Scenario of the spread of the invasive species *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) in Brazil

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Abstract

*Zaprionus indianus* was first recorded in Brazil in 1999 and rapidly spread throughout the country. We have obtained data on esterase loci polymorphisms (*Est2* and *Est3*), and analyzed them, using Landscape Shape Interpolation and the Monmonier Maximum Difference Algorithm to discover how regional invasion occurred. Hence, it was apparent that *Z. indianus*, after first arriving in São Paulo state, spread throughout the country, probably together with the transportation of commercial fruits by way of the two main Brazilian freeways, BR 153, to the south and the surrounding countryside, and the BR 116 along the coast and throughout the north-east.

Key words: *Zaprionus indianus*, esterase, landscape shape interpolation, Monmonier’s maximum difference algorithm.

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Introduction

*Zaprionus indianus* is an African species, that is now widespread throughout several tropical areas worldwide, probably as a result of the intense commerce of agricultural goods. In Brazil (Figure 1), this drosophilid was first reported by Vilela (1999) in Santa Isabel (São Paulo state), then throughout the state itself (Vilela et al., 2000), and afterwards other neighboring regions (Tidon et al., 2001; Tidon et al., 2003). Between 2000 and 2003, the species was progressively observed throughout Brazil as a whole (Castro and Valente, 2001; Santos et al., 2003; Kato et al., 2004; Mata et al., 2004; Loh and Bitner-Mathé, 2005; Mattos-Machado et al., 2005), in Uruguay (Göni et al., 2001, 2002), and more recently, in Central America and the United States (Linde et al., 2006).

Various tools have been employed for characterizing the species introduced into Brazil, such as alloenzyme polymorphisms (Mattos-Machado et al., 2005; Galego and Carareto, 2007), quantitative traits (David et al., 2006a,b) and chromosome inversions (Ananina et al., 2006). These studies indicated that the founder propagul were numerous. Vilela (1999) proposed that *Z. indianus* was maybe introduced by air transport from Africa. This proposal was thereafter endorsed by Tidon et al. (2003). Later, Galego and Carareto (2007) added weight to the concept of African introduction based on data from two polymorphic esterase loci, *Est2* and *Est3*, the first with two alleles (*Est2*<sup>F</sup> and *Est2*<sup>S</sup>), the second with four (*Est3*<sup>1</sup>, *Est3*<sup>2</sup>, *Est3*<sup>3</sup> and *Est3*<sup>4</sup>). Furthermore, they proposed that maritime introduction was more probably a result of an increase in the commerce of fruits between Africa and Brazil. Nevertheless, how *Z. indianus* was capable of spreading so rapidly countrywide remains a mystery.

We resorted to a landscape genetics approach as a tool to answer this question. This requires constructing a framework for testing the relative influence of landscape and the environmental features of gene flow and genetic discontinuities (Guillot et al., 2005), as well as that of genetic population structure (Manel et al., 2003; Holderegger and Wagner, 2006). It also provides insights into fundamental biological processes (Storfer et al., 2007), such as metapopulation dynamics, the identification of species distribution across specific geographical and anthropogenic barriers, and population connectivity. Several analyses can be performed using this approach, such as interpolation landscapes (Isaaks and Srivastava, 1989), which permit estimating data at unsampled locations by using a mathematical model of the spatial pattern of sampled values, as well as the Monmonier Maximum Difference algorithm (Monmonier, 1973), for identifying putative genetic barriers across landscapes.

Various molecular markers are applicable in landscape genetics, such as mtDNA (Liepelt et al., 2002), AFLP (Jacquemyn, 2004), microsatellites (Poissant et al., 2005) and allozyme polymorphisms (Hitchings and Bebee, 1997; Michels et al., 2001; Pfenniger, 2002; Arnaud, 2003; Hirao and Kudo, 2004). Since esterases appear to be the most polymorphic loci in Brazilian *Z.
indianus populations (Mattos-Machado et al., 2005; Galego et al., 2006; Galego and Carareto, 2007), Est2 and Est3 loci were chosen for inferring the spreading dynamics of Z. indianus regionwise.

Methods

Sampling

Specimens of Z. indianus were collected from 2004 to 2007, in 22 localities of Brazil (Table 1), 13 in the state of São Paulo (SP), three in Minas Gerais (MG), two in Rio Grande do Sul (RS), and one each in Santa Catarina (SC), Rio de Janeiro (RJ), Bahia (BA), and Brasilia (DF). Individuals were collected with traps containing enticing baits made up of banana and biological yeast, as described by Galego et al. (2006). Figure 1 shows the scatterplot of the locations of the populations sampled, with the enclosing convex polygon overlaid by the map of Brazil. Analysis was restricted to collections with more than 10 individuals. Collected individuals were maintained in mass culture with banana-agar medium. A random sample of 20 flies (10 males and 10 females, all 7 days old) of individuals emerging from eggs oviposited by females from nature, were used for esterase detection.

Polyacrylamide gel electrophoresis and esterase detection

Each individual fly was macerated in 15 μL of Tris-HCl 0.1 M, pH 8.8 (CR Ceron, MSc Dissertation, Universidade de São Paulo, 1988), whereupon the homogenate was applied to a 10% polyacrylamide gel. Electrophoresis was carried out in a Tris-glycine buffer pH 8.8 at 200 V for 3 h. A random sample of 20 individuals (10 males and 10 females) from each population was used. In the case of the EST2 system, which is restricted to males (Galego et al., 2006), only 10 individuals were analyzed. Detection of the esterases (EST) was undertaken as suggested by Galego et al. (2006). After detection, the gels were stored as described by Ceron et al. (1992).

Data analysis

Alloenzyme data were analyzed using the computer software programmes TFPGA version 1.3 (Miller, 1997), Genetic Analyses in Excel (GenAlEx) version 6 (Peakall and Smouse, 2006), and Alleles in Space -AIS- (Miller, 2005). Allele and genotype polymorphic-locus frequencies, observed (H₀) and expected (Hₑ) heterozygosity, and Hardy-Weinberg equilibrium, were all estimated by TFPGA. The estimation of genetic distances (Nei, 1972) and F₅₀ analysis were undertaken with GenAlEx. AIS analysis of Landscape Shape Interpolation (LSI) and the Monmonier Maximum Difference Algorithm (MMDA), was performed to evaluate inter-individual patterns of genetic and geographical variation. The calculated surface for LSI was based on the midpoints of edges derived from Delaunay triangulation (Watson, 1992; Brouns et al., 2003), and the heights on “pseudoslopes” from the genetic and geographical distance matrix (Miller, 2005). The LSI approach visualizes the graphical representation of the pattern of genetic distance across the whole landscape, and is a way of producing a 3-dimensional surface plot where the X and Y axes correspond to geographical locations, whereas surface heights (Z-axes) represent genetic distances. Basically, the figure contains an inferred graphical representation of patterns of diversity across the sampled landscape that (ideally) contains peaks in areas where there are large genetic distances. The initial construction is Delaunay triangulation (Watson, 1992; Brouns et al., 2003) based on connectivity networks of sampling areas and assigning genetic distances, whereupon interpolation procedure (a = 1, grid size = 50 x 50, raw Nei, 1972, genetic distance between points) can be applied.

Furthermore, the building of putative genetic barriers across landscapes, as determined by MMDA, is found in the connectivity network of all the sampled locations used in studies that are generated in three steps by Delaunay triangulation (Watson, 1992; Brouns et al., 2003). The first step is to identify the greatest genetic distance between any 2 locations joined in the connectivity network, thereby forming the initial barrier segment. Secondly, the initial
barrier is followed in one direction until encountering either an external edge of the connectivity network or an internal segment previously defined as a barrier segment. In essence, for each extension of the barrier, the movement is in the direction of the greatest genetic distance between locations. Finally, the initial barrier identified in Step 1 is followed in the opposite direction to that taken in Step 2, until, once again, encountering either an external edge of the connectivity network or an internal segment previously defined as a barrier segment.

Results

The analysis of Est2 allele frequency distribution in Brazilian populations of *Z. indianus* (Table 1) shows fixation of the alleles Est2S in 8 of the 22 populations studied, and Est2F in 3. Est2S frequency was the lowest in Alfenas (0.09), and that of Est2F in Onda Verde and Rio de Janeiro (0.08). The frequency of locus Est3 alleles (Table 1) varied considerably according to geographic location, the least frequent being Est31. Est31 frequency varied from 0 (Ilhabela) to 0.94 (Santa Maria), Est32 from 0.05 (Rio Claro and Porto Alegre) to 0.89 (Ilhabela), and Est34 from 0 (in several localities) to 0.30 (Onda Verde). The frequency of Est32, although not detected in Santa Maria, Onda Verde and Ilhabela, was the highest in Brasília (0.69).

The average observed ($H_O$) and expected ($H_E$) was greater in Est3 than in Est2 (Table 2). Est3 $H_O$ ranged from 0 (Ilhabela) to 0.80 (Onda Verde) and $H_E$ from 0.20 (Ilhabela) to 0.52 (Ilhabela).

Table 1 - Geographical coordinates of the *Zaprionus indianus* populations sampled and allele frequencies of Est3 and Est2 esterase loci. 1: Est31; 2: Est32; 3: Est33; 4: Est34; S: Est2S; F: Est2F; H_O: observed heterozygosity; H_E: expected heterozygosity; ne: not evaluated. *Mattos-Machado et al. (2005).

| Locality            | Latitude       | 1   | 2   | 3   | 4   | $H_O$ | $H_E$ | S   | F   | $H_O$ | $H_E$ |
|---------------------|----------------|-----|-----|-----|-----|-------|-------|-----|-----|-------|-------|
| State of São Paulo (SP) |                |     |     |     |     |       |       |     |     |       |       |
| Mirassol            | 49°30’ W/20°47’ S | 0.40 | 0.22 | 0.10 | 0.28 | 0.60 | 0.70 | 0.60 | 0.40 | 0.30 | 0.35 |
| Onda Verde          | 49°30’ W/20°62’ S | 0.30 | 0.00 | 0.30 | 0.40 | 0.80 | 0.66 | 0.92 | 0.08 | 0.17 | 0.15 |
| São José do Rio Preto | 49°22’ W/20°49’ S | 0.34 | 0.12 | 0.08 | 0.46 | 0.12 | 0.12 | 0.00 | 1.00 | 0.00 | 0.00 |
| Itatiba              | 46°50’ W/23°00’ S | 0.16 | 0.21 | 0.16 | 0.47 | 0.79 | 0.68 | 0.36 | 0.64 | 0.18 | 0.46 |
| Ilhabela             | 45°21’ W/23°46’ S | 0.00 | 0.00 | 0.11 | 0.89 | 0.00 | 0.20 | 1.00 | 0.00 | 0.00 | 0.00 |
| Paulo de Faria       | 49°30’ W/20°62’ S | 0.46 | 0.12 | 0.13 | 0.29 | 0.50 | 0.67 | 0.31 | 0.69 | 0.22 | 0.49 |
| São Paulo            | 46°50’ W/23°31’ S | 0.18 | 0.23 | 0.09 | 0.50 | 0.64 | 0.66 | 0.42 | 0.58 | 0.17 | 0.49 |
| Paraíbuna            | 45°41’ W/23°26’ S | 0.44 | 0.21 | 0.21 | 0.14 | 0.62 | 0.67 | 1.00 | 0.00 | 0.00 | 0.00 |
| Maresias             | 45°21’ W/23°21’ S | 0.50 | 0.17 | 0.04 | 0.29 | 0.42 | 0.63 | 1.00 | 0.00 | 0.00 | 0.00 |
| Rio Claro            | 44°08’ W/22°43’ S | 0.65 | 0.20 | 0.10 | 0.05 | 0.70 | 0.52 | 1.00 | 0.00 | 0.00 | 0.00 |
| Ibirá                | 49°14’ W/21°04’ S | 0.42 | 0.29 | 0.12 | 0.17 | 0.42 | 0.70 | 1.00 | 0.00 | 0.00 | 0.00 |
| Olimpia              | 48°54’ W/20°44’ S | 0.35 | 0.35 | 0.00 | 0.30 | 0.67 | 0.66 | 0.00 | 1.00 | 0.00 | 0.00 |
| Sud Menucci           | 50°55’ W/20°41’ S | 0.45 | 0.25 | 0.15 | 0.15 | 0.70 | 0.69 | 1.00 | 0.00 | 0.00 | 0.00 |
| Southern States      |                |     |     |     |     |       |       |     |     |       |       |
| Porto Alegre (RS)    | 51°13’ W/30°01’ S | 0.33 | 0.62 | 0.00 | 0.05 | 0.45 | 0.51 | 0.53 | 0.47 | 0.35 | 0.50 |
| Santa Maria (RS)     | 53°48’ W/29°41’ S | 0.94 | 0.00 | 0.00 | 0.06 | 0.12 | 0.12 | 1.00 | 0.00 | 0.00 | 0.00 |
| Florianópolis (SC)   | 48°32’ W/27°35’ S | 0.14 | 0.28 | 0.05 | 0.53 | 0.60 | 0.62 | 0.82 | 0.17 | 0.36 | 0.30 |
| Alfenas (MG)         | 46°10’ W/21°20’ S | 0.32 | 0.03 | 0.03 | 0.62 | 0.29 | 0.51 | 0.09 | 0.91 | 0.18 | 0.16 |
| Belo Horizonte (MG)  | 43°56’ W/19°55’ S | 0.52 | 0.16 | 0.00 | 0.32 | 0.56 | 0.60 | 0.71 | 0.29 | 0.29 | 0.41 |
| Córrego Danta (MG)   | 45°55’ W/19°24’ S | 0.18 | 0.28 | 0.00 | 0.54 | 0.53 | 0.60 | 0.00 | 1.00 | 0.00 | 0.00 |
| Poços de Caldas (MG)* | 46°33’ W/21°47’ S | 0.00 | 0.50 | 0.50 | 0.00 | ne  | ne   | ne  | ne  | ne   | ne   |
| Rio de Janeiro (RJ)  | 43°12’ W/22°54’ S | 0.50 | 0.17 | 0.23 | 0.10 | 0.73 | 0.66 | 0.92 | 0.08 | 0.12 | 0.12 |
| Northern States      |                |     |     |     |     |       |       |     |     |       |       |
| Brasília (DF)        | 47°55’ W/15°46’ S | 0.11 | 0.69 | 0.00 | 0.20 | 0.38 | 0.47 | 0.32 | 0.68 | 0.45 | 0.35 |
| Jequie (BA)          | 40°04’ W/13°51’ S | 0.30 | 0.45 | 0.10 | 0.15 | 0.60 | 0.68 | 1.00 | 0.00 | 0.00 | 0.00 |
| Lençóis (BA)*        | 41°23’ W/12°33’ S | 0.05 | 0.57 | 0.38 | 0.00 | ne  | ne   | ne  | ne  | ne   | ne   |
| Beberibe (CE)*       | 38°07’ W/04°10’ S | 0.21 | 0.45 | 0.24 | 0.10 | ne  | ne   | ne  | ne  | ne   | ne   |
Table 2 - Means (üler) and standard-errors (SE) of observed (H₀) and expected (Hₑ) heterozygosity in Brazilian populations of *Zaprionus indianus* and chi-squared comparison (*χ²*). ne: not evaluated.

| Geographic region | Est2 (üler ± SE) | Est3 (üler ± SE) | *χ²* |
|-------------------|-----------------|-----------------|-----|
| São Paulo state (SP) | H₀: 0.08 ± 0.03, Hₑ: 0.15 ± 0.06 | 0.54 ± 0.07, 0.58 ± 0.05 | 5.05* |
|                   |                  | 4.18*            |     |
|                   | *χ²*             | 0.42            | 0.04 |
| Southern (S)       | H₀: 0.19 ± 0.06 | 0.47 ± 0.08     | 1.19 |
|                   | Hₑ: 0.21 ± 0.07 | 0.52 ± 0.07     | 1.25 |
|                   | *χ²*             | 0.02            | 0.03 |
| Northeast (N)      | H₀: ne           | 0.49 ± 0.11     | ne  |
|                   | Hₑ: ne           | 0.57 ± 0.10     | ne  |
|                   | *χ²*             | ne              | 0.02 |
| Total              | H₀: 0.13 ± 0.03  | 0.51 ± 0.04     | 6.35** |
|                   | Hₑ: 0.17 ± 0.04  | 0.56 ± 0.04     | 5.93* |
|                   | *χ²*             | 0.25            | 0.10 |
| SPXS               | *χ²*             | 6.37**          | 1.04 |
| SPXN               | *χ²*             | Ne              | 0.51 |
| SXN                | *χ²*             | Ne              | ne  |

*p < 0.05; **p < 0.01; ***p < 0.001.

The polymorphism displayed by both alloenzyme markers demonstrated a significant geographical genetic structure among the 22 Brazilian populations of *Z. indianus* sampled in this study, as shown by the FST and Nei (1972) genetic distance values. The Est3 H₀ values of the Brazilian populations of *Z. indianus* (0.54) were almost the same as the three esterase H₀ of Indian population loci, each of which harboring 5 alleles, *i.e.*, 0.54 and 0.56 (Parkash et al., 1994) and 0.58 (Parkash and Yadav, 1993), respectively. However, the Est2 H₀ values from Brazilian populations (0.08) were smaller than an esterase locus with two alleles in Indian populations, viz., 0.17 (Parkash and Yadav, 1993) and 0.33 (Parkash et al., 1994). These differences could be attributed to genetic drift (sampling errors) or the founder effect.

**Discussion**

Originally from tropical Africa, historical records show that *Z. indianus* arrived in Brazil in 1998 (Vilela, 1999), and quickly spread throughout São Paulo (Vilela et al., 2000), Rio de Janeiro (Loh and Bitner-Mathé, 2005), and the southern (Toni et al., 2001; Castro and Valente 2001) and midwestern (Tidon et al., 2003) states. The remaining Brazilian regions were thereafter rapidly colonized (Santos et al., 2003; Kato et al., 2004; Mata et al., 2004; Mattos-Machado et al., 2005), 5 years after the first records in Para, one of the most northerly states in Brazil (Santos et al., 2003).

Pairwise genetic distance (Nei 1972) and FST (Weir and Cockerham, 1984) indices differed significantly from zero in several populations (Table S1). About 91% of the pairwise FST values were significantly different from zero. The overall FST value was 0.414 (*p < 0.001*), and the pairwise estimates of FST ranged from 0.003 (Sud Menucci versus Paraibuna) to 1.000 (Santa Maria versus Poços de Caldas).

Genetic boundaries depicted in Est2 and Est3 data are shown in Figure 1. The first boundary (A) separated the coastal populations (Rio de Janeiro, Maresias, Ilhabela and Florianópolis), the localities in south-eastern São Paulo state (Paraibuna, Itatiba, São Paulo and Rio Claro) and northern populations (Brasilia, Jequié, Lençóis and Beberibe), from the rest. The second boundary (B) enclosed Itatiba and São Paulo, thereby isolating both populations. The last (C), isolated Florianópolis, Maresias and Ilhabela and coincided with the geological formation composed of the Serra do Mar Range. Genetic Landscape Shape Interpolation analysis (Figure 2) generated peaks indicating the greatest genetic distances in populations from São Paulo, Itatiba and other localities of south-eastern São Paulo state, decreasing from there in direction to the north and south of Brazil.

**Figure 2** - Genetic Landscape Shape Interpolation (GLSI) analysis, using a 50 x 50 grid and a distance weighting parameter of 1. A: polygonal plot of GLSI, overlaid by the map of Brazil. The peaks indicate the localization of the highest genetic variability. B: a 3-dimensional surface-plot view. The dark zones indicate areas with the highest genetic variability. The geographic coordinates of regions with the highest and lowest genetic variability are indicated in the map and 3-dimensional plotting.
Allele frequencies were employed in the relatively promising, but little used, methodologies of spatial interpolation (Storfer et al., 2007) and the Monmonier algorithm. These approaches could be especially useful in the case of continuously distributed species, by representing allele frequency across a landscape surface, and identifying putative genetic barriers. Normally, mitochondrial DNA markers have been used in these analyses (Dupanloup et al., 2002). By using mtDNA HVRI polymorphism, it was thus possible to infer the action of a past specific barrier hindering gene flow between Italian and Balkanic populations of the European roe deer. Moreover, Manni et al. (2004) suggested that the Monmonier algorithm could also be applied in the identification of barriers by using geographical patterns of genetic, morphological and linguistic variation.

The application of these approaches to our data facilitated depicting the graphic pattern of the ratio between genetic and geographic distances (pseudoslope) throughout the sampled regions, with the surface edges corresponding to the highest ratios. All the edges were located in southeastern Brazil, specifically São Paulo state, thereby indicating the higher genetic structuring of these populations, possibly due to both early origin and low gene flow. Historical data reinforce the idea of the earlier arrival of Z. indianus in São Paulo state, whereas the 2 highest peaks in the graphical surface, isolated by A and B putative barriers, as inferred by MMDA analysis, suggest population isolation. Based on these clues, analysis of genetic data reinforces the hypothesis that São Paulo state was the center from which Z. indianus spread throughout Brazil. On the other hand, the northern and southern populations presented the lowest ratios between genetic and geographic distances, as shown by depressions in the graph-surface. This landscape indicated lower genetic structuring, probably due to a later invasion. This scenario agrees with the above-cited historical records.

By identifying 3 boundaries for gene flow through MMDA analysis, a putative scenario of the spread of Z. indianus in Brazil can be visualized (Figure 1). Boundary A separates the coastal populations from the remainder, boundary B isolates the towns of São Paulo and Itatiba, both located very close to Valinhos, where Z. indianus was first observed, whereas boundary C corresponds to a natural geological barrier, the Serra do Mar, a 1500 km long mountain range extending from Espírito Santo to Santa Catarina states. These boundaries separate two of the main highways in Brazil, the BR153 and BR116. The first is an important route for commercial interchange with inland Brazil (Confederação Nacional de Transportes a), whereas the second is coastal (Confederação Nacional de Transportes b). A similar manner of diffusion, due to the fruit trade, may have occurred in the Paleartic region (Yassin et al., 2009). However, in the Americas the spread was extremely fast (about six years, from São Paulo to Florida), in contrast to the Paleartic region, where it took more than 40 years for Z. indianus to spread from India to Egypt. The great difference in the pace of spread between Brazil/USA and India/Egypt can be attributed to the more developed freeway networks in Brazil than in the Paleartic region.

These findings suggest that the spreading of Z. indianus occurred from São Paulo, the state where commercial highway traffic is the heaviest, to the north and south of Brazil by way of both the BR153 and the BR116 highways. The landscape genetics approach hereby applied for characterizing the genetic structure of populations from an initial colonizer species soon after its introduction, as well as its relevance in offering the possibility of determining the source of invasion, and demographic parameters of the species, also offers a unique opportunity for accompanying the evolutionary dynamics of the invader species over time.

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Supplementary Material
The following online material is available for this article:
Table S1 - Pairwise values of $F_{ST}$ and genetic distance between the Brazilian populations of *Zaprionus indianus*.
This material is available as part of the online article from http://www.scielo.br/gmb.

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