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Geographic origin of the US and Brazilian *Aedes albopictus* inferred from allozyme analysis

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A survey of genetic variation using allozymes was conducted on 57 populations of *Aedes albopictus*, an Asian mosquito that was introduced into the US and Brazil in the mid-1980s. Our objective was to quantify the patterns of genetic variation among its populations and to use that information to trace genetically the geographic origin of the US and Brazilian populations. Populations from the various regions were genetically distinct from one another. Populations from within a region were genetically similar to one another, which resulted in a significant positive correlation between geographic and genetic distances. A discriminant analysis of allele frequencies separated populations from the various countries into nine non-overlapping clusters; the US, Japanese, Chinese and Brazilian populations formed closely placed, but distinct, clusters. The probability of assigning a population to the correct country was 98 per cent. The US and the Brazilian populations were closest in terms of genetic distance from the Japanese populations. Based on discriminant and genetic distance analyses, we conclude that the US and the Brazilian *Aedes albopictus* originated in Japan.

Keywords: *Aedes albopictus*, allozyme variation, allele frequencies, multiple discriminant analysis.

Introduction

Aedes albopictus (Skuse) (Diptera: Culicidae) is a mosquito native to Asia. It has expanded its range from its centre of origin in south Asia to include most of Asia, Hawaii, Madagascar, Mauritius and the South Pacific (reviews in Hawley, 1988 and Rai, 1991). *A. albopictus* was inadvertently introduced in the mid-1980s into the United States and Brazil (Forattini, 1986; Sprenger & Wuithiranyagool, 1986). Although a few small introductions into the US have been reported in the last few years (Eads, 1972; Reiter & Darsie, 1984), a large breeding population was discovered for the first time in and around Houston, Texas. Its distribution in the US is now widespread, covering about 18 southern, southeastern and midwestern states. The mosquito is considered to be a public health threat because it is an efficient vector of several viruses, including dengue (Rai, 1991). Laboratory studies have shown that the Houston strain is an efficient vector of all four dengue serotypes (Mitchell *et al.*, 1987).

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We have studied the population genetics of *A. albopictus* using a variety of genetic markers since its introduction into the US (Black *et al.*, 1988a and b, 1989; Kambhampati & Rai, 1991; Kambhampati *et al.*, 1990). These studies reveal that much genetic drift accompanies the establishment of populations. We have most recently sampled 57 populations from the native habitats as well as the US and Brazil. Our objective was to trace genetically the geographical origin of the US and the Brazilian populations using the information obtained from allele frequency variation. Because the ability to transmit dengue virus to a host has been shown to be a function of the geographical origin of *A. albopictus* (Boromisa *et al.*, 1987), the geographic origin of a given population is an important variable in the assessment of its potential danger to public health. We also wished to determine if the patterns of genetic variation observed in some of the US, Malaysian and Borneo populations (Black *et al.*, 1988a, and b; Kambhampati *et al.*, 1990) were representative of the species as a whole.

Studies on allozyme variation were previously conducted on the yellow fever mosquito, *Aedes aegypti* (Powell *et al.*, 1980; Scott & McClelland, 1975;

Tabachnick & Powell, 1979; Wallis *et al.*, 1983) which, like *A. albopictus*, is closely associated with humans. Powell *et al.* (1980) found that populations from a given region formed distinct clusters in a discriminant analysis of allele frequencies, with a 90 per cent probability of assigning a population to the correct group.

Materials and methods

Fifty-seven collections from nine countries (Fig. 1, Table 1), which represent the world-wide distribution of *A. albopictus*, were analysed for genetic variation. Eggs were collected either by placing ovitraps in the field or by capturing adult females which then oviposited in the laboratory. The eggs were shipped to the University of Notre Dame where they were reared to adulthood and used in the analysis. The adults were frozen at − 70°C until electrophoresis.

The protocols of the electrophoresis were described by Black *et al.* (1988b). Single mosquitoes were homogenized in 30 μl of grinding buffer and genetic variation was surveyed at eight enzymatic loci: aconitase (ACON), esterase (EST), α-glycerophosphate dehydrogenase (α-GPDH), hydroxyl acid dehydrogenase (HAD), isocitrate dehydrogenase (IDH), malic acid dehydrogenase (MDH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM). These eight loci were chosen because of the 25 loci examined in several populations, they were found to be consistently polymorphic and therefore of particular relevance to this study (Pashley & Rai, 1983; W. C. Black IV, unpublished data). The alleles were scored with reference to the mobility of the most common allele at a given locus, which was assigned a r.f. = 1.0.

Allele frequencies were analysed using BIOSYS-1 (Swofford & Selander, 1981). Variance in allele fre-

Fig. 1 A map to show the present distribution of *Aedes albopictus*. The circles represent the nine countries from which 57 populations were sampled.

Table 1 Populations of *Aedes albopictus* used in the study

Source	Collector(s)	Date collected	*n**
United States			
1 Memphis, TN	W. Black & J. Ferrari	June 1986	45
2 Jacksonville, FL	G. Gregory & B. Peacock	September 1986	20
3 New Orleans 1, LA	M. Andis & E. Bordes	June 1986	23
4 New Orleans 2, LA	M. Andis & E. Bordes	June 1986	19
5 New Orleans 3, LA	M. Andis & E. Bordes	June 1986	16
6 New Orleans 4 + 5, LA	M. Andis & E. Bordes	June 1986	23
7 Chambers Co., TX	D. Sprenger	September 86	38
8 Galveston Co., TX	D. Sprenger	September 86	31
9 Liberty Co., TX	D. Sprenger	September 86	11
10 Houston 32 + 34, TX	D. Sprenger	June 1986	43
11 Houston 61, TX	D. Sprenger	June 1986	39
12 Houston 203, TX	D. Sprenger	June 1986	37

Table 1 — *Continued*

Source	Collector(s)	Date collected	*n**
13 Houston 218, TX	D. Sprenger	June 1986	26
14 Houston 509, TX	D. Sprenger	June 1986	10
15 Evansville, IN	V. Dunn	September 1986	40
16 Indianapolis, IN	M. Sinsko	September 1986	36
17 Chicago, IL	B. Black	September 1987	43
18 East St. Louis, IL	C. Pumpuni	September 1988	36
19 Milford, DE	C. Stachecki	September 1987	29
20 New Hanover, NC	C. Apperson	August 1988	39
21 Savannah, GA	O. Fultz	July 1988	32
22 Oak Hill, OH	R. Berry	September 1987	24
23 Baltimore, MD	D. Engber	September 1987	36
24 Faustus, OH	R. Berry	September 1987	27
25 Rockingham, NC	D. Engber	July 1987	55
26 New Alsace, IN	S. Foster	August 1987	20
27 Lexington, KY	F. Cilek	August 1987	39
28 Carolina Beach, NC	D. Engber	July 1987	40
Brazil			
29 Cariacica City	F. Antunano	October 1986	64
30 Santa Tereza	F. Antunano	June 1987	65
31 Anchieta	F. Antunano	July 1987	40
32 Sao Paulo	F. Antunano	July 1987	40
Japan			
33 Nagasaki	M. Mogi	September 1988	40
34 Kogeshima	M. Mogi	August 1988	40
35 Setagaya	K. Kurihara	August 1988	38
36 Ebina	T. Tadano	August 1986	39
37 Zama	T. Tadano	August 1986	37
38 Saga	M. Mogi	August 1986	37
39 Seburi	M. Mogi	August 1988	40
Malaysia			
40 Kent Ridge, Singapore	K. Chan	August 1986	76
41 Amoy, Singapore	L. Jien	August 1988	40
42 Kuala Lumpur	H. Yong	August 1986	40
43 Kuala Lumpur — A	W. Hawley	December 1987	25
44 Kuala Lumpur — B	W. Hawley	December 1987	54
45 Kuala Lumpur — D	W. Hawley	December 1987	21
46 Telipot	W. Hawley	December 1987	43
47 Chenering	W. Hawley	December 1987	22
48 Wakaf Tapai	W. Hawley	December 1987	15
Borneo			
49 Kota Kinabalu	W. Hawley	December 1987	17
50 Serian	W. Hawley	December 1987	34
Sri Lanka			
51 Colombo	F. Amerisinghe	July 1986	34
India			
52 Sonapur	V. Sharma	July1986	37
53 Hardwar	V. Sharma	May 1988	39
Madagascar			
54 Tananarive	D. Fontenille	January 1988	24
People's Republic of China			
55 Hunan Province A	R. Xu	June 1989	40
56 Hunan Province B	R. Xu	June 1989	33
57 Haiku City	R. Xu	June 1989	34

*Mean number of mosquitoes sampled per locus.

quencies was partitioned using *F*-statistics (Wright, 1978). The allele frequencies were also analysed by a stepwise multiple-discriminant analysis using the program DISCRIMINANT in SPSSx (SPSS Inc., 1989).

Results

A complete list of allele frequencies is available from the authors upon request. All eight loci were polymorphic in one or more populations. The degree of polymorphism at a given locus was generally maintained across populations and the most common allele was the same in a majority of the populations. For example, the *α-Gpdh* locus was fixed for one allele (r.f. = 1.00) in 51/57 populations. The only populations polymorphic at this locus were from Malaysia and Borneo. However, whereas the latter had the 1.33 allele at a very low frequency, this allele was predominant in the two populations from Borneo. The most polymorphic locus was *Est*; however, no variation was observed at this locus in three US populations: Evansville, Jacksonville and Memphis. In most populations, three alleles were found at the *Pgm* locus. All four Brazilian populations were fixed for one allele (r.f. = 1.00) at the *Acon* locus. The number of alleles at the *Had* locus ranged from 1 to 6; however, no variation was detected in the populations from Sonapur and Kota Kinabalu. *Mdh* was relatively less polymorphic throughout the world. Three alleles were scored in some of the US and Japanese populations, whereas most others contained only two. The allele composition at the *Idh* locus was fairly uniform: most populations had the same three alleles. No variation was detected at this locus in samples from Kuala Lumpur-A and Wakaf Tapai.

Allele frequencies in each of the 57 populations were analysed using a stepwise multiple-discriminant analysis. All the populations from a given country were grouped together for the purposes of the analysis and countries in which only one location was sampled were

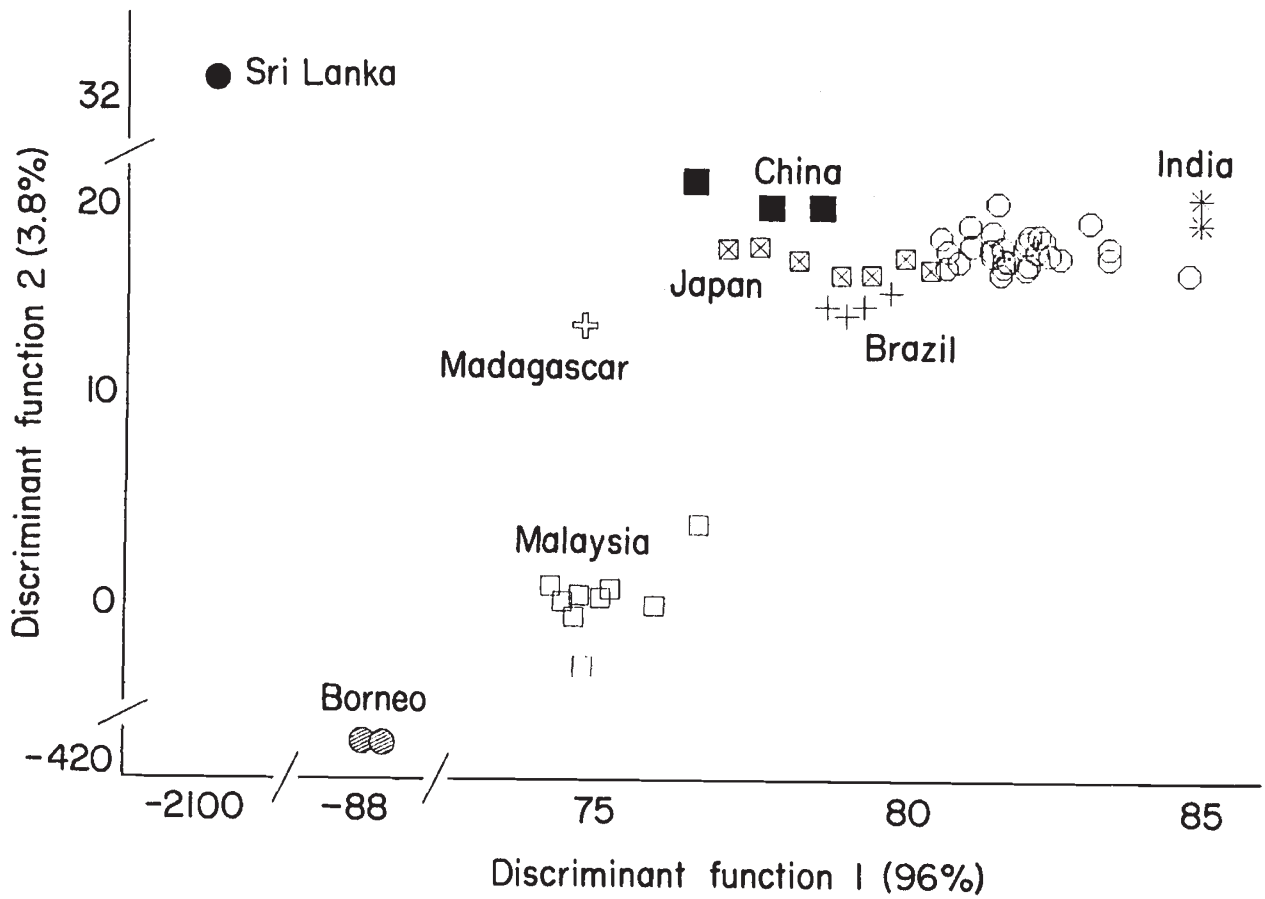

Fig. 2 The results of discriminant analysis, the input for which was the allele frequencies of 57 populations of *Aedes albopictus*. The percentage of variation in allele frequencies accounted for by the first and second discriminant functions is shown in parenthesis beside the *x*- and *y*-axis titles. Each country is represented by a different symbol and the name of the country is given near the respective clusters. The group centroids for populations from each country are given in Table 2.

input as separate groups for a total of nine groups. The overall percentage of populations correctly classified was 98.13 per cent. Only 1/57 populations was misclassified: Zama from Japan was classified into the US group. The results are shown in Fig. 2 and the mean location for each group in the two-dimensional space and the 95 per cent confidence intervals are given in Table 2. It is clear that populations from each country formed a distinct cluster with no overlap. Four groups, US, Japan, China and Brazil, formed closely placed, but distinct, clusters. The populations from India were placed close to the US–Japan–China–Brazil cluster. The populations from Malaysia formed a distinct cluster by themselves. The populations from Sri Lanka and Borneo were far removed from the other seven populations. The first discriminant function (DF) accounted for about 96 per cent of the total variance in allele frequencies and the second DF for about 3.8 per cent.

The populations from China, Japan, Brazil, the US, India and Madagascar were separated from one another primarily on the basis of DF1. Populations from Malaysia were separated from the above primarily along DF2. Sri Lanka and Borneo populations were separated from the rest along both DF1 and DF2. The standardized discriminant function coefficients for DF1 and DF2 are given in Table 3. The alleles that contributed the most to discrimination along DF1 were *Pgi* 1.13, *Had* 1.50, *Pgm* 0.80, *Gpdh* 1.00 and 1.33 and along DF2 *Gpdh* 1.00 and 1.33, *Had* 0.68, *Est* 1.12 and *Pgm* 0.86.

The US and the Brazilian populations were entered into the discriminant analysis as 'unknowns' to be classified into one of the seven groups that were established (Japan, Malaysia, China, India, Madagascar, Borneo, Sri Lanka). Of the 28 US populations, 16 (57 per cent) were classified into the Japan group, seven (25 per cent) into the China group and five (18 per cent) into the Malaysia group. Of the four Brazilian populations, three (75 per cent) were classified into the Japan group and one (25 per cent) into the Madagascar group.

Populations in proximity should be genetically more similar to one another through local selection and migration than to distant populations resulting in a positive correlation between geographical and genetic distances. However, such a correlation may not exist for a variety of reasons (e.g. genetic drift, long-distance migration, panmixia). The close clustering among populations of each country in the discriminant analysis suggested that such a correlation may exist for the *A. albopictus* populations. To determine if this indeed was the case, we undertook a correlation analysis between the geographical and the genetic distances. The geographical distance was calculated from the lati-

Table 2 Group centroids and their 95 per cent confidence intervals along DF1 and DF2 for populations of *Aedes albopictus* derived from a discriminant analysis of allele frequencies. See Fig. 2 for location of the mean positions in the two-dimensional space

	Group centroid		±95% CI	
Country	DF1	DF2	DF1	DF2
USA	81.65	17.18	0.19	0.16
Brazil	78.68	14.32	0.25	0.24
Japan	78.24	16.53	0.52	0.22
Malaysia	74.03	−0.68	0.29	0.61
Borneo	−88.71	−415.49	0.01	0.06
India	84.91	18.72	0.01	0.01
China	77.25	18.15	0.88	0.33
Sri Lanka*	−2093.19	32.35	—	—
Madagascar*	73.71	13.22	—	—

*Only one location was sampled.

Table 3 Standardized discriminant function coefficients derived from a discriminant analysis of allele frequencies. The values in the table show the contribution of the various alleles to discrimination among groups along DF1 and DF2

		Discriminant function coefficients	
Locus	r.f.	DF1	DF2
Pgm	0.80	5.98	−1.03
	0.86	0.95	1.82
	0.93	−1.46	−0.86
	1.07	−0.58	−0.55
Pgi	0.93	−1.65	0.59
	1.06	−0.21	0.31
	1.13	−17.37	0.10
Est	0.95	−0.08	−0.34
	1.00	−0.33	−0.44
	1.06	0.56	0.56
	1.12	−1.42	−2.66
Had	0.68	−1.81	−2.94
	1.20	−0.77	−0.11
	1.40	−0.71	−0.29
	1.50	−11.11	−1.56
Mdh	0.55	1.53	0.14
	0.63	−1.72	0.33
	1.00	−0.15	0.22
	1.50	−0.06	−0.12
Idh	0.70	0.36	1.16
	0.89	−0.21	0.25
	1.00	0.22	0.11
Gpdh	1.00	5.74	18.31
	1.33	4.96	15.93

Table 4 A matrix of Nei's unbiased genetic distances averaged by country for populations of *Aedes albopictus*. The standard errors are given immediately following the mean for the within country values

Country	1	2	3	4	5	6	7	8	9
1. USA	0.086 (0.008)								
2. Brazil	0.082	0.031 (0.006)							
3. Japan	0.064	0.060	0.041 (0.006)						
4. Malaysia	0.108	0.080	0.084	0.063 (0.006)					
5. Borneo	0.280	0.255	0.247	0.248	0.038 (0.001)				
6. Sri Lanka	0.096	0.074	0.068	0.064	0.198	*			
7. India	0.121	0.074	0.084	0.069	0.220	0.039	0.051 (0.001)		
8. Madagascar	0.119	0.058	0.102	0.125	0.284	0.113	0.109	*	
9. China	0.068	0.061	0.035	0.063	0.227	0.044	0.059	0.110	0.026 (0.006)

*Only one population sampled.
Underlined values are average distances for locations within a country.

tude and the longitude of each location and we used Nei's unbiased genetic distance as a measure of the genetic relatedness among populations. Only the distances between the Asian populations were included. The distances between the US and the Brazilian populations versus the remainder and the US versus Brazilian populations were excluded because of the colonization history. In the first instance, a total of 244 data points were included and the correlation was non-significant ($r=0.08$, $P>0.05$). In the second analysis, the distances between the two Borneo populations and the remainder were excluded because the allele frequencies at one locus (*Gpdh*) contributed disproportionately to differentiation among populations from Borneo and elsewhere and resulted in large genetic distances (see e.g. Tables 3 and 4, and Black *et al.*, 1988b). With the Borneo populations excluded, the correlation improved substantially and was significant ($n=202$, $r=0.34$, $P<0.001$, Fig. 3).

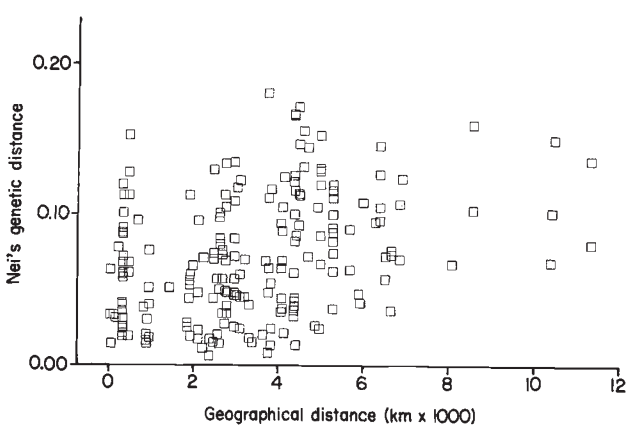

Fig. 3 A plot to show the relationship between geographical distance and genetic distance for Asian populations of *Aedes albopictus*. See text for further details. $r=0.34$, $P<0.001$.

Nei's (1978) unbiased genetic distances, averaged by country, are given in Table 4. The distances among populations within a country were in general smaller than those among countries, as expected. However, the average distances among populations from the US and from Malaysia were relatively large. The US populations were closest to those from Japan, followed by those from China. The Brazilian populations were closest to Madagascar followed by those from Japan and China. The distances between Borneo populations and the remainder were relatively large, which suggests extensive divergence in allele frequencies. Madagascar was also genetically well-differentiated from the remainder of the populations. The populations from India and Sri Lanka were separated from one another by a small genetic distance as were the Chinese and Japanese populations.

The allele frequencies were tested for deviation from Hardy–Weinberg expectations using a chi-square test. Of 333 tests, 61 were found to be significant at the 5 per cent probability level. This is significantly greater than expected with a type I error rate of 5 per cent ($\chi^2_{1,0.05}=118.13$, $P<0.001$). Both the native and the recently introduced strains deviated with equal frequency; however, all four Brazilian populations deviated from Hardy–Weinberg expectations at at least one locus. Of the 61 significant tests, 29 were at the *Est* locus, 15 at the *Pgm* locus, six at the *Acon* locus, four each at the *Idh* and the *Had* locus, two at the *Pgi* locus and one at the *Mdh* locus. Fifty-five of the 61 significant tests were the result of heterozygote deficiency and the remainder due to heterozygote excess. All but one heterozygote excess was at the *Pgm* locus.

Variance in allele frequencies was partitioned into variation among locations within cities, among cities within countries and among countries (Table 5). About 45 per cent of the total variance in allele frequencies

Table 5 Variance components and percentage contribution combined across loci for world populations of *Aedes albopictus*. See text for further details

Source	Variance	Percentage of total
Locations [cities (countries)]	0.228	44.88
Cities (countries)	0.168	33.07
Countries	0.112	22.05
Total	0.508	100.00

was attributable to variation among locations within cities, 33 per cent to variation among cities within countries and 22 per cent to among countries.

Discussion

The survey of allozyme variation at eight loci in 57 samples of *A. albopictus* revealed considerable genetic variation within and among populations. Two broad patterns emerged from the discriminant analysis of allele frequencies and the correlation analysis of geographical and genetic distances. The populations from a given region were similar to one another, while the populations from different countries were genetically distinct from one another. As a consequence, the probability of assigning a population to the correct region in the discriminant analysis was extremely high. Together, the results of the discriminant and the correlation analyses indicated that the populations within a given region are sufficiently similar to one another and the populations from different regions sufficiently genetically differentiated from one another to enable a strong inference of the geographical origin of a population. The populations from Japan, China, Brazil and the US formed closely placed, but distinct, clusters. Therefore, based on the results of the discriminant analysis and the genetic distance analysis, we conclude that the US populations originated in northern Asia (Japan or China), corroborating the observation by Hawley *et al.* (1987) based on photoperiodic responses. Fifty-seven per cent of the populations were assigned to Japan when the US populations were input as an unknown group, which strongly suggests that Japan is the most likely source among the north Asian populations. The pattern of the used-tire trade [the presumed mode of introduction (Craven *et al.*, 1988)] also points to Japan as the most likely source.

In the discriminant analysis, the Brazilian populations were placed close to those from Japan and when they were input as an unknown group, 3/4 populations were classified into the Japan group. This strongly suggests that the Brazilian populations also originated in Japan. The assignment of one population (Cariacica City) to Madagascar is probably an anomaly because the used-tire trade between Brazil and Madagascar is non-existent (Pumpuni, 1989) and the allele frequencies and composition in Brazilian populations do not suggest multiple origins (see below). The small genetic distance between Brazilian and Madagascar populations is primarily a result of similarities in allele frequencies between the population from Cariacica City and Madagascar at a single locus (*Mdh*).

Hawley *et al.* (1987) compared the photoperiodic responses of several populations and found that the Brazilian and the US populations differed from one another in one respect: under short-day conditions, the US populations initiated diapause (a characteristic of temperate populations) and the Brazilian populations did not (a characteristic of subtropical/tropical populations). This suggests that the Brazilian populations had a fundamentally different origin to those in the US. However, Pumpuni (1989) was able to select for a nearly diapause-free strain of *A. albopictus* from a diapausing stock in the laboratory in five generations and once achieved, the trait remained stable for several more generations. Such a phenomenon has also been reported for other insect species (Harvey, 1957; Henrich & Denlinger, 1983). Therefore, the possibility that the Brazilian populations originated in northern Japan and subsequently switched to a diapausing-free life-cycle cannot be ruled out.

Because the US and the Brazilian populations were sampled fairly soon after their introduction, inferences can be made with regard to the dynamics of the colonization of the Americas by *A. albopictus*. Although both the US and the Brazilian populations originated in the same region and colonized the respective continents at about the same time, the allozyme analysis suggests that they possess fairly distinct colonization histories. The US populations were in general comparable to those from other parts of the world in terms of allele composition and the colonization does not appear to have severely depleted most of the US populations of genetic variation. In particular, all the alleles present in the Japanese populations were also detected in the US populations. The exceptions were three populations: Memphis, Jacksonville and Evansville, which were fixed for one allele at the *Est* locus suggesting an initial bottleneck effect. The lack of a bottleneck effect in most of the other US populations suggests that the founder population may have been quite large.

In contrast, populations in Brazil appear to have been subjected to a bottleneck effect during the introduction. *Acon* and *Pgi* were both fixed for r.f. = 1.00 allele in all four Brazilian populations. The remaining loci were not visibly effected. The similarities in allele

composition and frequencies among the Brazilian populations suggest that the mosquito was probably introduced into one location in Brazil from one source, subsequently spreading to other locations.

The results of this study also confirm the observations by Black *et al.* (1988a and b) and Kambhampati *et al.* (1990) that the breeding structure of *A. albopictus* is characterized by local genetic drift. The partitioning of variance in allele frequencies indicates that almost one-half of the variation is due to the within-population component. The localized breeding structure was also evident in the Hardy–Weinberg tests; allele frequencies at a significant number of loci contained fewer than expected heterozygotes, which is indicative of inbreeding. A possible explanation for the drift and inbreeding is that the breeding sites of *A. albopictus* often show a discontinuous or patchy distribution and the adult mosquitoes are known to disperse an average of only about 104 m from their breeding sites (Bonnet & Worchester, 1946; Mori, 1979). These two factors in combination are likely to result in relatively small populations and localized breeding units. If the effects of local selection and migration are not strong, then a localized breeding structure with little or no gene flow could potentially preclude the emergence of distinct genetic relatedness patterns because each population has the opportunity to evolve independently of neighbouring populations. However, the genetic drift in *A. albopictus* does not appear to be overwhelming the local selection effects and the natural migration patterns. Both the discriminant and the correlation analysis indicated that the populations in proximity to one another are genetically similar.

In summary, the analysis of allozyme variation in 57 populations of *A. albopictus* revealed considerable variation within and among populations. In the discriminant analysis of allele frequencies and the correlation analysis of geographical and genetic distances, populations from the various regions were shown to be sufficiently genetically distinct from one another and populations from within a region sufficiently genetically similar to enable a strong inference of the geographical origin of a given population. The probability of assigning a population to the correct region was over 98 per cent. We conclude that the source of the US *A. albopictus* is northern Asia, which corroborates the earlier suggestion by Hawley *et al.* (1987). Among the northern Asian populations included in this study, Japan is the most likely source. In addition, our results strongly suggest that northern Asia, Japan in particular, is also the source of the Brazilian populations.

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