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Analysis of the drosophilid *Zaprionus indianus* introduction in Brazil: contribution of esterase loci polymorphisms.

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Introduction

The colonization of environmental niches by exotic species constitutes an unplanned experiment that provides historical information on ecology and evolution (Carroll and Boyd, 1992; Cox, 2004; Strauss *et al*., 2006). Such invasions allow comparing the biological responses to the new niche with those to the ancestral environments, by direct observation rather than by inference as it is usually done, as observed by Carroll (2007).

Recently, a drosophilid - *Zaprionus indianus* - was introduced in Brazil (Vilela, 1999). This is an African species (Tsacas, 1985) that had already spread over Asia several decades ago (Gupta, 1970; Amoudi, 1993; Parkash and Yadav, 1993a,b; Karan *et al*., 2000). Since then, a number of reports have indicated its occurrence in several regions of Brazil (Galinkin and Tidon, 2000; Moraes *et al*., 2000; Vilela *et al*., 2001; Toni *et al*., 2001; Tidon *et al*., 2003; Machado *et al*., 2005; Galego *et al*., 2006), in other South American countries (Goñi *et al*., 2001, 2002) and recently in the United States of America (Linde *et al*., 2006). In Brazil, *Z. indianus* was known as fig fly because of the loss of 50% of the fig crop in 1999 due to larvae infestation. The potential status of pest during the early period of its introduction stimulated Brazilian researchers to study *Z. indianus* aiming at understanding the biology, ecology, genetics, and population structure of this well succeeded invader. An adequate tool to investigate the genetic structure and the relationships between populations are the alloenzyme markers as, for instance, esterases, a multi-functional and heterogeneous group of enzymes that frequently show polymorphic loci in Drosophilidae (Brady and Richmond, 1990; Parkash and Yadav, 1993; Russell *et al.*, 1996; Dumancic *et al.*, 1997; Nascimento and Bicudo, 2002; Campbell *et al.*, 2003; Machado *et al*., 2005; Galego *et al.,* 2004, 2006).

In this study, populations from 22 regions of Brazil were sampled, so as to propose a model of *Z. indianus* introduction in the country, using as a reference the genetic structure of these populations by means of data on esterase polymorphisms. The polymorphic Est3 locus was used as marker, which present four alleles ($Est3^1$, $Est3^2$, $Est3^3$, $Est3^4$) in most Brazilian populations (Galego *et al.,* 2006).

Table 1. Populations of *Z. indianus* sampled for this study, with their regions, geographic location and sample collector.

Region	Locality	Geographic location	Collector
State of São Paulo (SP)	Mirassol	49º30´W/20º47´S	Galego, LGC
	Onda Verde	49º30´W/20º62´S	Granzotto, A
	São José do Rio Preto	49º22´W/20º49´S	Galego, LGC
	Itatiba	46º50´W/23º00´S	Galego, LGC
	Ilhabela	45º21´W/23º46´S	Fazza, AC
	Paulo de Faria	49º30´W/20º62´S	Granzotto, A
	São Paulo	46º50´W/23º31´S	Setta, N
	Paraibuna	45º41´W/23º26´S	Galego, LGC
	Maresias	45º21´W/23º21´S	Galego, LGC
	Rio Claro	44º08´W/22º43´S	Carareto, CMA
	Ibirá	49º14´W/21º04´S	Galego, LGC
	Olímpia	48º54´W/20º44´S	Galego, LGC
	Sud Menucci	50º55´W/20º41´S	Lofego, AC
Southeast (Except to SP) and Centerwest Regions (SE+MW)	Alfenas (MG)	46º10´W/21º20´S	Galego, LGC
	Belo Horizonte (MG)	43º56´W/19º55´S	Galego, LGC
	Córrego Danta (MG)	45º55´W/19º24´S	Galego, LGC
	Poços de Caldas (MG)	46º33W/21º47´´S	Machado et al. (2005)
	Rio de Janeiro (RJ)	43º12´W/22º54´S	Bitner-Mathé, B
	Brasília (DF)	47º55´W/15º46´S	Tidon, R
South Region (S)	Porto Alegre (RS)	51º13´W/30º01´S	Valente, V
	Santa Maria (RS)	53º48´W/29º41´S	Valente, V
	Florianópolis (SC)	48º32´W/27º35´S	Hoffman, P
Northeast Region (NE)	Jequié (BA)	40º04´W/13º51´S	Luizon, MR
	Lençóis (BA)	41º23´W/12º33´S	Machado et al. (2005)
	Beberibe (CE)	38º53´W/04º10´S	Machado et al. (2005)

SP: São Paulo state; SE: Southeast; MW: Mid-West; S: South; NE: Northeast.

Material and Methods

The collections of *Z. indianus* were performed with traps containing attractive baits made of banana and biological yeast, as described by Galego *et al*. (2006). The populations collected were joined in geographic groups (Table 1), as the Brazilian state of collection localities: Northeast, Mid-West, Southeast and South, with exception of the São Paulo state that was considered as a block out of Southwest region, on account of the great number of collections performed in this state. The Asia and Africa data used in the analyses were those published by Machado *et al*. (2005). The esterases were detected as described by Galego *et al.* (2006). The alloenzyme data were analyzed using the computer softwares TFPGA version 1.3 (Miller, 1997) and Genetix version 4.05.2 (Belkier *et al*., 1996). The allele and genotype frequencies of the polymorphic loci and the observed (H_O) and expected (H_E) heterozygosity were determined with TFPGA. UPGMA analysis (Swofford and Olsen, 1990) was performed using the same software. Genetic distances (Nei, 1978) were determined by Genetix. Excel and Minitab softwares were used for the multivariate analyses of genetic data.

Results and Discussion

Allele frequencies of Est3 locus (Figure 1) ranged between 0.471 (South) and 0.150 (Northeast) for Est3^1, 0.362 (Northeast) and 0.183 (São Paulo State) for Est3^2, 0.380 (Northeast) and 0.127 (Southeast + Mid-west) for Est3^3 and 0.345 (São Paulo State) to 0.108 (Northeast) for Est3^4. In Asia and Africa, the frequency of these alleles ranged from, respectively, 0.361 to 0.403 for Est3^1, 0.472 to 0.238 (Est3^2), 0.167 to 0.285 (Est3^3) and 0 to 0.074 (Est3^4). The absence of allele Est3^4 in the Asian populations suggests that the propagule of *Z. indianus* that invaded Brazil may have come from Africa rather than from Asia, since the African lineages present this allele, even if at a low frequency (0.074). Reinforcing this hypothesis, David *et al.* (2006b), who studied quantitative traits related to body size, suggested that the propagule that colonized Brazil might have come from high-latitude African regions, due to similarities between the Brazilian populations and those of those latitudes.

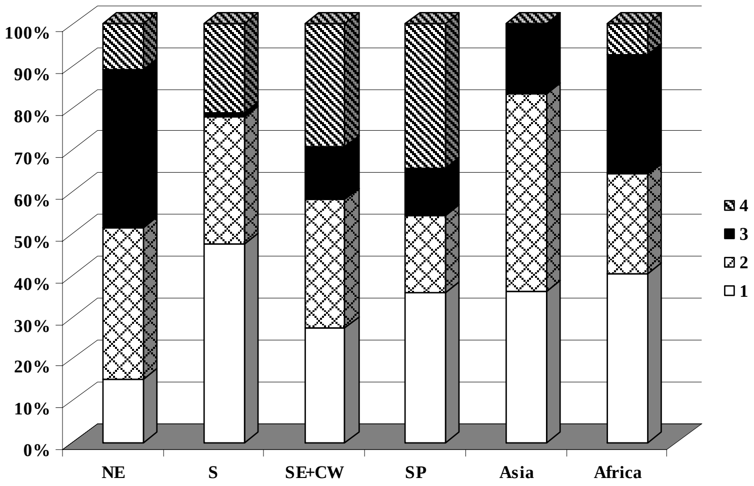

Figure 1. Allele distribution in the Est3 locus (Est3^1: 1, Est3^2: 2, Est3^3: 3, Est3^4: 4) from 21 Brazilian populations of *Z. indianus* clustered in four regions and Africa and Asia populations studied by Machado *et al.* (2005) (*). SP: São Paulo state; SE: Southeast; CW: Mid-west; NE: Northeast; S: South.

Multivariate analysis with genetic variables (Est3 allele frequencies, observed and expected heterozygosity) grouped samples of São Paulo, other Southeastern, Mid-western and Southern populations of *Z. indianus* together the African populations (Figure 2). The Northeastern and Asian populations showed less similarity to this cluster, and Asian is an outgroup of Brazilian and African populations. UPGMA analysis using the genetic distances (Figure 3) grouped the populations from Southeast together with those from Asia and Africa (ancestral populations). São Paulo city and Itatiba populations were closely related to African populations. The similarity showed by both analyses is congruent with the chronological reports of invasion along the Brazil: 1999, in São Paulo state, Southwestern and Mid-western regions (Vilela, 1999; Vilela *et al.*, 2000; Tidon *et al.*, 2003), 1999-2000 in Southern (De Toni *et al.*, 2001; Castro and Valente, 2001), and 2001 in Northeastern (Santos *et al.*, 2003; Machado *et al.*, 2006) regions.

The data obtained in the present study indicate that the invasion of the Brazilian territory by *Z. indianus* is according to the theoretical invasion scenario 1 proposed by Facon *et al.* (2006) in which a change in the migration regime, possibly as a result of human activity, is responsible for the process of invasion. Our data corroborate, too, the scenario suggested by Machado *et al.* (2005) and David *et al.* (2006b) that a single invasion occurred by a unique and numerous propagule, followed by rapid expansion. Their idea is that the propagule probably came from tropical Africa and, once in Brazil, spread from one locality to another by jumps, thanks to human transportation (David *et al*., 2006b). The African origin of Brazilian populations of *Z. indianus* was reinforced by analysis of chromosome inversion polymorphism (Ananina *et al*., 2006) and quantitative traits (David *et al*., 2006a,b).

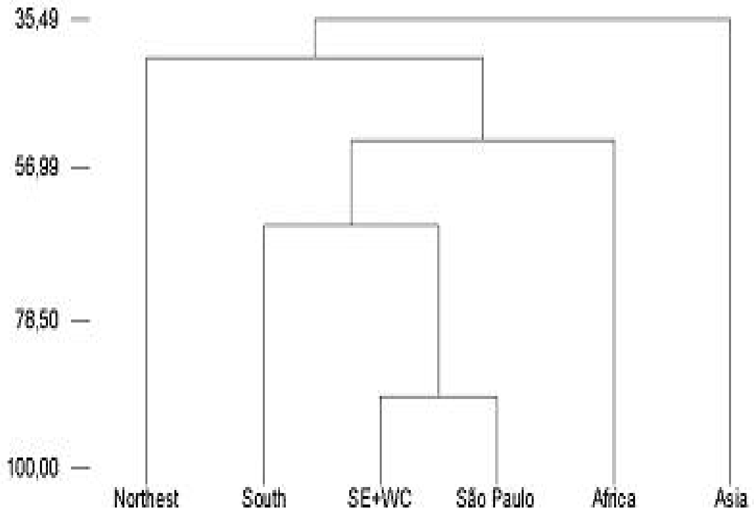

Figure 2. Multivariate analysis of *Z. indianus* populations using genetic variants regarding to Est3 locus (Est3 allele frequencies, observed and expected heterozygosity). Asia and Africa data from Machado *et al*. (2005). SE: Southest; CW: Mid-west.

The UPGMA and multivariate analyses here reported and particularly the common occurrence of allele Est3^4 in a population of African origin and in Brazilian populations corroborate with genetic data the hypothesis of African origin of *Z. indianus* that colonized Brazil. These analyses further indicate that the arrival of the invaders in Brazil occurred in the State of São Paulo, spreading over the country later on. Vilela *et al*. (2000) suggested that *Z. indianus* introduction occurred by plane, possibly due to presence of eggs and larvae in meals, but the conclusions of Machado *et al*. (2005), David *et al*. (2006a,b), and Ananina *et al*. (2006) indicate that the founder propagule was numerous. Accordingly, this hypothesis makes more probable the introduction by ships, considering that a numerous propagule demands a big reservoir as a fruit charges and the fruits international commercialization and transportation was mainly made by maritime waterway (França and Gondin, 1999, in http://www.bnb.gov.br/content/Aplicacao/ETENE/Rede_Irrigacao/ Docs/Fruticultura%20I-%20Uma%20visao%20geral%20do%20mercado.PDF). Taking also into consideration that Brazil imports fruit from Africa (http://www.mre.gov.br/index.php?option=comcontentandtask=categoryandsectionid=5andid=11andItemid=557), it is rather likely that the entrance has occurred by the Port of Santos, a port that accounts yearly for one fourth of all products traded by the country on the international market (Available at <http:// http://www.vivasantos.com.br/ 04/04a.htm>, accessed on 11/03/2007), which shows the importance of this port for the entrance of merchandise from other countries. Furthermore, a great part of the goods that arrive in Santos is distributed throughout the State of São Paulo and from there to the rest of the country.

It is probable that after the arrival, *Z. indianus* has spread over the State of São Paulo by highway transportation and from there to the whole country, mainly as a result of fruit commerce, as suggested by Tidon *et al*. (2003). The UPGMA and multivariate analyses support this scenario. In both, large blocks of populations from the State of São Paulo were grouped together with the ancestral populations from Africa. After its introduction, *Z. indianus* rapidly spread over the southeastern, southern and mid-western regions, as shown by the publication records (Vilela, 1999; Galinkin and Tidon, 2000; Moraes *et al*., 2000; Toni *et al*., 2001; Tidon *et al*., 2003), only reaching the northern and north-eastern regions later on. The structure of the Brazilian *Z. indianus* populations studied here does not suggest the occurrence of spontaneous and progressive spreading of the marginal populations. The UPGMA analyses clustered populations far from each other, which suggests that the main way of spreading of this species in Brazil continues to be the commercial food transportation, which is one of the two possibilities raised by David *et al.* (2006b). The analyses also show that the colonization was more pronounced and early in areas with heavier highway traffic. Genetic characterization of populations of a colonizer species soon after its introduction, as it is the case of *Z. indianus* in South America, besides its relevance in offering a possibility to determine the source of the invasion and demographic parameters of the species, also represents a unique opportunity to follow-up the evolutionary dynamics of the invader species over time.

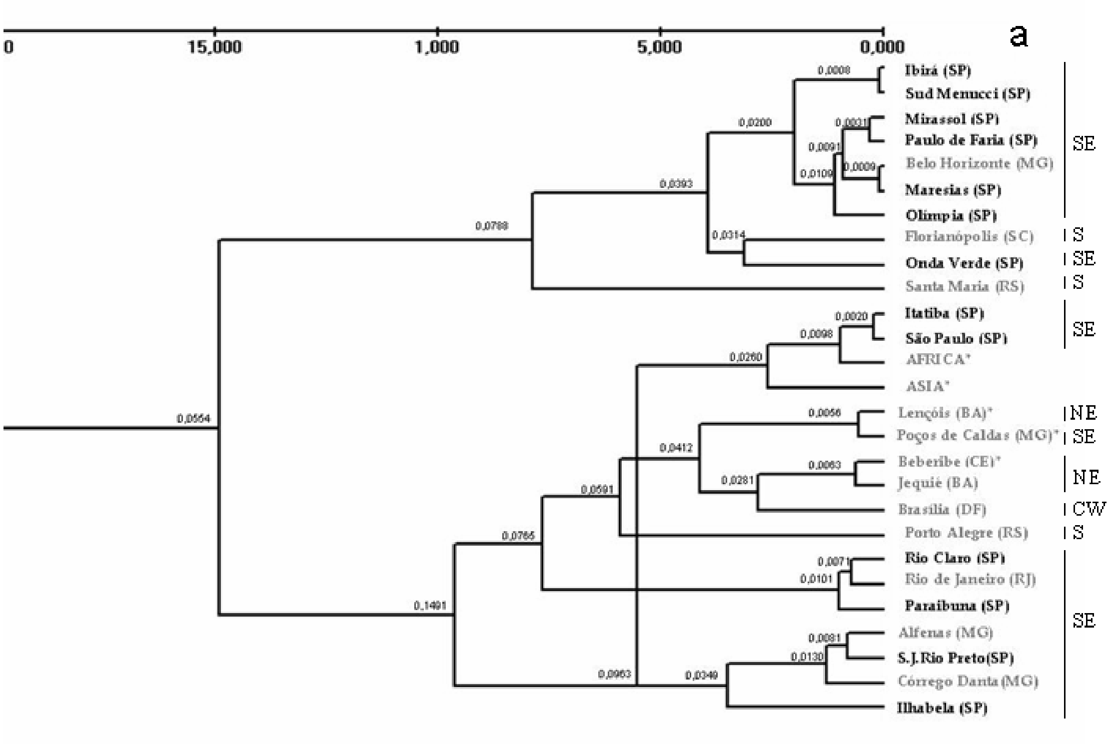

Figure 3. UPGMA analyses using the Nei´s genetic distances (1978) between 21 Brazilian populations and Asia and Africa populations of *Z. indianus*. The * indicate the populations studied by Machado *et al.* (2005). Localities in grey scale are outside São Paulo state. SP: São Paulo state; SE: Southest; CW: Mid-west; NE: Northest; S: South.

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Toxicological tests of tibolone in *Drosophila melanogaster* wild type and Oregon-*flare* strains.

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Introduction

For many years estrogen replacement has been used effectively to prevent osteoporosis and other climacteric complains such as vaginal dryness, hot flushes and mood symptoms that are related to the marked decline in plasma estradiol levels in postmenopausal women. Estrogen replacement, however, may lead to cell proliferation in the uterus and breast. In particular in the uterus, unopposed estrogen replacement treatment leads to an increased risk of developing uterine cancers. Therefore, an ideal therapy would be one that acts as an estrogen on the bone and the urogenital system, but not on the uterus or the breast (Bloom, 2006).