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Higher genetic variation estimated by microsatellites compared to isoenzyme markers in *Aedes aegypti* from Rio de Janeiro

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Aedes aegypti populations from five districts in Rio de Janeiro were analyzed using five microsatellites and six isoenzyme markers, to assess the amount of variation and patterns of gene flow at local levels. Microsatellite loci were polymorphic enough to detect genetic differentiation of populations collected at small geographic scales (e.g. within a city). *Ae. aegypti* populations were highly differentiated as well in the city center as in the outskirts. Thus, dengue virus propagation by mosquitoes could be as efficient in the urban area as in the outskirts of Rio de Janeiro, the main entry point of dengue in Brazil.

Key words: *Aedes aegypti* - microsatellites - isoenzymes - genetic differentiation

Dengue is the most important mosquito-borne disease in Brazil where it is essentially transmitted by *Aedes aegypti*. Rio de Janeiro is considered to be the main introduction location for dengue viruses which have caused most epidemics in Brazil with increasing incidence of severe cases (Nogueira et al. 2002, Teixeira et al. 2002). The urban mosquito *Ae. aegypti* is a major dengue vector in Brazil. It is present in high density at the vicinity of highly populated areas (Lourenço-de-Oliveira et al. 2004). The species shows high level of resistance to insecticides (Luz et al. 2003, Braga et al. 2004, Da-Cunha et al. 2005) and is highly susceptible to dengue viruses (Lourenço-de-Oliveira et al. 2004). Moreover, uncontrolled urbanization and intensive travel and migration are additional factors that favor both vector and virus dis-semination (Gubler 1998, Herrera et al. 2006).

Studies on *Ae. aegypti* population structure based on allozyme analysis have been reported for more than 40 years (Tabachnick et al. 1979). More recently, microsatellite markers have been extensively applied to study genetic variations or to detect gene flow (Bruford & Wayne 1993, Jarne & Lagoda 1996). Microsatellites are apparently neutral, codominant and highly polymorphic markers (Tautz 1989).

Field studies on genetic differentiation within vector populations can yield important information regarding to

evolution and population biology (Wang et al. 2001). Variations in isoenzymes and microsatellites among vector populations have provided evidence that water storage habits and human densities may affect their differentiation (Huber et al. 2002). Besides, microsatellite markers provide a sensitive measure of divergence and therefore can potentially distinguish populations that may have recently diverged (Lanzaro et al. 1995). Here, we report a genetic study on *Ae. aegypti* in Rio de Janeiro using both microsatellite and isoenzyme markers.

MATERIALS AND METHODS

Mosquito samples - *Ae. aegypti* was sampled in five districts in Rio de Janeiro in March 2003. Samples were divided into two groups according to the human density in the sampled district: (i) high human density (city center) - Pilares (PILR) and Tijuca (TIJU) (15,364.17 inhabitants/km² and 17,431.88 ha/km², respectively) and (ii) moderate human density (outskirt) - Barra da Tijuca (BART), Campo Grande (CAMG), and Taquara (TAQU) (1,726.46 ha/km², 2,182.58 ha/km² and 6,444.13 ha/km², respectively). To avoid collections of descendants from small number of *Ae. aegypti* females, 20 ovitraps (Reiter et al. 1991) were set per district during two consecutive weeks (Dibo et al. 2005). Districts are separated by a minimal distance of 8.2 km and a maximal of 32.4 km (see Costa-Ribeiro et al. 2006 for more details).

Mosquitoes were reared to the adult stage (F_0 generation) in insectaries under standardized conditions (25 \pm 1°C, 80 \pm 10% relative humidity and 12 h light/dark cycle) and subsequently, stored at -80°C. Mosquitoes from the same samples were analyzed by both the microsatellite and isoenzyme assays.

Microsatellites - Thirty mosquito adults from each district were analyzed using six microsatellite loci: C2A8, 34/72, T3A7, AED19, 38/38, and 9A89 (see Huber et al. 2001 for more details). DNA was extracted in DNAzol solution (Gibco BRL). PCR reactions containing 1X buffer (Eurobio), 1.2 mM MgCl₂, 60 μ M of each dNTP, 5 pmol of

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each primer, 0.25 units of Taq polymerase (Eurobio), and 2 µl of DNA were performed in a 9600 thermal cycler (Perkin-Elmer). The reaction conditions were 5 cycles (2 min at 96°C, 30 s annealing at locus-specific, annealing temperature (T_a) and 1 min 15 s extension at 72°C) followed by 25 cycles (30 s at 95°C, 30 s at T_a and 1 min 15 s at 72°C) and a final elongation step (5 min at 72°C). One primer (1.5 pmoles) was end-labeled with one of the three different fluorescent phosphoramidite dyes (FAM, HEX or NED) appropriate for ABI PRISM instruments. One microliter of each PCR reaction was diluted to 1:20 times with distilled water. Samples were prepared by adding 0.5 µl of internal size standard 400HD ROX (Perkin Elmer) and deionised formamide for a final volume of 20 µl.

Isoenzymes - Forty-eight adults from each district were analyzed for 10 enzyme systems: glucose phosphate isomerase (Gpi, EC 5.3.1.9.), glutamate oxaloacetate transaminases (Got1 and Got2, EC 2.6.1.1.), glycerol phosphate dehydrogenase (Gpd, EC 1.1.1.8.), hexokinases (Hk, EC 2.7.1.1.), malate dehydrogenase (Mdh, EC 1.1.1.37.), malic enzyme (Me, EC 1.1.1.40.), and phosphoglucumutase (Pgm, EC 2.7.5.1.). Each individual mosquito was ground in 25 µl of distilled water and centrifuged (12,000 g for 3 min at +4°C). The supernatant containing soluble proteins was loaded onto a 12.8% starch gel in Tris-Maleate-EDTA (pH 7.4) buffer and run for 4-5 h (Pasteur et al. 1988). A laboratory strain of *Ae. aegypti* named "Paea" (collected

in 1994 in Tahiti, French Polynesia) was used as a mobility control for isoenzyme polymorphism.

Genetic analysis - Hardy-Weinberg (HW) proportions were compared using GENEPOP software (version 3.4) (Raymond & Rousset 1995). Deviations from HW were tested using an exact test procedure with the alternative hypothesis H1 corresponding to deficits or excess in heterozygotes (Rousset & Raymond 1995). Linkage disequilibrium was tested between pairs of loci for each sample using Fisher's exact test on rank \times column contingency tables. F_{IS} , the inbreeding coefficient, and F_{ST} , the fixation index, were estimated as described by Weir and Cockerham (1984). Genetic differentiation across samples was estimated by calculating the P value associated to the F_{ST} estimate. Significance levels for multiple testing were corrected using sequential Bonferroni's procedures (Holm 1979). Genetic isolation by geographic distance was tested by estimating rank correlations between $F_{ST}/(1-F_{ST})$ calculated between pairs of samples and Ln distances (Slatkin 1993).

RESULTS

Microsatellite polymorphism - The locus 9A89 was monomorphic and 3-4 alleles were scored for the other five loci: three for 34/72, AED19, and 38/38, and four for C2A8 and T3A7 (Table I). The allelic frequencies varied for the loci C2A8 from 0.036 (allele 226) in CAMG to 0.717 (allele 229) in TIJU; for 34/72, from 0.017 (allele 89)

TABLE I
Allele frequencies, F_{IS} and deviations from Hardy-Weinberg expectations observed at five polymorphic loci in *Aedes aegypti* samples from Rio de Janeiro estimated by microsatellite analysis

	C2A8							34/72					
	220 ^a	223	226	229	F_{IS}	P	N	85	87	89	F_{IS}	P	N
BART	0.192	0.135	0.154	0.519	-0.2209	0.0189	26	0.120	0.720	0.160	0.0240	0.8443	25
PILR	0.103	0.103	0.155	0.638	-0.2431	0.4844	29	0.083	0.833	0.083	0.2162	0.1105	30
TIJU	0.100	0.117	0.067	0.717	-0.0741	0.5715	30	0.167	0.650	0.183	0.1769	0.4469	30
TAQU	0.150	0.150	0.133	0.567	-0.3376	0.0829	30	0.207	0.759	0.034	0.5591	0.0020^b	29
CAMG	0.089	0.179	0.036	0.696	0.3379	0.0167	28	0.167	0.817	0.017	0.0333	1	30
	T3A7							AED19					
	216	220	224	228	F_{IS}	P	N	172	175	178	F_{IS}	P	N
BART	0.018	0.161	0.821	0.000	0.5355	0.0081^b	28	0.929	0.071	0.000	-0.0588	1	28
PILR	0.000	0.483	0.517	0.000	0.8058	0.0000^b	30	0.880	0.120	0.000	-0.1163	1	25
TIJU	0.000	0.704	0.222	0.074	1.0000	0.0000^b	27	0.883	0.117	0.000	0.5272	0.0301	30
TAQU	0.000	0.333	0.567	0.100	0.8842	0.0000^b	30	0.750	0.196	0.054	0.1162	0.4927	28
CAMG	0.000	0.517	0.483	0.000	0.7321	0.0001^b	29	0.776	0.224	0.000	-0.2727	0.2871	29
	38/38						All loci						
	76	79	88	F_{IS}	P	N	F_{IS}	P					
BART	1.000	0.000	0.000	-	-	27	0.0210	0.0219^b					
PILR	0.931	0.000	0.069	0.4766	0.1043	29	0.2349	0.0002^b					
TIJU	0.000	0.000	1.000	-	-	28	0.3637	0.0000^b					
TAQU	0.000	0.000	1.000	-	-	27	0.2835	0.0000^b					
CAMG	0.000	1.000	0.000	-	-	30	0.2715	0.0003^b					

a : allele length estimated in base pairs; F_{IS} : the inbreeding coefficient; N: the sample size; P : the probability for rejecting Hardy-Weinberg equilibrium; significant P values (< 0.05) are in bold the significant values after correction using Bonferroni's method; b : heterozygote deficit; -: not determined.

in CAMG to 0.833 (allele 87) in PILR; for T3A7, from 0.018 (alleles 216) to 0.821 (allele 224) in BART; for AED19, from 0.054 (allele 178) in TAQU to 0.929 (allele 172) in BART; and for 38/38, from 0 to 1 (Table I).

For HW equilibrium, 90 tests were carried out (1 test for each locus in each sample). Significant deviations from Hardy-Weinberg equilibrium associated to deficits of heterozygotes were detected in the locus 34/72 in TAQU ($F_{IS} = 0.5591$, $P = 0.0020$) and in the locus T3A7 for all samples: BART ($F_{IS} = 0.5355$, $P = 0.0081$), PILR ($F_{IS} = 0.8058$, $P < 10^{-4}$), TIJU ($F_{IS} = 1$, $P < 10^{-4}$), TAQU ($F_{IS} = 0.8842$, $P < 10^{-4}$), and CAMG ($F_{IS} = 0.7321$, $P = 0.0001$). Tests considering all loci for each sample showed significant deviations from Hardy-Weinberg equilibrium due to heterozygote deficits (Table I). No linkage disequilibrium was detected among 34 comparisons tested. Genetic differentiation evaluated by estimating F_{ST} values was high and significant when considering mosquitoes from all five districts ($F_{ST} = 0.3304$, $P < 0.05$; Table II). When samples were grouped according to human density (city center versus outskirt), a higher genetic differentiation ($F_{ST} = 0.3792$, $P < 0.05$) was detected among samples from the outskirt (Table II). When testing isolation by geographic distance by estimating rank correlations between $F_{ST}/(1-F_{ST})$ calculated between pairs of samples and log geographical distances, no isolation was demonstrated ($P = 0.1522$) (data not shown).

Isoenzyme polymorphism - Six (Pgm, Pgi, Mdh, Hk2, Got1, and Got2) out of 10 loci investigated were polymorphic. 2-3 alleles were scored for each locus: 2 for Pgm, Mdh, Got1 and Got2, and 3 for Pgi and Hk2 (Table III). The allelic frequencies varied for the loci Pgm from 0.115 (100) in PILR to 0.958 (80) in BART; for Mdh, from 0.344 (110) in CAMG to 0.656 (100) in CAMG; for Hk2, from 0 to 0.778 (100) in BART; for Got1, from 0.085 (60) in CAMG to 0.915 (100) in BART and CAMG; and for Pgi and Got2, from 0 to 1 (Table III).

TABLE II

Differentiation of *Aedes aegypti* populations in Rio de Janeiro estimated using microsatellite and isoenzyme markers

Comparisons	Microsatellites	Isoenzymes
All samples		
N	5	5
F_{ST}	0.3304 ^a	0.0200 ^a
City centre		
N	2	2
F_{ST}	0.3510 ^a	0.0021
Outskirt		
N	3	3
F_{ST}	0.3792 ^a	0.0375 ^a

N: number of samples; a: $P < 0.05$.

TABLE III

Allele frequencies, F_{IS} and deviations from Hardy-Weinberg expectations observed at six polymorphic loci in *Aedes aegypti* samples from Rio de Janeiro estimated by isoenzyme analysis

	Pgm				Pgi				Mdh				All loci				
	80 ^a		100		80		100		80		100			80		100	
	<i>F</i> _{IS}	<i>P</i>	<i>N</i>		<i>F</i> _{IS}	<i>P</i>	<i>N</i>		<i>F</i> _{IS}	<i>P</i>	<i>N</i>			<i>F</i> _{IS}	<i>P</i>	<i>N</i>	
BART	0.958	0.042	-0.0330	1	48	0.010	0.990	0.000	0.0000	-	48	0.542	0.458	-0.3333	0.0396	48	
CAMG	0.781	0.219	0.2177	0.1952	48	0.000	1.000	0.000	-	-	48	0.656	0.344	0.2250	0.1964	48	
PILR	0.885	0.115	-0.1190	1	48	0.000	1.000	0.000	-	-	48	0.531	0.469	0.1319	0.3963	48	
TAQU	0.862	0.138	-0.1500	0.5737	47	0.000	0.979	0.021	-0.0108	1	48	0.531	0.469	0.2985	0.0465	48	
TIJU	0.938	0.062	-0.0562	1	48	0.000	1.000	0.000	-	-	48	0.628	0.372	0.0548	0.7601	47	
	80	100	110	<i>F</i> _{IS}	<i>P</i>	<i>N</i>	60	100	<i>F</i> _{IS}	<i>P</i>	<i>N</i>	80	100	<i>F</i> _{IS}	<i>P</i>	<i>N</i>	
BART	0.189	0.778	0.033	0.1427	0.4061	45	0.085	0.915	-0.0824	1	47	0.000	1.000	-	-	48	
CAMG	0.479	0.521	0.000	-0.7484	0.0000 ^c	48	0.085	0.915	0.4620	0.0237	47	0.000	1.000	-	-	48	
PILR	0.302	0.698	0.000	-0.2252	0.1718	48	0.115	0.885	-0.1190	1	48	0.000	1.000	-	-	48	
TAQU	0.304	0.696	0.000	-0.3250	0.0361	46	0.094	0.906	-0.0930	1	48	0.000	1.000	-	-	48	
TIJU	0.255	0.745	0.000	-0.3333	0.0226	47	0.094	0.906	-0.0930	1	48	0.021	0.979	1.0000	0.0105 ^b	48	

a: relative mobility of the allele compared to the control (see text); F_{IS} : the inbreeding coefficient; N: the sample size; P : the probability for rejecting Hardy-Weinberg equilibrium: significant P values (< 0.05) are in bold; b: heterozygote deficit; c: heterozygote excess; Pgm: phosphoglucose mutase; Mdh: malate dehydrogenase; Hk2: hexokinase 2; Got1: glutamate-oxaloacetate transaminase 1; Got2: glutamate-oxaloacetate transaminase 2; in bold, the significant values after correction using Bonferroni's method; -: not determined.

For Hardy-Weinberg equilibrium, 150 tests were carried out. Only two significant deviations from Hardy-Weinberg equilibrium were observed: a heterozygote deficit in TIJU ($F_{IS} = 1$, $P = 0.0105$) and a heterozygote excess in CAMG ($F_{IS} = -0.7484$, $P < 10^{-4}$) (Table III). When considering all loci in each sample, a significant heterozygote excess was observed in CAMG ($F_{IS} = -0.0856$ and $P < 10^{-4}$) (Table III). No linkage disequilibrium was detected among 42 comparisons. Differentiation was significant when considering all samples ($F_{ST} = 0.0200$, $P < 0.05$), and samples from the outskirts ($F_{ST} = 0.0375$, $P < 0.05$) (Table III). No correlation was detected when testing genetic isolation by geographic distance (data not shown).

DISCUSSION

As expected, microsatellite markers displayed higher polymorphism when compared to isoenzyme loci. For microsatellite loci, five among six loci were polymorphic whereas for isoenzyme loci, only six among ten were polymorphic.

Microsatellite loci in *Ae. aegypti* populations from Rio de Janeiro were less polymorphic when compared with Asian populations (Huber et al. 2002, 2004, Paupy et al. 2004). Low abundance and limited polymorphism in microsatellites have been already described for *Ae. aegypti* (Fagerberg et al. 2001, Ravel et al. 2001, 2002). In all samples, heterozygote deficits were observed due to the locus T3A7. This may be related to four main factors: (i) the locus is under selection, (ii) null alleles may be present, (iii) inbreeding may be common in populations, or (iv) the presence of population substructure which leads to a Wahlund's effect. The locus T3A7 was probably subjected to mutations in its flanking regions which resulted in inhibition of primer annealing with the complementary DNA sequences. The failure in detection of microsatellite PCR products generates null alleles and thus, a deficit in heterozygotes (Lanzaro et al. 1995). Inbreeding could not explain heterozygote deficits as deficits were not observed in all loci. The Wahlund effect which describes deviations due to the pooling of subpopulations in HW equilibrium could be one explanation. However, the less polymorphic isoenzyme loci only detected two samples with a deviation from HW equilibrium due to a heterozygote excess or deficit.

Isoenzyme markers revealed a larger genetic differentiation among samples from the outskirts whereas samples from the city center were not differentiated. This pattern contrasted with those observed in Vietnam (Tran Khanh et al. 1999).

Ae. aegypti females tend to lay eggs in artificial containers at the vicinity of human habitations. So, dispersal varies upon the distribution of breeding sites and human density for blood feeding (Tsuda et al. 2001). Gene flow contributes to homogenize populations and thus reduces genetic differences among them. In Rio de Janeiro, samples were collected at the end of the rainy season, in March, when a large number of unintentional containers such as cans, tires and bottles were productive

in larvae and pupae (Honório et al. 2006). This type of breeding sites more abundant in highly populated areas predominates in the city center where low genetic differentiation or deficits among mosquito population was detected using isoenzyme markers. Conversely, microsatellite markers were polymorphic enough to differentiate populations within the city center whereas isoenzymes failed to detect any differentiation.

Dengue is endemic in Rio de Janeiro since the introduction of dengue virus in 1986 (Lourenço-de-Oliveira et al. 2004). Variations in dengue incidence are observed within the city depending on vector distribution and vector control implementation (Luz et al. 2003). Our results demonstrated microsatellite markers to be more adapted to evaluate genetic structure of samples collected within a small geographic scale like a city. The same pattern of genetic differentiation of *Ae. aegypti* populations in the city center and the outskirts suggested that the dengue virus propagation by mosquitoes could be as efficient in urban area as in the outskirts of Rio de Janeiro.

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REFERENCES

- Braga IA, Lima JBP, Soares SS, Valle D 2004. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoas, Brazil. *Mem Inst Oswaldo Cruz* 99: 199-203.
- Bruford MW, Wayne RK 1993. Microsatellites and their application to population genetic studies. *Curr Opin Genet Dev* 3: 939-943.
- Costa-Ribeiro MCV, Lourenço-de-Oliveira R, Failloux AB 2006. Geographic and temporal genetic patterns of *Aedes aegypti* populations in Rio de Janeiro, Brazil. *Trop Med Int Hlth* 11: 1276-1285.
- Da-Cunha MP, Lima JBP, Brogdon WG, Moya GE, Valle D 2005. Monitoring of resistance to the pyrethroid cypermethrin in Brazilian *Aedes aegypti* (Diptera: Culicidae) populations collected between 2001 and 2003. *Mem Inst Oswaldo Cruz* 100: 441-444.
- Dibo MR, Chiaravalloti-Neto F, Battigaglia M, Mondini A, Favaro EA, Barbosa AAC, Glasser CM 2005. Identification of the best ovitrap installation sites for gravid *Aedes (Stegomyia) aegypti* in residences in Mirassol, state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 100: 339-343.
- Fagerberg AJ, Fulton RE, Black IV WC 2001. Microsatellite loci are not abundant in all arthropod genomes: analyses in the hard tick, *Ixodes scapularis* and the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol* 10: 225-236.
- Gubler DJ 1998. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 11: 480-496.
- Herrera F, Urdaneta L, Rivero J, Zoghbi N, Ruiz J, Carrasquel G, Martinez JA, Pernalet M, Villegas P, Montoya A, Rubio-Palis Y, Rojas E 2006. Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. *Mem Inst Oswaldo Cruz* 101: 625-633.

- Holm S 1979. A simple sequentially rejective multiple test procedure. *Scand J Stat* 6: 65-70.
- Honório NA, Cabello PH, Codeço CT, Lourenço-de-Oliveira R 2006. Preliminary data on the performance of *Aedes aegypti* and *Aedes albopictus* immatures developing in water-filled tires in Rio de Janeiro. *Mem Inst Oswaldo Cruz* 101: 225-228.
- Huber K, Luu Le L, Chanta N, Failloux AB 2004. Human transportation influences *Aedes aegypti* gene flow in Southeast Asia. *Acta Trop* 90: 23-29.
- Huber K, Luu Le L, Tran Huu H, Tran Khanh T, Rodhain F, Failloux AB 2002. Temporal genetic variation in *Aedes aegypti* populations in Ho Chi Minh City (Vietnam). *Heredity* 89: 7-14.
- Huber K, Mousson L, Rodhain F, Failloux AB 2001. Isolation and variability of polymorphic microsatellite loci in *Aedes aegypti*, the vector of dengue viruses. *Mol Ecol Notes* 1: 219-222.
- Jarne P, Lagoda PJJ 1996. Microsatellites, from molecules to populations and back. *Trends Ecol Evol* 11: 424-429.
- Lanzaro GC, Zheng L, Toure YT, Traore SF, Kafatos FC, Vernick KD 1995. Microsatellite DNA and isozyme variability in a West African population of *Anopheles gambiae*. *Insect Mol Biol* 4: 105-112.
- Lourenço-de-Oliveira R, Vazeille M, Filippis AMB, Failloux AB 2004. *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to dengue and yellow fever viruses. *Trans R Soc Trop Med Hyg* 98: 43-54.
- Luz PM, Codeço CT, Massad E, Struchiner CJ 2003. Uncertainties regarding dengue modeling in Rio de Janeiro, Brazil. *Mems Inst Oswaldo Cruz* 98: 871-878.
- Nogueira RMR, Miagostovich MP, Schatzmayr HG 2002. Dengue viruses in Brazil. *Dengue Bull* 26: 77-83.
- Pasteur N, Pasteur G, Bonhomme F, Catalan J, Britton-Davidian J 1988. *Practical Isozyme Genetics*, John Wiley and Sons/Ellis Horwood Ltd., England.
- Paupy C, Chanta N, Huber K, Lecoz N, Reynes J-M, Rodhain F, Failloux AB 2004. Influence of breeding sites features on genetic differentiation of *Aedes aegypti* populations analyzed on a local scale in Phnom Penh municipality of Cambodia. *Am J Trop Med Hyg* 71: 73-81.
- Ravel S, Hervé JP, Diarrassouba S, Kone A, Cuny G 2002. Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera: Culicidae) from Côte d'Ivoire: evidence for a microgeographic genetic differentiation of mosquitoes from Bouaké. *Acta Trop* 82: 39-49.
- Ravel S, Monteny N, Velasco Olmos D, Escalante Verdugo J, Cuny G 2001. A preliminary study of the population genetics of *Aedes aegypti* (Diptera: Culicidae) from Mexico using microsatellite and AFLP markers. *Acta Trop* 78: 241-250.
- Raymond M, Rousset F 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86: 248-249.
- Reiter P, Amador MA, Anderson RA, Colon N 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *J Am Mosq Control Assoc* 7: 52-55.
- Rousset F, Raymond M 1995. Testing heterozygote excess and deficiency. *Genetics* 140: 1413-1419.
- Slatkin M 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47: 264-279.
- Tabachnick WJ, Munstermann LE, Powell JR 1979. Genetic distinctness of sympatric joins of *Aedes aegypti* in East Africa. *Evolution* 33: 287-295.
- Tautz D 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res* 17: 6463-6471.
- Teixeira MG, Costa MCN, Guerra Z, Barreto ML 2002. Dengue in Brazil: Situation-2001 and trends. *Dengue Bull* 26: 70-76.
- Tran Khanh T, Vazeille-Falcoz M, Mousson L, Tran Huu H, Rodhain F, Nguyen Thi H, Failloux AB 1999. *Aedes aegypti* in Ho Chi Minh city (Vietnam): Susceptibility to dengue 2 Virus and genetic differentiation. *Trans R Soc Trop Med Hyg* 93: 581-586.
- Tsuda Y, Takagi M, Wang S, Wang Z, Tang L 2001. Movement of *Aedes aegypti* (Diptera: Culicidae) released in a small isolated village on Hainan island, China. *J Med Entomol* 38: 93-98.
- Wang R, Zheng L, Touré YT, Dandekar R, Kafatos F 2001. When genetic distance matters: Measuring genetic differentiation at microsatellite loci in whole-genome scans of recent and incipient mosquito species. *Proc Nat Acad Sci USA* 98: 10769-10774.
- Weir BS, Cockerham CC 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.