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Esterase Profile in *Drosophila mercatorum pararepleta* (Diptera; Drosophilidae), a Non-cactophilic Species of the repleta Group: Development Patterns and Aspects of Genetic Variability

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Luciana Paes de Barros Machado, Natalia Silva Alves, Jaqueline de Oliveira Prestes, Gabriela Ronchi Salomón, Daiane Biegai, Thais Wouk, and Rogério Pincela Mateus (2017) Esterases are a diversified group of isozymes that performs several metabolic functions in *Drosophila*. In the *D. repleta* group, this class of enzymes was well described in cactophilic species, existing a lack of studies considering substrate specificity and life cycle expression in the non-cactophilic species. The larvae of cactophilic species of the *D. repleta* group develop in rotting cacti cladodes, but adults are generalists. Thus, different patterns expression can be found for esterases throughout development. In this work we analyzed esterase profile and substrate specificity during development, and genetic variability aspects in *D. mercatorum pararepleta*, a non-cactophilic and generalist species of *D. repleta* group that was understudied hitherto. Samples of 3rd (F3) and 104th (F104) generations of three *D. mercatorum pararepleta* strains, obtained after collections in xerophytic enclaves of southeastern Brazil (ITI and SER in São Paulo state and RIP in Paraná state), and of D33 strain (obtained from Cristalina-GO, Midwest of Brazil, and established in the laboratory in 1987) were analyzed. Eight esterase loci, EST-1 to EST-8, were detected. EST-1 and EST-2 were adult exclusive. Only EST-3 and EST-8 were monomorphic; all the others presented between two (EST-6) and six (EST-7) alleles. EST-7 was the only dimeric locus and also the only one that showed to be a preferably β-esterase regarding affinity to α- and β-naphthyl acetates as substrates. The other seven loci were divided into three classes: α-esterase exclusive (EST-2); preferably α-esterase (EST-3, EST-4, EST-5 and EST-8); and α/β-esterase (EST-1 and EST-6). The EST-3, EST-5 and EST-6 loci were not detected in all samples, suggesting that they could have become pseudogenes due to the mutation accumulation after the gene duplication. The allele frequency of EST-7 locus, which showed the highest number of alleles, in adults of D33 and SER-F3 evidenced a higher variability and diversity in the oldest strain (six alleles, Ho = 0.46) than in the youngest (five alleles, Ho = 0.26). Moreover, the analysis of SER-F104 revealed that this locus became monomorphic. The higher variability in the strain established in the laboratory at least two decades ago, together with the allele fixation in the SER-F104, indicate that the SER strain probably suffered a more severe action of founder effect/bottleneck when it was established in the laboratory and, therefore, even if the maintenance afterwards was performed using a high number of individuals, it did not assured the conservation of the existing genetic variability.

Key words: Esterase, *D. mercatorum pararepleta*, Development expression, Genetic variability, Substrate affinity.

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BACKGROUND

Esterases constitute a diversified group of isozymes in insects that plays important roles in cell metabolism of insecticides (Feyereisen 1995; Silva and Lapenta 2011), in digestive and reproductive pathways (Gilbert 1981; Richmond and Senior 1981; Karotam et al. 1993; Argentine and James 1995; Nisbet and Billingsley 2000) and also in the metamorphosis process, controlling the levels of juvenile hormone (Gu and Zera 1994; Campbell et al. 2001; Kethidi et al. 2005; Mackert et al. 2008). In *Drosophila* (Diptera; Drosophilidae), esterases have also been applied within phylogenetic and molecular evolution studies (Zouros et al. 1982; Lapenta et al. 1995, 1998; Nascimento and Bicudo 2002; Mateus et al. 2009, 2011; Robin et al. 2009; Silva et al. 2009; Lopes et al. 2014).

Several works about differential patterns of temporal (throughout development - Zouros et al. 1982; Bélo et al. 2004; Mateus et al. 2011), spatial (different body parts - Kambysellis et al. 1968; Zouros et al. 1982; Lapenta et al. 1998; Mateus et al. 2005; Cavasini et al. 2008; Silva et al. 2009, 2010) and among genera (Lapenta et al. 1998; Nascimento and Bicudo 2002) esterase expression in *Drosophila* species have demonstrated that gene duplication is a recurrent mode of evolution observed in this group of enzymes, which can result in functional divergence of the copies and/or redundancy and acquirement of pseudogene status (Zouros et al. 1982; Pen et al. 1990; Mateus et al. 2009, 2011; Robin et al. 2009; Lopes et al. 2014). The esterase specificity studies, using α- and β-naphthyl acetates as substrates, showed that these enzymes can display interspecific variation and intrastrain polymorphism, confirming their utility as a tool for diagnosis and differentiation among species and strains, and also for estimative of evolutionary changes in a locus (McReynolds 1967; Zouros and van Delden 1982; Lapenta et al. 1998; Silva et al. 2009).

The knowledge of isoenzymatic polymorphisms can also be relevant for studies about maintenance of genetic variability in captive or threatened species kept in laboratories (Nei et al. 1975; Leberg 1992). Information about the number of alleles per locus and about polymorphic loci with high variability, such as esterases, could be used to make these enzymes a molecular marker to evaluate bottleneck/founder effect, and heterozygosity measures could be applied to evaluate the loss of variation through generations in laboratory stocks of insects (Nei et al. 1975; Briscoe et al. 1992; Takasusuki et al. 2002). The use of these estimates is very important to understand the captivity over genetic variability, which has implications on the knowledge about organism management and conservation.

The *repleta* group of *Drosophila* is composed of species that are phytophagous. Half of species of this group have their larvae developing in rotting cacti cladodes, while the adults are generalists. The other half has generalist larvae and adults (Starmer and Gilbert 1982; Pereira et al. 1983; Starmer et al. 1986; Morais et al. 1994). This food diversity requires several types of enzymes, including esterases, in order to metabolize distinct sources of resources (Jimenez and Gilliam 1990). Therefore, the expression of these enzymes could be affected depending on tissue, development time, and host exploitation (Kircher 1982; Matzkin et al. 2006; Bono and Markow 2009).

Drosophila mercatorum pararepleta (Dobzhansky and Pavan 1943; Wharton 1944) is a non-cactophilic species of the *repleta* group, which is found in natural environments with open vegetation in Brazil. The other subspecies, *D. m. mercatorum* (Patterson and Wheeler 1942; Wharton 1944), has an anthropic association and a cosmopolitan distribution. In South America it was never registered in Brazil, only in Peru, Colombia and Venezuela (Pereira 1979; Tidon-Sklorz and Sene 1999). Few studies regarding esterase patterns were conducted on *D. m. pararepleta* (Pereira 1979; Zouros et al. 1982; Thomé 2005). However, there is a lack of knowledge specially considering expression during development and substrate specificity, not only for *D. mercatorum*, but also for non-cactophilic species of *repleta* group in general. Therefore, in this work we analyzed the esterase profile throughout development and alpha/beta substrate specificity, using laboratory strains of *D. m. pararepleta*. We also estimated the genetic variability of one polymorphic locus in different generations. Our results showed one major beta esterase locus, and the larval beta esterase previously detected for *D. m. mercatorum* was not observed. However, two exclusive loci were observed in adults, one alpha exclusive and the other an alpha/beta esterase. After more than 100 generations, we detected the complete loss of polymorphism in a beta esterase locus in one strain, probably because of severe bottleneck effect when the strain was established and subsequent genetic drift, contributing to the extinction of the less frequent alleles.

MATERIALS AND METHODS

Esterase Expression Throughout Development

The analysis of esterase expression patterns during development was performed using four strains of *Drosophila mercatorum pararepleta* collected in south and southeast of Brazil. Three of these strains were obtained during summer of 2007: 1) SER – collected in Serrana/SP (21°15'S, 47°34'W); 2) ITI – obtained from Itirapina/SP (22°16'S, 47°48'W); and 3) RIP – collected in Rio do Poço, Guarapuava/PR (25°28'S, 51°87'W). These samples were collected using traps containing fermented banana and orange bait hung 1.5 m above ground (Sene et al. 1981). Inseminated females from nature were placed into vials containing banana/agar culture medium, where isofemale lines were established. The fourth strain, D33 (from Cristalina/GO), was established in 1987 and was provided by Laboratório de Genética Evolutiva (USP, Ribeirão Preto/SP). The analyses of esterase expression during development were performed in 2007 using flies of the third generation (F3 hereafter) of SER, ITI and RIP, and of the 241st generation (F241 hereafter) of D33 (20 years after establishment). For each strain, 40 adults (20 females and 20 males), 10 first instar larvae, 10 third instar larvae, and 20 pupae were stored at negative 20°C. PAGE 10% was performed and esterase activity, using α and β-naphthyl acetates, was revealed as described by Mateus et al. (2009, 2011). The esterase loci were identified with numbers, and the alleles with letters, designating the faster loci and allele as EST-1A, and so on.

Substrate Specificity of Esterases

The analysis of esterase substrate specificity (α and/or β) were performed in adults, first and third instar larvae from the same strains described above, using the methodology described by Mateus et al. (2009, 2011), with modifications: samples were macerated in 40 μL of buffer (0.1 M Tris-HCl, pH 8.8), quickly centrifuged, and then, three aliquots of 10 μL of supernatant were applied in three different parts of the gel. After electrophoresis, the three parts of the gel were separated and one was stained only with α-naphthyl acetate, the second only with β-naphthyl acetate, and the third, as control, with both substrates.

Allele Frequency of EST-7 Esterase Locus

The allele frequency of the EST-7 locus was performed using samples from F3 (analyzed in 2007) and F104 (analyzed in 2015) of SER, and from F241 (also analyzed in 2007) of D33. SER was compared to D33 because both strains presented similar expression pattern during development (results below). The EST-7 locus was selected to estimate the allele frequency because of its highest number of alleles. It is important to point out that the strains were maintained in several vials, avoiding inbreeding, mixing flies from different vials every transfer to new vials. In this analysis, 120 samples from these two strains were used: 20 males and 20 females of D33, SER-F3, and SER-F104. PAGE 10% was performed according to Mateus et al. (2009, 2011). The software TFPGA (Miller 1997) was used to obtain EST-7 allele frequency and mean heterozygosity (observed – Ho; expected – He).

RESULTS

Esterase Expression during Development

The pattern of esterase expression profile during development of four *Drosophila mercatorum pararepleta* strains is summarized on table 1. Eight loci were found in the adults (EST-1 to EST-8). The same pattern was observed for larvae and pupae, with exception of EST-1 and EST-2, which were exclusive of adults (Fig. 1). EST-3 was not expressed on larvae and pupae of RIP (Fig. 1), and presented very low expression frequency during the entire development of ITI. EST-5 was not observed on larvae of RIP (Fig. 1), and EST-6 was not detected on adults of ITI (Fig. 2). The other loci (EST-4, EST-7 and EST-8) showed no variation among strains regarding expression during development. In general, D33 and SER presented the most similar esterase expression pattern during development among all strains.

Among these eight loci, two were monomorphic (EST-3 and EST-8), one was dimorphic (EST-6), and five were polymorphic (three alleles - EST-5; four alleles - EST-1 and EST-2; five alleles - EST-4; and six alleles - EST-7). Only locus EST-7 demonstrated to be dimeric, while the other seven were monomeric.

Substrate Specificity

All loci presented activity when α-naphthyl acetate was the only substrate provided. EST-2 was the only locus not detected when β-naphthyl acetate was alone in the solution, indicating that this is a α-naphthyl acetate exclusive locus (Fig. 3). One locus was preferably β-esterase, EST-7, as it was stained in pink when in contact with both substrates (control). Four loci (EST-3, EST-4, EST-5 and EST-8) were preferably α-esterase (staining in black on control experiment). Two loci (EST-1 and EST-6) stained in purple in the control experiment, suggesting that these loci have similar activity on α and β substrates, being called α/β-esterase (Table 1 and Fig. 3). No variation during development and among strains was observed regarding esterase substrate affinity.

Allele Frequency of EST-7 Esterase Locus

The analyzed samples from D33, after 20 years in laboratory, presented higher estimates of variability (six alleles) and genetic diversity (Ho = 0.46) for EST-7 locus than F3 of SER (five alleles, Ho = 0.26) (Table 2). After eight years of SER F3 analysis, about 104th generation, EST-7 locus became monomorphic in this strain

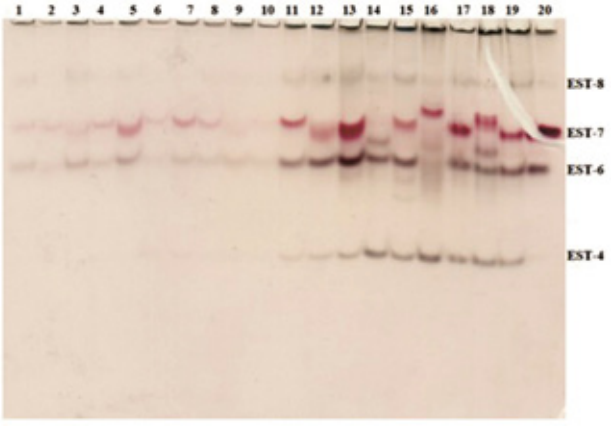

Fig. 1. Esterase profile of 1st instar (samples 1 to 10) and 3rd instar (samples 11 to 20) larvae of *Drosophila mercatorum pararepleta* from Rio do Poço strain (RIP). It can be observed the absence of EST-1 and EST-2 (which are adult exclusive), and also EST-3 and EST-5 (which are not detected only in this strain).

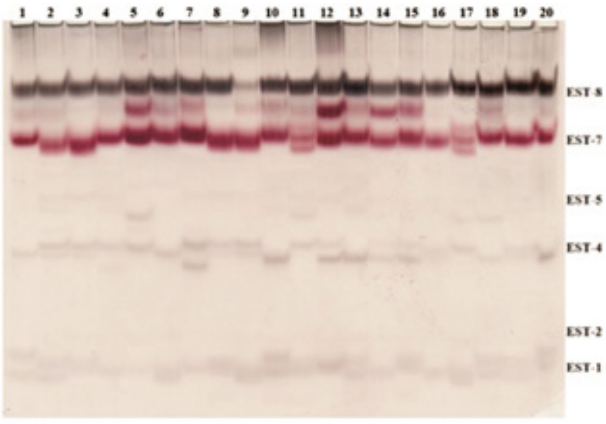

Fig. 2. Esterase profile in 20 adults of *Drosophila mercatorum pararepleta* from Itirapina strain (ITI). It can be observed that no sample showed the presence of EST-3 (which has low frequency in this strain) and EST-6 (which was not detected in this strain).

Table 1. Esterase expression during development in *Drosophila mercatorum pararepleta* strains. Loci in bold had expression only in adults

				RIP				ITI				SER				D33			
Loci	Sub	AN	ESS	L	P	♂	♀	L	P	♂	♀	L	P	♂	♀	L	P	♂	♀
EST-1	mono	04	α/β	-	-	X	X	-	-	X	X	-	-	X	X	-	-	X	X
EST-2	mono	04	α ex	-	-	X	X	-	-	X	X	-	-	X	X	-	-	X	X
EST-3	mono	01	α	-	-	X	X	↓	↓	↓	↓	X	X	X	X	X	X	X	X
EST-4	mono	05	α	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EST-5	mono	03	α	-	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EST-6	mono	02	α/β	X	X	X	X	X	X	-	-	X	X	X	X	X	X	X	X
EST-7	di	06	β	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EST-8	mono	01	α	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

L = larvae; P = pupae. RIP = Rio do Poço (Guarapuava/PR); ITI = Itirapina/SP; SER = Serrana/SP; D33 = Cristalina/GO. Sub = number of esterase subunities; mono = monomeric; di = dimeric. AN = allele number. ESS= esterase substrate specificity; α = preferably α-esterase; α ex = α-esterase exclusive; β = preferably β-esterase; α/β = similar affinity with both substrate, α/β-esterase. - = no esterase expression; X = esterase expression; ↓ = low frequency of esterase expression (less than 50% of sampled individuals).

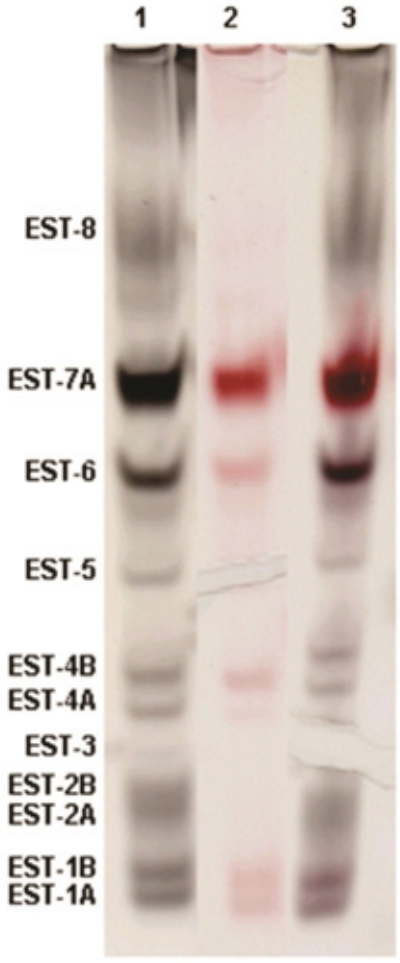

Fig. 3. General pattern of esterase profile in adults of *Drosophila mercatorum pararepleta*. 1 = Esterase activity on only α- naphthyl acetate substrate; 2 = Esterase activity on only β- naphthyl acetate substrate; 3 = Esterase activity on α- and β-naphthyl acetate substrates (control).

Table 2. Allele frequencies and mean heterozygosities (expected = He, observed = Ho) of EST-7 locus of *Drosophila mercatorum pararepleta* strains

Alleles	D-33	SER-F3	SER-F104
1	0.14	0.02	-
2	0.27	0.18	-
3	0.11	0.70	1.00
4	0.21	-	-
5	0.25	0.09	-
6	0.02	0.01	-
	He = 0.79	He = 0.46	He = 0.00
	Ho = 0.46	Ho = 0.26	Ho = 0.00

D-33 = Cristalina/GO; SER = Serrana/SP; F3 = 3rd generation; F104 = 104th generation.

DISCUSSION

Esterase comprise a multifunctional and heterogeneous group of hydrolytic enzymes that preferably acts on esters of carboxylic acid, and also on substrates that contain amide bonds, which are widely distributed in nature (Walker and Mackness 1983). The molecular heterogeneity of esterases in *Drosophila* (Diptera; Drosophilidae) species reflects a multiloci structure that act over several types of substrate. These enzymes have been detected with differential expression in all developmental stages and in several tissues of these insects (Lapenta et al. 1998; Mateus et al. 2005; Cavasini et al. 2008; Silva et al. 2009, 2010), with variation among genera, intra and interstrains (Lapenta et al. 1995, 1998; Nascimento and Bicudo 2002). These features show the importance of this type of enzyme in the normal development and reinforcing the relevance of studies in this area. Despite this group of enzymes is well investigated in cactophilic species of *Drosophila repleta* group, the knowledge about the expression pattern during development in non-cactophilic species of this group has been poorly investigated.

The esterase analyses using strains of *Drosophila mercatorum pararepleta*, a non-cactophilic specie of *repleta* group, showed no genus specificity. However, two loci (EST-1 and EST-2) were exclusive of the adult phase. Differences in the expression during development were detected for some loci (EST-3, EST-5 and EST-6) (Table 1). These variations in the esterase expression, which apparently do not disturb the normal development of these organisms, suggest that these loci are non-specific and/or pseudogenes. The null alleles of these loci could be due to the accumulation of mutations that resulted in activity loss, but not affected viability, as they are probably extra copies that arose through gene duplication (Zhang 2003).

Esterase profile analysis of *Drosophila mercatorum pararepleta* were previously performed by Pereira (1979) and Thomé (2005), using adults of natural populations from Midwest of Brazil (Brasília/DF). Both studies applied distinct esterase detection methodology from those used here, and we, furthermore, also analyzed larvae and pupae. Pereira (1979) used starch gel electrophoresis analysis and found four esterase loci, two α and two β-esterase. Thomé (2005) used PAGE, as we did in this work. However, the esterase staining was performed only in the presence of α-naphthyl acetate and a different protocol for naming the

alleles was employed, based on Pereira (1979) work. Only one locus (EST-1) was named, and all variations were designated as alleles of this locus. Interestingly, if our protocol for naming the alleles is employed in the Thomé (2005) analyses, the same eight loci are detected, despite that author has stained only α-esterases. This is possible because we did not detected exclusive β-esterases; on the other hand, the lower loci number observed by Pereira (1979) must be due to the use of starch gel electrophoresis instead of PAGE, which results in better detection of esterase loci (Silva et al. 2009, 2010). Pereira (1979) found two dimeric loci (one α, EST-1, and one β, EST-3), while we detected only one (EST-7, β-esterase), however it presented higher allele number (six) than the EST-1 locus (five) of Pereira (1979).

Zouros and van Delden (1982), similarly to our work, did not find distinct age-specific α and β-esterases profiles for *Drosophila mojavensis*, a cactophilic species of *repleta* group. Considering substrate specificity, *Drosophila mercatorum pararepleta* presented only one exclusive α-esterase locus (EST-2 - Fig. 3). Single substrate enzymes usually have one fixed allele, which is maintained by directional selection, while multiple substrate enzymes recognize several substrates, and, therefore, these loci present different alleles, being this polymorphism a result of stabilizing selection (Gillespie and Langley 1974; Zouros and van Delden 1982). Thus, despite EST-2 has shown to be a α-esterase, we have strong evidence that all esterase loci of *D. m. pararepleta* are multiple substrates, including EST-2 that presented four alleles (Table 1).

Two major β-esterases can be found in several *Drosophila* species (Pereira 1979; Zouros and van Delden 1982; Zouros et al. 1982; Lapenta et al. 1995, 1998; Robin et al. 2009; Silva et al. 2009; Mateus et al. 2011). One is widespread in the *repleta* group (EST-5) and, therefore, it has been used in evolutionary and phylogenetic studies (Zouros et al. 1982; Lopes et al. 2014). The other, also known as larval esterase (EST-4), is expressed specifically at the late larval stage and shows a possible function related to juvenile hormone degradation, important event in the larva-pupa transition (Zouros et al. 1982; East 1982; Flatt et al. 2005; Mateus et al. 2011). Zouros et al. (1982) stated that the high level of variation of EST-4 expression among species, including being totally absent in some, such as in the *D. m. pararepleta* strains analyzed here, is not representative of the phylogenetic relationship among species.

Zouros et al. (1982) analyzed the β-esterases in the *repleta* group, including a strain identified by the authors as *D. m. mercatorum*, the other subspecies of *D. mercatorum*, and they detected a larval β-esterase in this species. However, this strain is from southeast coast of Brazil (Angra dos Reis/RJ), and Pereira (1979) and Tidon-Sklorz and Sene (1999) suggest that this subspecies does not occur in Brazil. Therefore, if we consider that the strain analyzed by Zouros et al. (1982) is actually *D. m. pararepleta*, then it is possible to infer that there is differential interstrain expression of EST-4.

The analysis of mean observed heterozygosity (Ho) in the EST-7 locus using the three different approaches allowed us to covered the effect of bottleneck in short, medium and long term. Despite the higher number of allele per locus in D33 (six) than in SER-F3 (five), surprisingly the D33 heterozygosity was approximately 50%, and closer to the mean heterozygosity of natural populations (data from Pereira 1979 and Thomé 2005). On the other hand, the Ho of SER-F3 was almost half of that found for D33, and the analysis of this strain eight years later showed that the EST-7 locus became monomorphic.

Measures of variability, such as the number of polymorphic loci and alleles, and of diversity, such as heterozygosity, are sensible indexes to the differences pre and post-bottleneck in the populations. For this reason, the analysis of this marker is important for the maintenance of captive and threatened populations in the laboratory (Nei et al. 1975; Briscoe et al. 1992; Leberg 1992). After bottleneck, the levels of Ho of allozymes in *Drosophila* can be severely reduced in a couple of years, even in populations maintained with large sizes (Briscoe et al. 1992). However, the number of polymorphic loci and alleles per locus are better indicators of a bottleneck effect than Ho (Leberg 1992) as the mean number of alleles per locus increases faster than the mean heterozygosity after the population size is reacquired. However, the number of alleles per locus loss is greater as the more severe is the bottleneck, independently of the populational growth a posteriori, with the mean number of allele per locus increasing more rapidly than mean heterozygosity when the population size is reestablished. However, the loss of allele number per locus will be greater as more severe is the bottleneck, independently of the later population growth. On the other hand, the Ho decrease will depend on the population growth, being weaker when the bottleneck intensity is light

and population growth is fast (Nei et al. 1975).

Thus, the reduction of genetic diversity is directly related to bottleneck severity and subsequent population growth, and maintaining captured populations as large populations is not sufficient to ensure the essential genetic variability of natural populations (Nei et al. 1975; Briscoe et al. 1992). Takasusuki et al. (2002) proposed that outbreending (for at least four generations) would overlap the effect of the bottleneck on genetic diversity in *Diatraea saccharalis* (Lepidoptera), and they suggested that genetic drift and overdominance should be responsible for heterozygosity excess detected in EST-3 locus. However, this suggestion may not be adequate because the recently bottleneck effect itself, suffered by the lineages of this insect, may have been responsible for the heterozygosity excess, since populations that passed through a recent bottleneck should experience reduction in allele number before heterozygosity loss, in loci that the variation is under the Infinite Allele Model (IAM), such as allozymes (Maruyama and Fuerst 1985). Considering our results, the SER strain suffered a much more pronounced bottleneck when established in the laboratory than D33, since even the subsequent population growth did not prevent the fixation of a single allele, within the five initial, in the EST-7 locus. The fixation of this allele may have occurred by the action of genetic drift, eliminating, generation after generation, the less frequent alleles. If directional selection was the factor responsible for fixing the remaining allele of the EST-7 locus, the same pattern should have been observed in D33 that was maintained in the same laboratory conditions as SER.

CONCLUSIONS

The analysis of esterase profile during life cycle of *Drosophila mercatorum pararepleta* strains revealed two adult exclusive loci (EST-1 and EST-2), no gender specificity, and no development changes of esterase specificity on α and β-naphthyl acetate substrates (Table 1 and Fig. 3). Only one β-esterase locus was observed (EST-7), differently of the other *Drosophila* species (Pereira 1979; Zouros and van Delden 1982; Zouros et al. 1982; Robin et al. 2009; Silva et al. 2009; Mateus et al. 2011). If the specimens from Angra dos Reis/RJ (southeast coast of Brazil, Zouros et al. 1982) really belong to the *D. m. mercatorum* subspecies, the presence of a larval β-esterase could be diagnostic for this subspecies, and the absence would be characteristic of *D. m. pararepleta*. Nevertheless, if the specimens collected in RJ were, in fact, individuals of the same subspecies analyzed here (as we believe, accordingly to drosophilid distribution studies by Tidon-Sklorz and Sene 1999), this support that the presence/absence of larval esterase does not have any relation to the phylogeny of the r*epleta* group (Zouros et al. 1982).

The intra and interstrain null alleles suggest that these non-specific esterases might have lost their activity due to the accumulation of mutations in the extra copies, which were originated by gene duplication events (Robin et al. 2009; Lopes et al. 2014). Therefore, analyses of esterase genetic variability of *D. m. pararepleta* populations should take into account the occurrence of null alleles in these loci. Our work also showed that esterase variability analyses using laboratory strains must be performed as soon as their were established, since the genetic diversity and variability might be significantly reduced as a result of founder effect/bottleneck suffered in the strain establishment. Depending on the intensity of these events, a significant population increase and practices that minimize inbreeding may not be enough to prevent the effects of these events (Nei et al. 1975; Briscoe et al. 1992).

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