkaline phosphatase activation patterns in ontogeny of *Anastrepha fraterculus*.

Ontogenetic development										
	Larval (z value)				Pupal (z value)		Adult (males and females) (z value)			
Isozymes	1.18	0.90	0.55	0.20	Isozymes	0.51*	Isozymes	0.87	0.51	0.37**
	Stages				Stages (in hours)		Stages (in days)			
1st	-1.53[a]	1.22[a]	0.00[a]	-1.22[a]	24	-1.39[a]	4	-2.89[a]	-3.40[a]	-1.70[a]
2nd	3.06[b]	-3.06[b]	2.45[b]	3.06[b]	144	-2.73[b]	15	0.68[b]	-0.68[b]	-1.70[a]
3rd	-1.53[a]	1.84[a]	-2.45[c]	-1.84[a]	168	-1.22[a]	30	0.68[b]	1.36[c]	-1.70[a]
					192	0.03[c]	60	-1.46[a]	1.36[c]	1.70[b]
					216	1.61[d]	100	2.99[c]	1.36[c]	3.40[c]
					312	3.34[e]				
	H = 13.64	H = 11.08	H = 8.96	H = 12.67		H = 22.13		H = 19.74	H = 16.77	H = 18.46
	P = 0.001	P = 0.004	P = 0.012	P = 0.002		P = 0.001		P = 0.001	P = 0.002	P = 0.001

Samples with same letters do not present significant differences with Kruskal-Wallis and Tukey test.
*The other pupal isozymes did not present significant differences in pattern activity.
**This isozyme occurred only in males.

Ceratitis capitata, the pupal isozymes retain some of their activity during the first two days of the mature fly. At the adult stage, the appearance of a specific isozyme with electrophoretic variants is noted (Bourtzis *et al.*, 1993). In *A. fraterculus*, only one pupal isozyme persisted throughout adult life and two new electromorphs appeared at this last stage. The presence of one very active isozyme in 30-, 60- and 100-day old adults (males and females), and one isozyme that occurs only in 60- and 100-day old males, with strong and very strong activity, respectively, appears to be important at the end of the lifespan of *A. fraterculus*, particularly since males live twice the time of females in this species (Martins, 1986). These results clear the way for investigations about the role of APH in the adult of this and other species.

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RESUMO

Onze eletromorfos de fosfatase alcalina (APH) foram detectados durante o desenvolvimento ontogenético de *Anastrepha fraterculus* (Diptera: Tephritidae). Isozimas específicas foram encontradas em cada estágio analisado (embrionário, larval, pupal e adulto) e algumas isozimas são compartilhadas por dois estágios. Uma isozima específica do estágio adulto (Aph 0.37) ocorreu somente em machos com 60 e 100 dias de idade. Não foi observada diferença no padrão de ativação de APH em larvas de terceiro estágio alimentadas em quatro tipos de frutos (mamão papaia, goiaba, banana e kiwi).

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