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ALLOZYMIC POLYMORPHISM IN *AEDES AEGYPTI* POPULATIONS FROM ARGENTINA

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ABSTRACT. In Argentina, reinfestation with *Aedes aegypti* was registered in 1986. At present, the mosquito is well established in 10 provinces, from Buenos Aires up to the country's northern frontiers. This paper presents estimates of genetic variability in *Ae. aegypti* populations from central Argentina and determinations of genetic distances among them. We analyzed allozymic frequencies at 11 loci in samples from 3 localities. The proportion of polymorphic loci varied between 27.3 and 63.6. Expected mean heterozygosity ranged from 0.090 to 0.161 and Rogers' similarity among samples ranged between 0.909 and 0.958. The lack of relationship between genetic and geographic distances is in agreement with a recent colonization of the studied area. The mean Wright's coefficient F_{ST} value (0.065) indicates low levels of genetic differentiation among populations from different localities. Given the recent reinfestation with this mosquito in Argentina, the high levels of polymorphism found could indicate multiple introductions of representative samples from genetically different subpopulations.

KEY WORDS *Aedes aegypti*, allozymic polymorphism, heterozygosity, genetic differentiation, dispersal

INTRODUCTION

In the Americas, the introduced species *Aedes aegypti* (L.) is the primary vector of viruses causing urban yellow fever and dengue in humans. By the beginning of this century, *Ae. aegypti* was found from the southern USA to Buenos Aires, Argentina. In 1965, 17 out of 49 countries in the Americas reported the eradication of the mosquito. However, because of economical, political, and technical problems, most of these countries suffered reinfestation (Nelson 1986).

In Argentina, reinfestation started in 1986, in Misiones Province. At present, the mosquito is well established in 10 provinces, from Buenos Aires to the northern frontiers of the country (Boffi 1998). Buenos Aires Province was reinfested in 1991 and the Federal District was reinfested in 1995. High infestation levels in the autumn (35% in 1996 and 18% in 1997) have been found in houses in Buenos Aires Province and the Federal District (Boffi 1998, Schweigmann and Boffi 1998). Reappearance of *Ae. aegypti* in Córdoba Province was 1st detected in 1995 (Avilés et al. 1997).

Aedes aegypti aegypti is a domestic subspecies strongly associated with human habitats. Eggs are laid mainly in artificial containers, rarely situated more than 100 m from human dwellings. The capacity of eggs to resist desiccation for long periods is the principal obstacle for mosquito control. This property allows reinfestation from eggs preserved in dry sites, including those localities from which larvae and adults have been eliminated (Forattini 1965).

Evidence indicating that variation in vector competence among different populations is genetically determined (Aitken et al. 1977) has encouraged studies on intraspecific polymorphism in this mosquito. Knowledge of geographic patterns of genetic variability can provide "markers" to identify possible biotypes or subspecies and their relationship with ecological, behavioral, or other attributes of epidemiologic relevance.

Tabachnick et al. (1979) analyzed, using electrophoretic zymograms, polymorphism in both domestic and sylvan forms of *Ae. aegypti* populations from Asia, Africa, and the Americas. Genetic distance between these forms suggests that gene flow is restricted, probably because of the existence of different genotypes related to habitat preferences. Moreover, samples from human housing separated by 1 km or less showed significant differences in allele frequencies, indicating restricted gene flow.

Tabachnick and Powell (1979) and Powell et al. (1980) studied polymorphism at 22 enzyme-coding loci in 34 populations of *Ae. aegypti* representing the worldwide distribution of the species. Genetic differences among populations enable inferences on the geographic origin of a sample and would be related to the time of introduction of *Ae. aegypti* into the different continents. An allozymic survey of populations from San Pablo, Brazil (Dinardo-Miranda and Contel 1996) revealed higher genetic variability than that reported by other authors.

In order to contribute to the knowledge on colonization and dispersal patterns in Argentine populations of this species, we have estimated levels of genetic variability and determined genetic distances among different populations of *Ae. aegypti* using allozymes as genetic markers.

MATERIALS AND METHODS

Samples of 4th-stage larvae of *Ae. aegypti* were collected in urban areas of Villa María City, 32°24′S, 63°15′W (Córdoba Province); Zárate City, 34°15′S, 59°10′W (Buenos Aires Province); and

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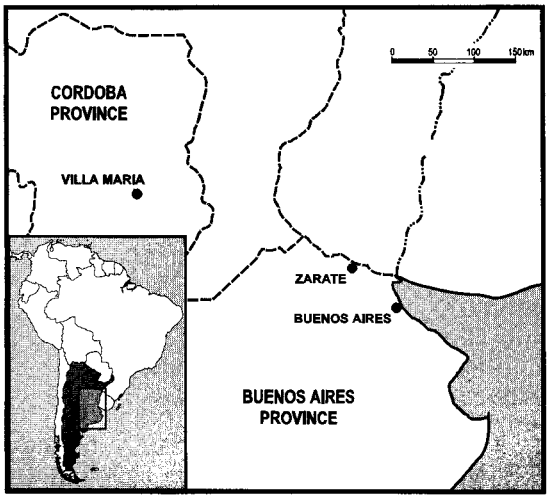

Fig. 1. Location of *Aedes aegypti* collection sites.

Buenos Aires City, 34° 36'S, 58° 23'W (Federal District) (Fig. 1), in February and March 1996. By that time, these were the only 3 out of many sampling places in central Argentina where mosquitoes of the species were found. Larvae were transported alive to the laboratory and reared until adults emerged. They were frozen and stored at −30°C until electrophoresis was performed. Individual mosquitoes were homogenized in an all-glass microgrinder with 15 μl of distilled water. The resulting suspension was adsorbed in a 2.5-mm Whatman 3MM paper wick (Whatman International Ltd., Maidstone, England), inserted into a 12% starch gel block, and electrophoresed at 4°C. The following proteins were analyzed, utilizing 2 buffer systems: Tris–borate–ethylenediaminetetraacetic acid, pH 8.6, for the separation of superoxide dismutase (EC 1.15.1.1., *Sod*), malic enzyme (EC 1.1.1.4, *Me*), leucine aminopeptidase (EC 3.4.11.1, *Lap*), and 6-phosphogluconate dehydrogenase (EC 1.1.1.43, *6Pgdh*); and Tris citric, pH 7, for glucose-phosphate isomerase (EC 5.3.1.9, *Gpi*), isocitrate dehydrogenase (nicotinamide adenine dinucleotide phosphate [NADP] EC 1.1.1.42, *Idh*), malate dehydrogenase (EC 1.1.1.37, *Mdh*), phospho-glucomutase (EC 5.4.2.2, *Pgm*), glycerophosphate dehydrogenase (EC 1.1.1.8, *Gpdh*), and hexokinase (EC 2.7.1.1, *Hk*) (de Sousa et al. 1996). Staining procedures were those indicated by Tabachnick and Powell (1979) and Tabachnick et al. (1979). Twenty-six to 28 individuals from each collection site were studied for each buffer system.

Criteria for genetic interpretation of zymograms were similar to those applied by other authors to polymorphisms detected in *Aedes* mosquitoes (Tabachnick et al. 1979, Tabachnick and Powell 1979, Munstermann 1988). Locus and allele designation were as used by Tabachnick et al. (1979) for *Ae. aegypti*. For each sample, mean number of alleles per locus (A), proportion of polymorphic loci (P), and expected and observed mean heterozygosity (H_e and H_o, respectively) were calculated. Expected genotypic frequencies according to the Hardy–Weinberg equilibrium were estimated using Levene's (1949) correction for small samples. Goodness of fit was tested by a chi-square test. Genetic similarity among populations was calculated with Nei's identity (I_N; Nei 1972) and Rogers' (1972) indices. Population differentiation was also estimated by F_{ST} values (Wright 1951). F_{ST} is a measure of the variance of gene frequencies between populations. Significance of the observed values was tested with a chi-square heterogeneity test. All calculations were performed with the BIOSYS 2 program (Black 1997).

Table 1. Allelic frequencies of polymorphic loci in 3 populations of *Aedes aegypti* from Argentina.

Locus	Allele	Population		
		Zárate	Villa María	Buenos Aires
Gpdh	100	0.933	1.000	1.000
	72	0.067	0.000	0.000
Idh-2	100	0.940	1.000	0.654
	80	0.060	0.000	0.346
Pgm	100	0.288	0.375	0.200
	120	0.712	0.625	0.800
6Pgdh	100	0.981	1.000	0.920
	70	0.019	0.000	0.080
Gpi	105	0.050	0.000	0.016
	100	0.950	1.000	0.952
	93	0.000	0.000	0.032
Me	95	0.000	0.000	0.018
	100	1.000	1.000	0.929
	105	0.000	0.000	0.054
Mdh	84	0.192	0.107	0.060
	100	0.769	0.893	0.940
	120	0.038	0.000	0.000
Lap-1	113	0.125	0.196	0.375
	100	0.875	0.804	0.625

RESULTS

Three out of 11 loci analyzed were monomorphic in all populations: *Lap-2, Sod,* and *Hk* (a locus was considered polymorphic when the frequency of the most common allele was < 0.99). Table 1 shows allele frequencies for the polymorphic loci. No departures from Hardy–Weinberg equilibrium were detected at any of these loci. The value of A varied between 1.3 and 1.8 and P ranged between 27.3 and 63.6. Values H_e ranged between 0.090 and 0.161. The population from Villa María presented the lowest values for the 3 indices (Table 2).

Nei's identity showed marked homogeneity among populations, with the same value (0.98) between Zárate and Buenos Aires cities and between Buenos Aires and Villa María; I_N was 0.997 between Zárate and Villa María. Rogers' similarity

Table 2. Sample size, mean number of alleles per locus (A), percentage of polymorphic loci (P; 0.99 criterion), and mean heterozygosity per locus (H), in populations of *Aedes aegypti.*

Population	n[1]	A	P	H	
				Observed	Expected
Zárate	26	1.7 ± 0.2	63.6	0.125 ± 0.046	0.127 ± 0.046
Villa María	28	1.3 ± 0.1	27.3	0.091 ± 0.050	0.090 ± 0.050
Buenos Aires	27	1.8 ± 0.2	63.6	0.153 ± 0.049	0.161 ± 0.055

[1] n, sample size for each buffer system.

values were: 0.909 for the comparison Zárate vs. Buenos Aires, 0.915 for Villa María vs. Buenos Aires, and 0.985 for Villa María vs. Zárate. Because this index allowed a better discrimination of populations, it was used to construct the dendrogram shown in Fig. 2, using the unweighted pair-group method with arithmetic mean (UPGMA) method (Sokal and Sneath 1963). In 3 of the polymorphic loci (*Gpdh, Idh-1,* and *Lap-1*) F_{ST} values were significantly different from zero, as tested by a chi-square contingency test ($P < 0.05$), indicating differences in allele frequencies among populations (Table 3).

DISCUSSION

In loci *Pgm, Gpi, Me,* and *Mdh,* the relative mobility of alleles detected in our samples was similar to those reported by Tabachnick and Powell (1979) for populations from Asia, Africa, and part of the Americas. In contrast, *Idh* and *6Pgdh* in Argentinian samples presented alleles not found in those populations. The locus *Gpdh* showed polymorphism in the sample from Zárate; this locus was reported as monomorphic in most of the populations studied by other authors (Tabachnick and Powell 1979, Dinardo-Miranda and Contel 1996).

Values of P and H_e for Zárate and Buenos Aires samples (P = 0.64% and H_e = 0.127 and 0.16, respectively) were similar to those reported for populations from Puerto Rico (H = 0.163; Wallis et al. 1984) and for populations from different continents (P = 58%, H = 0.152; Tabachnick and Powell 1979). A higher degree of heterozygosity (H = 0.192) was found in populations from Brasilia (Brazil) on the basis of 15 allozymic loci (Dinardo-Miranda and Contel 1996). These authors proposed that this high level of polymorphism is the result of multiple introductions of *Ae. aegypti* in the area.

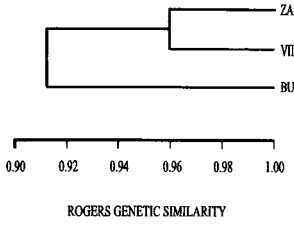

Fig. 2. Phenogram constructed using the UPGMA method, based on Rogers' genetic similarity values among samples of *Aedes aegypti.*

The Villa María sample showed the lowest indices of genetic variability. Reinfestation with *Ae. aegypti* in Argentina was detected in 1986. In spite of epidemiologic surveillance in Córdoba Province, the finding of the 1st individuals of *Ae. aegypti* in Villa María dates from February 1995 (1 larva in 1 of 5 sampling sites). In less than a month, several larvae were found in 8 of 13 sites, indicating a rapid spread of an original stock of a few individuals (Avilés et al. 1997). The low polymorphism in this population probably is due to founder effect in a recent colonization process.

The mean F_{ST} value of 0.065 indicates low levels of genetic differentiation among localities. However, significant values were found in 3 out of 8 polymorphic loci: *Gpdh, Idh-2,* and *Lap-1.* The fact that one or a few loci behave differently from the majority is usually interpreted as the action of natural selection on these loci or others closely linked to them (Slatkin 1987). Although we have no evidence to support this statement, it is interesting to point out that Powell et al. (1980) and Tabachnick et al. (1979) have also reported significant allele frequency differences in the locus *Idh-2* of *Ae. aegypti* from Asia and Africa. The authors proposed that the variation would be the result of selection in conjunction with limited gene flow between domestic and sylvan populations.

Genetic distances among the 3 populations analyzed here (Rogers' D = between 0.091 and 0.042) are low, with the highest value between Buenos Aires City and Zárate, which are the 2 closest localities. This result would indicate that time has not been sufficient for isolation by distance to become apparent (Slatkin 1993) and would be in agreement with a recent colonization of the studied area.

In spite of the recent reinfestation with the mos-

Table 3. Values of Wright's coefficient F_{ST} in population samples of *Aedes aegypti.*[1]

Locus	F_{ST}	Locus	F_{ST}
Gpdh	0.045*	*Gpi*	0.019
Idh-1	0.195**	*Me*	0.039
Pgm	0.025	*Mdh*	0.037
6Pgdh	0.036	*Lap-1*	0.062*
Mean	0.065		

[1] Chi-square heterogeneity test. *$P < 0.05$; **$P < 0.01$.

quito in this country, the levels of polymorphism found in populations from central Argentina were relatively high. This genetic heterogeneity could be the result of multiple introductions of different subpopulation samples of the gene pool of the species. Polymorphism at loci controlling vectorial capacity probably also is well represented in populations introduced in Argentina. Reiter (1998) demonstrated that the traffic of used tires is a major factor in the introduction of *Aedes albopictus* (Skuse) into the United States and Brazil and in the expansion of the species' range. Given that *Ae. aegypti* also prefers used tires as oviposition sites (Nelson et al. 1986), the commercial trade from neighboring countries possibly favors a continued dispersal of this species to Argentina.

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