



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

Luís Gustavo da Conceição Galego and Claudia Marcia Aparecida Carareto

Departamento de Biologia, Universidade Estadual Paulista “Júlio de Mesquita Filho”, São José do Rio Preto, SP, Brazil.

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.

Received: March 3, 2010; Accepted: March 30, 2010.

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 (Toni *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 (Goñi *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^F and

Est2^S), the second with four (Est3¹, Est3², Est3³ and Est3⁴). 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 Beebe, 1997; Michels *et al.*, 2001; Pfenninger, 2002; Arnaud, 2003; Hirao and Kudo, 2004). Since esterases appear to be the most polymorphic loci in Brazilian *Z.*

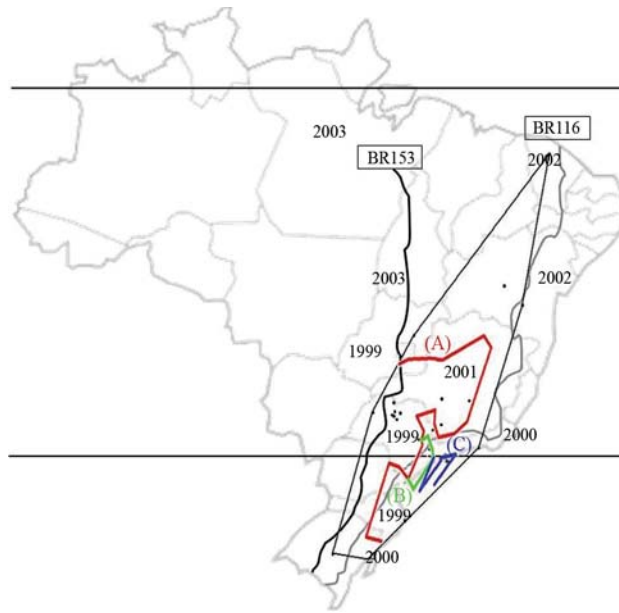


Figure 1 - Delaunay triangulation (fine black line) and genetic boundaries in heavy red (A), green (B) and blue (C) lines obtained with the Monmonier maximum difference algorithm. Note that (A) separates the coastal populations, the localities in south-eastern São Paulo state and northern populations from the rest, (B) encompasses the two south-eastern São Paulo populations, thereby isolating them, and (C) isolates coastal populations of the Brazilian south-east and south. The irregular parallel lines across the map of Brazil indicate the BR153 (black line) and BR 116 (gray line) freeways. The numbers indicate the years when *Zaprionus indianus* was first recorded in the various Brazilian states.

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 ovoposited by females from nature, were used for esterase detection.

Polyacrylamide gel electrophoresis and esterase detection

Each individual fly was macerated in 15 μ L 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 (H_O) and expected (H_E) heterozygosity, and Hardy-Weinberg equilibrium, were all estimated by TFPGA. The estimation of genetic distances (Nei, 1972) and F_{ST} 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-axis) 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 Est2^S in 8 of the 22 populations studied, and Est2^F in 3. Est2^S frequency was the lowest in Alfenas (0.09), and that of Est2^F 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 Est3³. Est3¹ frequency varied from 0 (Ilhabela) to 0.94 (Santa Maria), Est3⁴ from 0.05 (Rio Claro and Porto Alegre) to 0.89 (Ilhabela), and Est3³ 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 (Ilha-

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: Est2^S; F: Est2^F; H_O: observed heterozygosity; H_E: expected heterozygosity; ne: not evaluated. *Mattos-Machado *et al.* (2005).

Locality	Latitude	Est3						Est2			
		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
Paraibuna	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
Olímpia	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
Jequié (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 (\bar{X}) and standard-errors (SE) of observed (H_O) and expected (H_E) heterozygosity in Brazilian populations of *Zaprionus indianus* and chi-squared comparison (χ^2). ne: not evaluated.

Geographic region		Est2 ($\bar{X} \pm SE$)	Est3 ($\bar{X} \pm SE$)	χ^2
São Paulo state (SP)	H_O	0.08 \pm 0.03	0.54 \pm 0.07	5.05*
	H_E	0.15 \pm 0.06	0.58 \pm 0.05	4.18*
	χ^2	0.42	0.04	
Southern (S)	H_O	0.19 \pm 0.06	0.47 \pm 0.08	1.19
	H_E	0.21 \pm 0.07	0.52 \pm 0.07	1.25
	χ^2	0.02	0.03	
Northeast (N)	H_O	ne	0.49 \pm 0.11	ne
	H_E	ne	0.57 \pm 0.10	ne
	χ^2	ne	0.02	
Total	H_O	0.13 \pm 0.03	0.51 \pm 0.04	6.35**
	H_E	0.17 \pm 0.04	0.56 \pm 0.04	5.93*
	χ^2	0.25	0.10	
SPXS	χ^2	6.37**	1.04	
SPXN	χ^2	Ne	0.51	
SXN	χ^2	Ne	ne	

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

bela) to 0.70 (Mirassol and Ibirá). The average Est3 H_O in populations from São Paulo state (0.54 \pm 0.07), and the southern (0.47 \pm 0.08) and northern (0.49 \pm 0.11) states did not differ significantly ($X^2 = 0.10$). On the other hand, Est2 H_O ranged from 0 (11 populations) to 0.45 (Brasília), and H_E from 0 (11 populations) and 0.50 (Porto Alegre). The average Est2 H_O in populations from São Paulo state (0.08 \pm 0.03) and southern regions (0.19 \pm 0.06) were not significantly different. Almost all the populations, except those from Ibirá and Maresias, were estimated to be under Hardy-Weinberg equilibrium, with $p < 0.05$.

Pairwise genetic distance (Nei 1972) and F_{ST} (Weir and Cockerham, 1984) indices differed significantly from zero in several populations (Table S1). About 91% of the pairwise F_{ST} values were significantly different from zero. The overall F_{ST} value was 0.414 ($p < 0.001$), and the pairwise estimates of F_{ST} 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 (Brasília, 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.

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).

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 F_{ST} and Nei (1972) genetic distance values. The Est3 H_O values of the Brazilian populations of *Z. indianus* (0.54) were almost the same as the three esterase H_O 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 H_O 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.

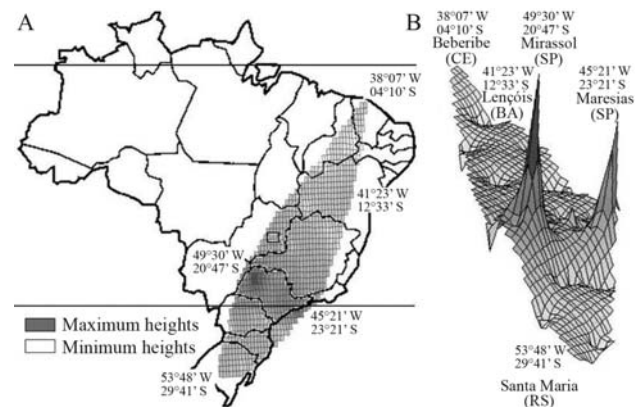


Figure 2 - Genetic Landscape Shape Interpolation (GLSI) analysis, using a 50 x 50 grid and a distance weighting parameter of 1. A: polygonal plot of GLSI, overlaid by the map of Brazil. The peaks indicate the localization of the highest genetic variability. B: a 3-dimensional surface-plot view. The dark 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 Palearctic 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 Palearctic 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 Palearctic 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.

Acknowledgments

This work was supported by FAPESP (04/05235-7) and CNPq.

References

- Ananina G, Rohde C, David JR, Valente VL and Klaczko LB (2006) Inversion polymorphism and a new polytene chromosome map of *Zaprionus indianus* Gupta (1970) (Diptera, Drosophilidae). *Genetica* 131:117-125.
- Arnaud JF (2003) Metapopulation genetic structure and migration pathways in the land snail *Helix aspersa*: Influence of landscape heterogeneity. *Landscape Ecol* 18:333-346.
- Brouns G, De Wulf A and Constaes D (2003) Delaunay triangulation algorithms useful for multibeam echosounding. *J Surv Eng* 129:79-84.
- Castro FL and Valente VLS (2001) *Zaprionus indianus* invading communities in the southern Brazilian city of Porto Alegre. *Drosophila Inf Serv* 84:15-17.
- Ceron CR, Santos JR and Campos Bicudo HEM (1992) The use of gelatin to dry cellophane wound slab gels in an embroidering hoop. *Rev Bras Genet* 15:201-203.
- David JR, Araripe LO, Bitner-Mathe BC, Capy P, Goñi B, Klaczko LB, Legout H, Martins MB, Voudibio J, Yassin A, *et al.* (2006a) Sexual dimorphism of body size and sternopleural bristle number: A comparison of geographic population of an invasive cosmopolitan drosophilid. *Genetica* 128:109-122.
- David JR, Araripe LO, Bitner-Mathe BC, Capy P, Goñi B, Klaczko LB, Legout H, Martins MB, Voudibio J, Yassin A, *et al.* (2006b) Quantitative trait analyses and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. *Heredity* 96 53-62.
- Dupanloup I, Schneider S and Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Mol Ecol* 11:2571-2581.
- Galego LGC and Carareto CMA (2007) Analysis of the drosophilid *Zaprionus indianus* introduction in Brazil: Contribution of esterase loci polymorphisms. *Drosophila Inf Serv* 90:79-84.

- Galego LGC, Ceron CR and Carareto CMA (2006) Characterization of esterases in a Brazilian population of *Zaprionus indianus* (Diptera, Drosophilidae). *Genetica* 126:89-99.
- Goñi B, Fresia P, Calviño M, Ferreira MJ, Valente VLS and Bassoda Silva L (2001) First record of *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) in southern localities of Uruguay, South America. *Drosophila Inf Serv* 84:61-65.
- Goñi B, Martinez ME, Techera G and Fresia P (2002). Increased frequencies of *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) in Uruguay. *Drosophila Inf Serv* 85:75-80.
- Guillot G, Estoup A, Mortier F and Cosson JF (2005) A spatial statistical model for landscape genetics. *Genetics* 170:1261-1280.
- Hirao AS and Kudo G (2004) Landscape genetics of alpine-snowbed plants: Comparisons along geographic and snowmelt gradients. *Heredity* 93:290-298.
- Hitchings SP and Beebe TJ (1997) Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: Implications for biodiversity conservation. *Heredity* 79:117-127.
- Holderegger R and Wagner HH (2006) A brief guide to landscape genetics. *Landscape Ecol* 21:793-796.
- Isaaks EH and Srivastava RM (1989) *An Introduction to Applied Geostatistics*. Oxford University Press, New York, 592 pp.
- Jacquemyn H (2004) Genetic structure of the forest herb *Primula elatior* in a changing landscape. *Mol Ecol* 13:211-219.
- Kato CM, Foureaux LV, César RA and Torres MP (2004) Ocorrência de *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) no estado de Minas Gerais. *Ciênc Agrotec* 28:454-455 (Abstract in English).
- Liepert S, Bialozyt R and Ziegenhage B (2002) Wind-dispersed pollen mediates postglacial gene flow among refugia. *Proc Natl Acad Sci USA* 99:14590-14594.
- Linde K, Steck GJ, Hibbard K, Birdsley JS, Alonso LM and Houle D (2006) First records of *Zaprionus indianus* (Diptera, Drosophilidae), a pest species on commercial fruits from Panama and the United States of America. *Fla Entomol* 89:402-403.
- Loh R and Bitner-Mathe BC (2005) Variability of wing size and shape in three populations of a recent Brazilian invader *Zaprionus indianus* (Diptera, Drosophilidae) from different habitats. *Genetica* 125:271-281.
- Manel S, Schwartz ML, Luikart G and Taberlet P (2003) Landscape genetics: Combining landscape ecology and population genetics. *Trends Ecol Evol* 18:189-197.
- Manni F, Guerard E and Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier's algorithm. *Hum Biol* 76:173-190.
- Mattos-Machado T, Solé-Cava AM, David JR and Bitner-Mathe BC (2005) Allozyme variability in an invasive drosophilid, *Zaprionus indianus* (Diptera, Drosophilidae): Comparison of a recently introduced Brazilian population with Old World populations. *Ann Soc Entomol Fr* 41:7-13.
- Miller MP (1997) Tool for population genetic analyses – TFGPA - 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.
- Miller MP (2005) Alleles in space (AIS): Computer software for the joint analysis of interindividual spatial and genetic information. *J Hered* 96:722-724.
- Michels E, Cottenie K, Neys L, DeGalas K, Coppin P and DeMeester L (2001) Geographical and genetic distances among zooplankton populations in a set of interconnected ponds: A plea for using GIS modeling of the effective geographical distance. *Mol Ecol* 10:1929-1938.
- Monmonier MS (1973) Maximum-difference barriers: An alternative numerical regionalization method. *Geogr Anal* 5:245-261.
- Nei M (1972) Genetic distance between populations. *Am Nat* 106:283-292.
- Parkash R and Yadav JP (1993) Geographical clinal variation at seven esterase encoding loci in Indian populations of *Zaprionus indianus*. *Hereditas* 119:161-173.
- Parkash R, Yadav JP and Vashist M (1994) Electrophoretic and cryptic genic variability in natural populations of *Zaprionus indianus*. *Proc Indian Nat Sci Acad B*60:75-82.
- Peakall R and Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288-295.
- Pfenninger M (2002) Relationship between microspatial population genetic structure and habitat heterogeneity in *Pomatia elegans* (O.F. Muller 1774) (Caenogastropoda, Pomatiidae). *Biol J Linn Soc Lond* 76:565-575.
- Poissant J, Knight TW and Ferguson MM (2005). Nonequilibrium conditions following landscape rearrangement: The relative contribution of past and current hydrological landscapes on the genetic structure of a stream-dwelling fish. *Mol Ecol* 14:1321-1331.
- Santos JF, Rieger TT, Campos SRC, Nascimento ACC, Félix PT, Silva SVO and Freitas FMR (2003) Colonization of Northeast Region of Brazil by the drosophilid flies *Drosophila malerkotliana* and *Zaprionus indianus* a new potential insect pest for Brazilian fruitculture. *Drosophila Inf Serv* 86:92-95.
- Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, Dezzani R, Delmelle E, Vierling L and Waits LP (2007) Putting the “landscape” in the landscape genetics. *Heredity* 98:128-142.
- Tidon R, Leite DF and Leão BFD (2003) Impact of the colonization of *Zaprionus indianus* (Diptera, Drosophilidae) in different ecosystems of the neotropical region: 2 years after the invasion. *Biol Conserv* 112:299-305.
- Toni DC, Hofmann PRP and Valente VLS (2001) First register of *Zaprionus indianus* (Diptera, Drosophilidae) in the state of Santa Catarina, Brazil. *Biotemas* 14:71-85.
- Vilela CR (1999) Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? *Drosophila Inf Serv* 82:37-39.
- Vilela CR, Teixeira EP and Stein CP (2000) Mosca-africano-do-figo, *Zaprionus indianus* (Diptera, Drosophilidae). In: Vilela E, Zucchi RA and Cantor F (eds) *Histórico e Impacto das Pragas Introduzidas no Brasil*. Editora Holos, São Paulo, pp 48-52.
- Watson DF (1992) *Contouring: A guide to the analysis and display of spatial data*. Pergamon Press, New York, 321 pp.
- Weir BS and Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:13.
- Yassin A, Borai F, Cappy P, David JR, Elias E, Riad SA, Shalaby HG, Serour S and Abou-Youssef AY (2009) Evolutionary genetics of *Zaprionus*. II. Mitochondrial DNA and chromosomal variation of the invasive drosophilidae *Zaprionus indianus* in Egypt. *Mitochondrial DNA* 20:34-40.

Internet Resources

- Confederação Nacional de Transportes (CNT)a, <http://www.cnt.org.br/informacoes/pesquisas/rodoviaria/2007/arquivos/pdf/ligacao057.pdf> (March 20, 2010).
- Confederação Nacional de Transportes (CNT)b, <http://www.cnt.org.br/informacoes/pesquisas/rodoviaria/2007/arquivos/pdf/ligacao048.pdf> (March 20, 2010).
- Mata RM, Kanegae MF and Tidon R (2004) Diagnóstico ambiental do Parque estadual do Jalapão mediante a análise da fauna de Drosophilídeos (Insecta, Díptera). XXV Congresso Brasileiro de Zoologia, Abstract, <http://www.zoologia.bio.br/congressos/CBZ/resumos/XXVCBZcompleto-111.html> (April 5, 2009).

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>.

Associate Editor: Fábio de Melo Sene

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table S1 - Pairwise values of F_{ST} (Weir and Cockerhan 1984), above diagonal, and genetic distance (Nei 1972), below diagonal, between the Brazilian populations of *Zaprius indianus*. Significant values are indicated in **bold** typeface. *São Paulo state*: IB (Ibirá), IL (Ilhabela), IT (Itaitiba), MA (Maresias), MI (Mirassol), OL (Olimpia), OV (Onda Verde), PF (Paulo de Faria), PR (Paraibuna); RC (Rio Claro); SJ (São José do Rio Preto); SU (Sud Mennucci) and SP (São Paulo); *other states of Southeast*: AL (Alfenas), BH (Belo Horizonte), CD (Córrego Danta), PC* (Poços de Caldas) and RJ (Rio de Janeiro); *South states*: FL (Florianópolis), PA (Porto Alegre) and SM (Santa Maria); and *North and Mideast states*: BR (Brasília), JE (Jequié), LE* (Lençóis) and BE* (Beberibe). *Machado *et al.* 2005.

	IB	IL	IT	MA	MI	OL	OV	PF	PR	RC	SJ	SU	SP	AL	BH	CD	RJ	BR	FL	PA	SM	JE	LE*	BE*	PC*
IB	****	0.226	0.460	0.015	0.099	0.767	0.086	0.458	0.013	0.039	0.797	0.006	0.425	0.727	0.105	0.798	0.033	0.511	0.100	0.232	0.117	0.013	0.704	0.581	0.752
IL	0.296	****	0.354	0.195	0.267	0.827	0.111	0.527	0.281	0.376	0.734	0.274	0.388	0.559	0.266	0.716	0.319	0.664	0.070	0.560	0.439	0.263	0.963	0.851	0.948
IT	0.272	0.299	****	0.452	0.181	0.160	0.336	0.075	0.498	0.562	0.116	0.493	0.011	0.085	0.260	0.107	0.449	0.182	0.256	0.282	0.619	0.473	0.713	0.605	0.718
MA	0.015	0.285	0.277	****	0.093	0.753	0.076	0.428	0.031	0.038	0.762	0.019	0.411	0.667	0.067	0.780	0.045	0.570	0.101	0.287	0.078	0.053	0.830	0.654	0.879
MI	0.108	0.233	0.068	0.110	****	0.382	0.117	0.148	0.115	0.128	0.394	0.105	0.142	0.352	0.023	0.411	0.087	0.287	0.111	0.130	0.176	0.126	0.703	0.530	0.749
OL	0.504	0.634	0.133	0.507	0.245	****	0.741	0.089	0.795	0.770	0.028	0.777	0.120	0.089	0.444	0.026	0.706	0.162	0.630	0.318	0.767	0.772	0.734	0.597	0.801
OV	0.060	0.118	0.179	0.059	0.068	0.449	****	0.385	0.080	0.147	0.677	0.090	0.345	0.574	0.128	0.720	0.083	0.608	0.099	0.399	0.206	0.133	0.830	0.710	0.787
PF	0.278	0.386	0.024	0.273	0.064	0.099	0.203	****	0.466	0.456	0.075	0.457	0.040	0.081	0.183	0.115	0.385	0.227	0.378	0.249	0.449	0.500	0.788	0.600	0.813
PR	0.012	0.344	0.286	0.031	0.115	0.519	0.058	0.282	****	0.023	0.817	0.003	0.464	0.765	0.122	0.846	0.009	0.569	0.160	0.267	0.094	0.035	0.704	0.590	0.722
RC	0.046	0.483	0.334	0.048	0.133	0.538	0.099	0.301	0.030	****	0.813	0.017	0.500	0.770	0.102	0.856	0.021	0.605	0.233	0.272	0.030	0.075	0.779	0.612	0.827
SJ	0.517	0.580	0.116	0.512	0.249	0.027	0.422	0.093	0.529	0.559	****	0.807	0.099	0.029	0.446	0.022	0.725	0.255	0.615	0.433	0.792	0.826	0.843	0.696	0.862
SU	0.006	0.343	0.286	0.020	0.112	0.513	0.063	0.280	0.003	0.023	0.526	****	0.452	0.749	0.105	0.828	0.014	0.551	0.146	0.249	0.083	0.028	0.718	0.588	0.754
SP	0.258	0.315	0.003	0.260	0.056	0.118	0.180	0.013	0.273	0.311	0.110	0.270	****	0.077	0.205	0.093	0.410	0.154	0.256	0.223	0.542	0.441	0.719	0.577	0.752
AL	0.464	0.462	0.067	0.447	0.199	0.070	0.351	0.060	0.483	0.521	0.038	0.478	0.064	****	0.378	0.047	0.688	0.315	0.498	0.477	0.738	0.773	0.952	0.769	0.969
BH	0.103	0.240	0.107	0.090	0.010	0.283	0.069	0.086	0.111	0.115	0.284	0.104	0.088	0.226	****	0.482	0.094	0.408	0.140	0.210	0.104	0.159	0.853	0.621	0.912
CD	0.525	0.579	0.115	0.528	0.260	0.027	0.447	0.112	0.548	0.587	0.022	0.543	0.110	0.050	0.305	****	0.772	0.172	0.572	0.382	0.867	0.800	0.768	0.650	0.819
RJ	0.038	0.212	0.217	0.045	0.058	0.436	0.034	0.203	0.027	0.038	0.444	0.050	0.203	0.402	0.056	0.469	****	0.539	0.188	0.247	0.076	0.064	0.711	0.579	0.722
BR	0.320	0.489	0.072	0.354	0.124	0.134	0.302	0.085	0.345	0.392	0.172	0.340	0.062	0.166	0.185	0.142	0.281	****	0.391	0.095	0.723	0.446	0.423	0.397	0.532
FL	0.077	0.106	0.137	0.082	0.056	0.394	0.044	0.188	0.101	0.148	0.386	0.097	0.136	0.308	0.067	0.371	0.077	0.214	****	0.265	0.328	0.104	0.739	0.628	0.786
PA	0.173	0.382	0.102	0.203	0.048	0.221	0.182	0.097	0.189	0.213	0.266	0.183	0.083	0.249	0.086	0.253	0.134	0.052	0.130	****	0.377	0.188	0.439	0.360	0.559
SM	0.211	0.748	0.434	0.170	0.209	0.624	0.182	0.368	0.185	0.097	0.637	0.171	0.402	0.610	0.167	0.687	0.109	0.533	0.254	0.343	****	0.186	0.951	0.717	1.000
JE	0.013	0.333	0.279	0.052	0.120	0.510	0.079	0.296	0.032	0.086	0.534	0.027	0.266	0.488	0.127	0.530	0.052	0.293	0.080	0.156	0.295	****	0.601	0.523	0.662
LE	0.557	0.735	0.448	0.601	0.439	0.572	0.541	0.467	0.560	0.617	0.597	0.566	0.445	0.601	0.499	0.588	0.516	0.418	0.514	0.403	0.757	0.533	****	0.077	0.035
BE	0.514	0.672	0.408	0.540	0.390	0.523	0.494	0.412	0.518	0.554	0.543	0.519	0.400	0.535	0.432	0.541	0.470	0.406	0.470	0.377	0.667	0.504	0.516	****	0.148
PC	0.571	0.736	0.451	0.615	0.450	0.590	0.534	0.474	0.566	0.631	0.603	0.577	0.454	0.608	0.513	0.603	0.521	0.452	0.527	0.437	0.771	0.552	0.508	0.528	****