Allozyme variability in an invasive drosophilid, *Zaprionus indianus* (Diptera: Drosophilidae): comparison of a recently introduced Brazilian population with Old World populations

Taissa de MATTOS MACHADO [1], Antonio M. SOLÉ-CAVA [2], Jean R. DAVID [3] & Blanche C. BITNER-MATHÉ [1]*

(1) Laboratório de Genética de Populações de *Drosophila*, Departamento de Genética, Universidade Federal do Rio de Janeiro, Bloco A, CCS, Ilha do Fundão. 21941-490, Rio de Janeiro, Brazil.
(2) Laboratório de Biodiversidade Molecular, Departamento de Genética, Universidade Federal do Rio de Janeiro, Brazil.
(3) Laboratoire Populations, Génétique et Evolution, Centre National de la Recherche Scientifique (CNRS), France.

Abstract. Colonizing species often go through genetic bottlenecks when new territories are invaded. The South American continent has been recently colonized by a generalist African drosophilid, *Zaprionus indianus*, which has become an agricultural pest in Brazil in the last five years. In this paper we used allozyme electrophoresis to estimate levels of genetic differentiation of *Z. indianus* collected from sites 4 300 km apart in Brazil. We also compared the level of polymorphism of the Brazilian populations with that found in laboratory strains from Africa and Asia, to verify if a significant decrease in gene variability has taken place during the invasion process. The populations were polymorphic for three out of the 11 loci investigated. Genetic distances and F_{ST} indices among Brazilian populations were small and generally non significant, suggesting a colonization from one single propagule followed by a rapid demographic expansion. Ancestral and old populations from Africa and Asia were slightly more heterozygous than those from Brazil. Compared to other drosophilids, *Z. indianus* appears to be characterized by a low proportion (25%) of polymorphic loci. We suggest that the propagule introduced to Brazil had a sufficient size to carry almost all the polymorphism from the (unknown) origin population, although not the precise allelic frequencies.

Résumé. **La variabilité des allozymes chez un drosophilide invasif, *Zaprionus indianus* (Diptera: Drosophilidae): comparaison d'une population brésilienne récemment introduite avec les populations de l'Ancien Monde**. Les espèces colonisantes passent souvent par des goulets d'étranglement lorsque de nouveaux territoires sont envahis. Le continent sud-américain a été récemment colonisé par un drosophilide généraliste africain, *Zaprionus indianus*, qui est devenu une nuisance pour l'agriculture au Brésil au cours des 5 dernières années. Dans cet article, nous avons utilisé l'électrophorèse des alloenzymes pour estimer le niveau de différenciation de populations de *Z. indianus* récoltées dans des sites brésiliens dont les plus éloignés sont distants de 4 300 km. Nous avons aussi comparé le polymorphisme des populations brésiliennes avec celui trouvé dans des souches de laboratoire de *Z. indianus* récoltées en Afrique et en Asie, afin de savoir si une diminution de la variabilité génétique a eu lieu lors de la colonisation. Les populations ont été trouvées polymorphes pour trois des 11 locus analysés. Entre les populations brésiliennes, les distances génétiques et les indices F_{ST} se sont avérés petits et généralement non significatifs, suggérant une colonisation par une seule propagule, suivie par une expansion démographique rapide. Les populations, ancestrales et anciennes, de l'Afrique et de l'Asie ont été trouvées, en moyenne, plus hétérozygotes que celles du Brésil. Comparé aux autres drosophilides, *Z. indianus* semble caractérisé par une faible proportion (25%) de locus polymorphes. Nous suggérons que la propagule, qui a été introduite au Brésil, avait une taille suffisante pour apporter pratiquement tout le polymorphisme qui était présent dans la population (inconnue) d'origine, bien que les fréquences alleliques n'aient pas été conservées.

The intense international commerce of agricultural goods has resulted in the spreading of many insect species across their natural dispersal boundaries. The organisms that survive the transportation from their original ecosystems and get established in new habitats can be a threat to endemic species and may become devastating agricultural pests (Vermeij 1996; Kolar & Lodge 2001).

Invasive species have particular genetic responses to the evolutionary forces related to the impact of the

* Corresponding author. E-mail : bcbitner@biologia.ufrj.br
Accepté le 25/10/2004.

foundation event. These characteristics make the evolutionary study of these populations very interesting (Holland 2000; Huey *et al.* 2000; Pascual *et al.* 2001). Furthermore, the genetic characterization of species is very important for the determination of invasion sources and demographic parameters (Carey 1991; Davies *et al.* 1999).

The genus *Zaprionus* Coquillet 1901 has currently 2 subgenera and more than 40 species recognized (Chassagnard & Kraaijeveld 1991). *Zaprionus indianus* Gupta 1970 is a drosophilid native to tropical Africa, which has spread through many countries in Asia and South America. Probably, this range expansion was made possible by the association of a high adaptability to new environments (by selection or ecological plasticity) and the widespread routes of fruit transportation.

Invasive species of drosophilids usually present a high ecological versatility (Brncic & Budnik 1987; Parsons 1987; David & Tsacas 1981). *Z. indianus* has been reared from many fruits types (Vilela *et al.* 2001), which indicates that it is ecologically very versatile. The first occurrence of this species in Brazil was observed in 1999 (Vilela 1999), in the city of Santa Isabel, state of São Paulo. At the time of this first observation, *Z. indianus* was detected breeding in immature fruits of figs (*Ficus carica* L.; Moraceae) and was responsible for the loss of 40% of the fig harvest in that year in the Valinhos area (São Paulo), one of the main fig producers in Brazil (Stein *et al.* 1999). The capability to breed in immature fruits might give this species a dispersal advantage when compared to other drosophilids that only breed on decomposing fruits (Stein *et al.* 1999; Vilela 2001). *Z. indianus* was subsequently observed throughout Brazil (Vilela 2001; Tidon *et al.* 2003; Toni *et al.* 2001), Argentina (Misiones, 27° S, 55° W; F.J. Krsticevic, personal comunication) and Uruguay (Goñi *et al.* 2001).

Only on very rare occasions it is possible to follow the changes in genetic composition of invading populations from the beginning of their introduction, as was the case of the *D. subobscura* Collin 1936 introduction to the American Continent (Ayala *et al.* 1989; Balanya *et al.* 1994; Pascual *et al.* 2001). The goal of this work was to characterize the genetic composition of distant populations of *Zaprionus indianus* in Brazil, only two years after its first observation in Brazil, using the classical technique of allozyme polymorphism (Ayala 1972; David 1982; Powell 1997).

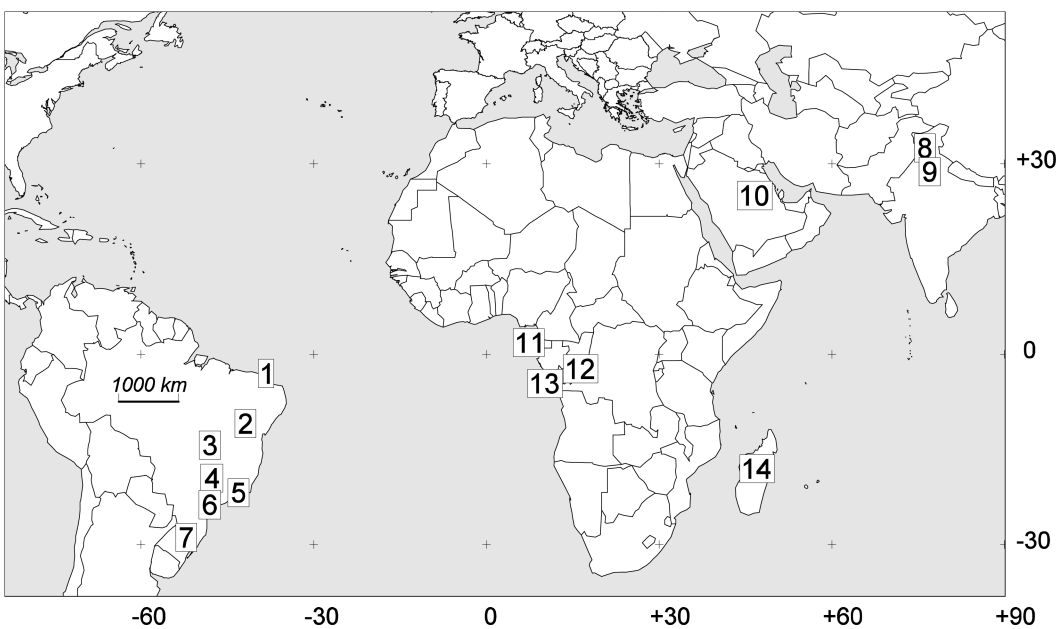

Figure 1
Partial map of world showing the location of sampling sites. 1, Beberibe (CE, Brazil, 4°10' S 38°07' W). 2, Lençóis (BA, Brazil, 12° 33' S 41° 23' W). 3, Brasília (DF, Brazil, 15° 46' S 47° 55' W). 4, Poços de Caldas (MG, Brazil, 21° 47' S 46° 33' W). 5, Rio de Janeiro (RJ, Brazil, 22° 54' S 43° 12' W). 6, São Paulo (SP, Brazil, 23° 31' S 46° 50' W). 7, Porto Alegre (RS, Brazil, 30° 01'S 51° 13' W). 8, Chandigarh (CH, India, 30° 44' N 76° 45' E). 9, Delhi (DH, India, 28° 37' N 77° 10' E). 10, Riyadh (SA, Saudi Arabia, 24° 38' N 46° 43' E). 11, São Tomé (ST, 1° 00' N 7° 00' E). 12, Brazzaville (BZ, Congo, 4° 16' S 15° 17' E). 13, Pointe Noire (PN, Congo, 4° 48' S 11° 51' E). 14, Madagascar (MA, 18° 55' S 47° 31' E).

Material and methods

1. Collection of samples

Population samples (fig. 1) were obtained, between March/2001 and July/2002, in the cities of Rio de Janeiro (RJ, Brazil, 22° 54' S 43° 12' W), São Paulo (SP, Brazil, 23° 31' S 46° 50' W), Poços de Caldas (MG, Brazil, 21°47'S 46°33'W), Lençóis (BA, Brazil, 12° 33' S 41° 23' W), Beberibe (CE, Brazil, 4° 10' S 38° 07' W), Brasília (DF, Brazil, 15° 46' S 47° 55' W) and Porto Alegre (RS, Brazil, 30° 01' S 51° 13' W). After collection, the individuals were preserved in liquid nitrogen until required for electrophoresis.

The samples from African and Asiatic populations were obtained from laboratory lines founded in pool and maintained in population bottles in the laboratory for several years. These samples were obtained from collections in the cities of Chandigarh (CH, India, 30° 44' N 76° 45' E), Delhi (DH, India, 28° 37' N 77° 10' E), Riyadh (SA, Saudi Arabia, 24° 38' N 46° 43' E), Pointe Noire (PN, Congo, 4° 48' S 11° 51' E), Brazzaville (BZ, Congo, 4° 16' S 15° 17' E), and in the islands of Madagascar (MA, 18° 55' S 47° 31' E) and São Tomé (ST, 1° 00' N 7° 00' E).

2. Electrophoresis and data analysis:

Individual flies were homogenized with no more than an equal volume of distilled water and analyzed by horizontal 12.5% starch gel electrophoresis, using a Tris-citrate, pH 8.0 buffer (0.25 Tris, 0.06 M citric acid; Ward & Bearmore 1977; for futher details see Solé-Cava *et al.* 1985). Fifteen enzyme systems were assayed, of which ten, coding for 11 *loci*, gave reproducible results: alpha-glycerophosphate dehydrogenase (α-*Gpd*), Acid phosphatase (*Acp-2*), alpha-esterases (*Est-3*), Hexokinases (*Hk-1*, *Hk-2*), Isocitrate dehydrogenase (*Idh*), Malate dehydrogenase (*Mdh*), Malic enzyme (*Me-1*), Phosphogluconate dehydrogenase (*Pgd*), Phosphoglucose isomerase (*Pgi*) and Phosphoglucomutase (*Pgm-2*).

Allozyme data were analyzed using the Genetix programme (Bellkir *et al.* 1996). Genotype frequencies were used to estimate gene frequencies, fits to Hardy-Weinberg equilibrium (Exact test with Bonferroni transformation of significant levels for multiple tests, Lessios 1992), and to estimate levels of genetic polymorphism (heterozygosity (*H*), percentage of polymorphic loci (*P*), mean number of allele per locus of each population (*A*).

Pairwise genetic distances (*D*) (Nei 1978) and F_{ST} (Weir & Cockerman 1984) were used to analyze the genetic divergence among the Brazilian populations. Confidence levels of those measurements were obtained through statistical ressampling via Monte Carlo simulations.

RESULTS

1. Gene frequencies and genetic variability of populations:

Of the 11 *loci* analyzed, 6 were monomorphic for all populations: α-*Gpd, Mdh, Pgi, Pgd, Me-1*and *Hk-1.* The allele frequencies per locus for each of the polymorphic loci, the mean observed (H_O) and expected (H_E) heterozygosities and the percentage of polymorphic loci of each population are given in Tables 1 and 2 for Brazilian populations (*Acp-2, Est-3, Pgm-2, Hk-2, Idh*) and for Asian and African populations (*Acp-2, Est-3, Pgm-2*), respectively. Mean expected heterozygosities (H_E) of Brazilian populations ranged from 0.043 in Lençóis (BA) to 0.091 in Poços de Caldas (MG). No significant deviations from Hardy-Weinberg expectations were observed. H_E in non-Brazilian populations ranged from 0.044 in Delhi (DH) to 0.159 in Pointe Noire (PN).

Table 3 shows the pairwise genetic distances and F_{ST} between Brazilian populations. Only five F_{ST} values were significantly different from zero. The highest genetic differentiation (F_{ST} = 0.105) was found between the populations from Poços de Caldas (MG) and Brasília (DF). There was no correlation with geographic distance.

As seen in Table 2, variations among laboratory strains from Asia and Africa were pronounced. For example, at the *Est-3* locus, 3 populations were monomorphic, but one for the allele C and one for D. We consider that such major difference reflect a genetic drift in the laboratory, so that calculating F_{ST} indices would be meaningless.

When large populations are split into several small ones, inbreeding and drift make levels of gene variation decrease in each sub-population. However, those populations evolve approximately as a Markov chain, so that the mean gene frequencies, considering all sub-populations, remain roughly the same. We thus considered that the best possible approximation of the polymorphism in Africa and Asia would be obtained by pooling the data of Pointe Noire, Brazzaville, São Tomé and Madagascar on the one hand and those of Chandigarh, Delhi and Riyadh on the other. For each group, we estimated allele frequencies and heterozygosities taking into account the arithmetic means (to diminish the effects of unequal sampling) of the allele frequencies of the populations of each group. The same procedure was applied to the Brazilian populations.

The pooled sample of Brazil had only half the variability of the African or Asian samples (Table 4). Furthermore, we observed important changes in the frequency of some alleles in the sample of Brazilian population compared to the other two groups (Table 1 and 2). For example, the frequencies of the allele A in the *Acp-2* locus, the allele D in the *Est-3* locus and the alleles C and D in the *Pmg-2* locus ranged from zero to 0.052 in the pooled Brazilian population. Conversely, the frequency of these alleles ranged from 0.125 to 0.379 in the sample of Asia and from 0.227 to 0.403 in that from Africa.

In the *Hk-2* and *Idh* loci, the Brazilian population showed alleles that were not present in the Asian or

Table 1. Allele frequencies of polymorphic loci analyzed in Brazilian populations of *Z. indianus*. *n* is the number of individuals sampled. H_O and H_E, direct count and Hardy-Weinberg expected mean heterozygosities, respectively (standard deviation values given between brackets). *A* is the mean number of alleles per locus, and *P* is the percentage of polymorphic loci for each population.

Loci		R J	SP	BA	MG	DF	RS	CE	**Total**
Acp-2	(*n*)	24	20	14	18	48	27	8	**159**
	A	0.042	0.025	0.000	0.028	0.083	0.111	0.000	**0.041**
	B	0.958	0.975	1.000	0.972	0.917	0.889	1.000	**0.959**
Est-3	(*n*)	28	18	22	19	27	40	20	**174**
	A	0.054	0.000	0.000	0.000	0.000	0.000	0.175	**0.033**
	B	0.643	0.500	0.614	0.500	0.722	0.650	0.425	**0.579**
	C	0.232	0.500	0.386	0.500	0.259	0.225	0.250	**0.336**
	D	0.071	0.000	0.000	0.000	0.019	0.125	0.150	**0.052**
Pgm-2	(*n*)	24	20	23	26	59	29	18	**199**
	A	0.000	0.000	0.000	0.000	0.000	0.017	0.000	**0.002**
	B	1.000	1.000	1.000	1.000	0.975	0.983	1.000	**0.994**
	C	0.000	0.000	0.000	0.000	0.025	0.000	0.000	**0.004**
	D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Hk-2	(*n*)	25	20	19	25	47	25	20	**181**
	A	0.000	0.000	0.000	0.200	0.011	0.000	0.000	**0.030**
	B	0.000	0.000	0.000	0.060	0.000	0.000	0.000	**0.009**
	C	1.000	1.000	1.000	0.740	0.989	1.000	1.000	**0.961**
Idh	(*n*)	25	20	20	27	49	25	20	**186**
	A	0.000	0.000	0.000	0.019	0.000	0.000	0.000	**0.003**
	B	1.000	1.000	1.000	0.981	1.000	1.000	1.000	**0.997**
H_O		0.043	0.045	0.054	0.077	0.065	0.062	0.041	**0.055**
		(0.119)	(0.133)	(0.178)	(0.155)	(0.147)	(0.137)	(0.136)	**(0.013)**
He		0.055	0.050	0.043	0.091	0.058	0.067	0.064	**0.065**
		(0.158)	(0.150)	(0.143)	(0.182)	(0.126)	(0.159)	(0.212)	**(0.163)**
A		1.4	1.2	1.1	1.5	1.5	1.4	1.3	**1.3**
P		18.2	18.2	9.11	36.4	27.3	27.3	9.11	**27.3**

African samples. However, in both cases the alleles were very rare (0.003 to 0.030), so that their absence in the non-Brazilian samples might be just a sampling artifact, caused by the more intensive sampling in Brazil (mean sample size, n = 180) than in the other two areas (Africa; n = 57; Asia; n = 40; Table 4).

DISCUSSION AND CONCLUSIONS

Native to tropical Africa, *Z. indianus* has extended its continental geographic range twice, first to the Indian subcontinent (Gupta 1970) and then, very recently (Vilela 1999), to South America. The date of the Indian colonization is not known but presumably it is not very recent since, after their latitudinal spread, the Indian populations seem to have had enough evolutionary time to establish clines for several morphometric traits (Karan *et al.* 2000). Genetic data from *D. subobscura* show that more than 10 years can be necessary to establish a significant latitudinal cline in body size (Huey *et al.* 2000).

The date of arrival of *Z. indianus* in Brazil (1998) is well documented, as well as its rapid appearance in many states (Vilela 1999; Toni *et al.* 2001; Tidon *et al.* 2003). Our samples, collected in 2002 in very distant localities and between 4 and 30 degrees of latitude South, are genetically very similar. When genetic distance and F_{ST} indices are calculated among Brazilian populations (table 3) few are significant, and likely a consequence of founder effect and genetic drift in natural populations. In other words, we consider that our data suggest a single introduction in Brazil, followed by an extremely

Table 2. Allele frequencies of polymorphic loci analyzed from African and Asiatic samples of *Z. indianus*. *n* is the number of individuals sampled. H_O and H_E, direct count and Hardy-Weinberg expected mean heterozygosities, respectively (standard deviation values given between brackets). *A* is the mean number of alleles per locus, and P is the percentage of polymorphic loci for each population.

Loci		CH	DH	SA	**Asia Total**	PN	ST	MA	BZ	**África Total**
Acp-2	(*n*)	10	19	8	**37**	16	11	19	9	**55**
	A	0.000	0.000	0.375	**0.125**	0.719	0.364	0.105	0.222	**0.353**
	B	1.000	1.000	0.625	**0.875**	0.281	0.636	0.895	0.778	**0.647**
Est-3	(*n*)	10	28	6	**44**	18	14	17	6	**55**
	A	0.000	0.000	0.000	**0.000**	0.194	0.000	0.147	0.000	**0.074**
	B	0.000	0.000	0.500	**0.167**	0.417	0.464	0.235	0.000	**0.285**
	C	1.000	0.000	0.417	**0.472**	0.306	0.393	0.235	0.000	**0.238**
	D	0.000	1.000	0.083	**0.361**	0.083	0.143	0.383	1.000	**0.403**
Pgm-2	(*n*)	10	17	10	**37**	14	17	17	13	**61**
	A	0.250	0.000	0.000	**0.083**	0.036	0.000	0.000	0.000	**0.009**
	B	0.000	0.412	0.800	**0.404**	0.214	0.559	0.941	0.115	**0.457**
	C	0.550	0.588	0.000	**0.379**	0.286	0.382	0.059	0.500	**0.307**
	D	0.200	0.000	0.200	**0.133**	0.464	0.059	0.000	0.385	**0.227**
H_O		0.027	0.011	0.132	**0.057**	0.083	0.102	0.052	0.042	**0.070**
		(0.090)	(0.035)	(0.257)	**(0.066)**	(0.148)	(0.205)	(0.142)	(0.139)	**(0.028)**
H_E		0.054	0.044	0.123	**0.137**	0.159	0.146	0.093	0.085	**0.163**
		(0.179)	(0.146)	(0.219)	**(0.259)**	(0.281)	(0.253)	(0.218)	(0.197)	**(0.285)**
A		1.2	1.1	1.4	**1.2**	1.6	1.4	1.4	1.3	**1.4**
P		9.1	9.1	27.3	**27.3**	27.3	27.3	27.3	18.2	**27.3**

rapid geographic expansion. Such a scenario is not a rule for all invasive species. For example, in the case of *D. melanogaster* Meigen 1830, North America was probably colonized at least twice by genetically different populations (David & Capy 1988).

The comparison of the Brazilian population with ancestral African and old Asiatic populations was initially difficult since only laboratory mass cultures were available. These cultures, kept sometimes for more than 5 years in the laboratory, certainly underwent inbreeding and genetic drift. This is clear if we consider, for example, the reduced polymorphism of *Acp-2* and *Est-3 loci* in Asia (Table 2). This was, at least partially, overcome by the use of the average gene frequencies from samples of each region to estimate a mean level of polymorphism on each continent. In those pooled samples, we found only 3 polymorphic *loci*. Rare alleles at the *Hk-2* and *Idh* loci, observed in Brazilian populations, might have been lost due to genetic drift. The number of polymorphic loci found in the Brazilian populations was also three, using the 99% rule (Powell 1997).

The average numbers of alleles per locus in Asia and Africa (1.4 and 1.2 respectively) were the same as in Brazil (1.3) but the average heterozygosities were higher in the Old World pooled samples (0.137 and 0.163 versus 0.065). Morphological data (unpublished results) indicate that the Brazilian population has probably originated from high latitude in Africa, but the genetic data are not informative concerning the precise origin of the invaders, and other traits and populations should be investigated for that purpose.

Finally, we may compare the level of polymorphism of *Z. indianus* to that found in other drosophilid species. Among the 38 species listed in a recent review on drosophilid gene variation (Powell 1997), 21 had fewer than 50% polymorphic loci and only four had levels of polymorphism lower than 30%. To the species list of Powell (1997), we may still add *D. sechellia* Tsacas & Bächli 1981, a specialized species endemic to the Seychelles (Cariou *et al*. 1990). This species is remarkable by its low proportion of polymorphic loci (9%) and low heterozygosity (0.027). These features are probably related to its very small population size and have been confirmed through the analysis of microsatellite loci (Harr *et al.* 1998).

Z. indianus apparently belongs to the group of drosophilids with low polymorphism, with a mean percentage of polymorphic loci of 27.3% This propor-

Table 3. Pairwise values of F_{ST} (Weir and Cockerman, 1984) (above diagonal) and unbiased genetic distance (Nei, 1978) (below diagonal) between Brazilian populations of *Zaprionus indianus*. Significant values are indicated in **bold** typeface.

Population	1	2	3	4	5	6	7
1- CE	****	0.059	0.071	**0.087**	0.023	0.054	0.042
2 - BA	**0.004**	****	0.017	0.059	0.010	0.001	0.031
3 - DF	**0.006**	0.001	****	**0.105**	– 0.005	0.066	– 0.001
4 - MG	**0.009**	0.005	**0.009**	****	**0.093**	0.037	**0.098**
5 - RJ	0.002	0.001	0.000	**0.009**	****	0.054	– 0.007
6 - SP	0.004	0.000	0.004	0.003	0.004	****	**0.068**
7 - RS	**0.004**	0.002	0.000	**0.010**	0.000	**0.005**	****

tion becomes even smaller (25%) if we include the *Adh* (alcohol dehydrogenase) locus, which was not analysed in the Brazilian populations but which was found to be monomorphic in Old World populations (J.R. David, unpublished). Recently, Harry *et al*. (1999) observed that the distribution of *Z. tuberculatus* Malloch 1932 is extending in the Middle East. In this species, among 18 allozyme loci investigated, seven (39%) were found to be polymorphic, again a fairly low proportion for a drosophilid. Such a low level of polymorphism found in *Zaprionus* is unexpected and quite surprising for two species that are widespread and abundant across tropical Africa (Tsacas *et al.* 1981) and needs to be confirmed. From ecological observations, we would expect African populations to have very large effective population sizes, and, hence, high levels of polymorphism. Another invasive cosmopolitan species, *D. kikkawai* Burla 1954 also seems to have a low polymorphism. Ancestral populations of *D. kikkawai* from India were investigated for allozyme loci and most of them were found to be monomorphic (J.R. David and P. Gibert, unpublished). A high level of genetic polymorphism does not appear to be a prerequisite for becoming a successful invader. On the other hand, a low polymorphism in a very abundant and widespread species is difficult to explain and, in this respect, deserves further investigations with other genetic markers.

Table 4. Mean Hardy-Weinberg expected heterozygosities for pooled gene frequency data (unweighted means) for the three macro-geographic regions of Brazil, Asia and Africa. n = mean sample size over the 5 loci analyzed for each region.

Locus	Brazil	Asia	Africa
Acp-2	0.079	0.219	0.457
Est-3	0.548	0.619	0.69
Pgm-2	0.012	0.668	0.645
HK-2	0.076	0.000	0.000
Idh	0.006	0.000	0.000
Mean *H*	**0.065**	**0.137**	**0.163**
Mean n	180	39.6	56.6

Acknowledgments – We are grateful to C. Lazosky for helping with interpretation of gels; R Tidon, V. Valente-Gaiesky, L. Futuro and D. Lachaise for samples. This study was supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Comité français d'Évaluation de la Coopération Universitaire avec le Brésil (COFECUB). This is paper n° 2 of a French-Brazilian cooperation program on *Zaprionus*.

REFERENCES

Ayala F.J., Powel R., Tracey M.L., Mourao C.A., PerezSal S. 1972. Enzyme variability in the *Drosophila-willistoni* group: 4. Genic variation in natural populations of *Drosophila willistoni*. *Genetics* **70**: 113-139.

Ayala F.J., Serra L., Prevosti A. 1989. A Grand experiment in evolution: the *Drosophila subobscura* colonization of the Americas. *Genome* **31**: 246-255.

Balanya J., Segarra C., Prevosti A., Serra L. 1994. Colonization of America by *Drosophila subobscura*: The founder event and a rapid expansion. *Journal of Heredity* **85**: 427-432.

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F. 1996. *GENETIX 4.04, Logiciel sous Windows pour la génétique des populations*. Montpellier, France.

Brncic D., Budnik M. 1987. Some interactions of colonizing species *Drosophila subobscura* with local *Drosophila* fauna in Chile. *Genét. Ibér.* **39**: 249-267.

Carey J.R. 1991. Establishment of the Mediterranean fruit fly in California. *Science* **253**: 1369-1373.

Cariou M.L., Solignac M., Monnerot M., David J.R. 1990. Low allozyme and mtDNA variability in the island endemic species *Drosophila sechellia* (*Drosophila melanogaster* complex). *Experientia* **46**: 103-104.

Chassagnard M., Kraaijeveld A.R. 1991. The occurrence of *Zaprionus sensu stricto* in the paleartic region (Diptera: *Drosophilidae*). *Annales de la Société Entomologique de France (n.s.)* **27**: 495-496.

David J.R. 1982. Latitudinal variability of *Drosophila melanogaster*: Allozyme frequencies divergence between European and Afrotropical populations. *Biochem. Genet*. **20**: 747-761.

David J.R., Capy P. 1988. Genetic variation of *Drosophila melanogaster* natural populations. *Trends Genet*. **4**: 106-111.

David J.R., Tsacas J. 1981. Cosmopolitan, subcosmopolitan, and widespread species: Different strategies within the *Drosophila* family. *C. R. Soc. Biogeog*. **27**: 11-26.

Davies N., Vilabllanca F.X., Roderick G.K. 1999. Bioinvasions of the Medfly *Ceratits capitata*: source estimation using DNA sequences at multiple intron loci. *Genetics* **153**: 351-360.

Goñi B., Fresia P., Calviño M., Ferreiro M.J., Valente V.L.S., Silva L. 2001. First record of *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) in southern localities of Uruguay, South America. *Drosophila Information Service* **84**: 61-65.

Gupta J.P. 1970. Description of a new species of *Phorticella zaprionus* (Drosophilidae) from India. *Proc. Ind. Natl. Sci. Acad.* 36: 62.

Harr B., Weiss S., David J.R., Brehm G., Schlotterer C. 1998. A microsatellite-based multilocus phylogeny of the *Drosophila melanogaster* species complex. *Cur. Biol.* **8**: 1183-1186.

Harry M., Rashkovetsky E., Pavlicek T., Baker S., Derzhavets E.M., Capy P., Cariou M.L., Lachaise D, Asada N., Nevo E. 1999. Fine-scale biodiversity of Drosophilidae in "Evolution Canyon" at the Lower Nahal Oren microsite. *Israel. Biologia* **54**: 685-705.

Holland B.S. 2000. Genetics of marine bioinvasions. p. 63-71 *in*: **Solé-Cava A.M., Russo C.A.M., Thorpe J.P** (eds.), *Marine genetics*. Kluwer Academic Publishers, Dordrecht.

Huey R.B., Gilchrist G.W., Carlson M.L., Berrigan D., Serra L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**: 308-309.

Karan D., Dubey S., Moreateau B., Parkash R., David J. R. 2000. Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus. Genetica* **108**: 91-100.

Kolar C.S, Lodge D.M. 2001. Progress in invasion biology: predicting invaders. *Trends Ecol. Evol.* **16**: 199-204.

Lessios H.A. 1992. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Mar. Biol*. **112**: 517-523.

Nei M. 1978. Estimation of heterozygosity from a small number of individuals. *Genetics* **89**: 583-590.

Parsons P.A. 1987. Features of colonizing animals: phenotypes and genotypes. p. 133-154 *in*: **Gray A.J., Crawley M.J., Edwards P.J.** (eds.), *Colonization, Succession and Stability*. Blackwell Scientific Publications, Oxford.

Pascual M., Aquadro C.F., Soto V., Serra, L. 2001. Microsatellite variation in colonizing and paleartic populations of *Drosophila subobscura. Mol. Biol. Evol*. **18**: 731-740.

Powell J.R. 1997. *Progress and prospects in Evolutionary Biology: the Drosophila model*. Oxford University press, New York, 562 p.

Solé-Cava A.M., Thorpe J.P., Kaye J.G. 1985. Reproductive isolation with little genetic divergence between *Urticina* (= *Tealia*) *felina* and *U. eques* (Antozoa; Actiniaria). *Mar. Biol*. 85: 279-284.

Stein C.P., Texeira E.P., Novo J.P.S. 1999. Mosca do Figo – *Zaprionus indianus*. [online]. http://www.iac.br/~cenfit/artigos/zaprionus

Tidon R., Leite D.F., Leão B.F.D. 2003. Impact of the colonization of *Zaprionus* (Diptera, Drosophilidae) in different ecosystems of the Neotropical Region: 2 years after the invasion. *Biol. Cons.* **112**: 299-305.

Toni D.C., Hofmann P.R.P., Valente V.L.S. 2001. First register of *Zaprionus indianus* (Diptera; Drosophilidae) in the state of Santa Catarina. *Biotemas* **14**: 71-85.

Tsacas L., Lachaise D., David J.R. 1981. Composition and biogeography of the Afrotropical drosophilid fauna. *In*: **Ashburner M., Carson H. L. & Tompson Jr.** (eds.), *The Genetics and Biology of Drosophila,* Vol. 3(a) p. 197-259. Academic Press, New York.

Vermeij G.J. 1996. An agenda for invasion biology. *Biol. Cons.* **78**: 3-9.

Vilela C.R. 1999. Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region? *Drosophila Information Service* **82**: 37-39.

Vilela C.R., Texeira E.P., Stein C.P. 2001. Mosca-Africana-do-Figo, *Zaprionus indianus* (Diptera: Drosophilidae), p. 48-52 *in*: Vilela E., Zucchi R.A., Cantor F. (eds.), *Histórico e Impacto das Pragas Introduzidas no Brasil*. Editora Holos, São Paulo.

Ward R.D., Beardmore J.A. 1977. Protein variation in the plaice (*Pleuronectes platessa*). *Gen. Res* **3**: 45-62.

Weir B.S., Cockerman C.C. 1984. Estimating F statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.